

P001

## The Effect of the Introduction of the Criteria for the Use of Intravenous Immunoglobulin (IVIg) in Australia on the Supply of IVIg in Western Australia

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### Aim

To review the use of IVIg in Western Australia since the introduction of "The Criteria".

### Method

Review of IVIg requests and dosing information for WA patients using the ARCBS national STARS database and according to "The Criteria", which identify conditions for which IVIg is funded under the National Blood Agreement.

### Results

Extensive communications between ARCBS and treating clinicians by letter, e-mail, facsimile and telephone were necessary to discuss eligibility and dose. 150 patients from the initial 363 were non-conforming and of these 92 ceased treatment as they no longer qualified; 54 of these were "IgG subclass deficiencies" and none has needed to restart IVIg. 265 new patients commenced IVIg. 343 patients (280 long-term) are on treatment. 30 over-weight patients had lean-body weight dose adjustment using a formula with individual clinician agreement.

A total of 2132 Kg of IVIG were supplied nationally in 2007/8 and 2364 Kg in 2008/9 an increase of 11%. In WA 187 Kg were supplied in each year an increase of 0%

### IVIg Supplied (grams per 1000 population)

	2004/ 05	Var.	2005/ 06	Var.	2006/ 07	Var.	2007/ 08	Var.	2008/ 09	Var.
National	72.1	7%	81.5	13%	91.5	12%	100.6	10%	109.1	8%
WA	74.2	17%	78.8	6%	85.2	8%	87.8	3%	84.6	-4%

Variance compared with previous year actual supply

Source: ARCBS STARS database/Trend and Analysis report.

### Conclusion

"The Criteria" and introduction of lean body weight-based dosing have proved invaluable tools in enabling eligible patients to receive IVIg in the lowest effective doses. These measures have aided the ARCBS WA TMS team in working closely with local clinicians to minimise the inexorable increase in use of this scarce resource.

*No conflict of interest to disclose*

**P002**

## **Review of Massive Transfusion at a Regional Australian Hospital**

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### **Aim**

To undertake a retrospective critical review of massive transfusion practice at the LGH with a view to compliance with the massive transfusion protocol.

### **Methods**

The main objective of the massive transfusion protocol is to provide appropriate early blood component therapy in an effort to prevent exsanguination, coagulopathy and thrombocytopenia.

The application of the protocol is guided by the results of the diagnostic testing (FBC; Coagulation profile etc) together with the clinical parameters. The protocol is applied for the duration of the massive transfusion episode and is monitored and adjusted by the clinical haematologist in collaboration with the treating physicians as required.

A review of patient and laboratory records was undertaken to establish the compliance, utility and efficacy of the massive transfusion protocol.

### **Results**

In a 6 month period 12 cases of massive transfusion were treated at the LGH. The majority of the patients treated were male (10 of 12) and the average age was 64 years. A total of 185 units of packed cells and 85 units of FFP were transfused. The overall ratio of packed cells transfused to FFP was 1.0 to 1.1 and was consistent through the episodes examined.

### **Conclusions**

Our review showed that of the 12 cases in question the intervention was performed in an appropriate time frame with the appropriate product support and complied closely with the massive transfusion protocol.

The aim of the massive transfusion protocol was achieved and patient outcomes were improved as a result.

*No conflict of interest to disclose*

P003

**Idiopathic Thrombocytopenia Purpura (ITP) and Intravenous Immunoglobulin (IVIg) –What We Have Found in Victoria**

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**Introduction**

ITP is an established condition for which IVIg can be used under the '*Criteria for the clinical use of intravenous immunoglobulin in Australia*'. The Criteria permit a dosage range of 1-2g/kg. IVIg is a precious resource made from volunteer plasma donations. ARCBS Transfusion Nurses (TN) play an important role in IVIg management.

**Aim**

- Review the number of ITP patients treated in Victoria, the dose/patient for each ITP classification and response to therapy
- Establish the resources required for review of IVIg use in this condition.

**Method**

The qualifying and review criteria for ITP require pre-treatment platelet count, and response to therapy; including platelet increment. TNs undertake telephone follow-up with treating clinical and laboratory staff to confirm platelet increment, and response to bleeding at or after 48hours. Platelet counts are recorded in the ARCBS national database (STARS). TNs keep a log of all calls and time taken.

**Results**

Data for December 2008 – June 2009 indicate a total of 182 patients treated for ITP in Victoria, most commonly for refractory ITP (51%, 91patients). Sixty two (34%) patients received multiple doses. The average IVIg dose was 0.9g/kg, for both adults and children. Follow-up platelet counts were available in 68% treated patients and of these 86.3% showed response and 13.7% showed no response. For this period, 195 follow-up telephone calls were made for follow-up of this indication alone.

**Conclusions**

The data available from Victoria suggest that a dose of around 1g/kg IVIg is effective in most patients with ITP. Close working relationships and good communication between ARCBS TNs and local clinicians facilitate individual patient management to support minimum effective IVIg dosing and careful review. These data may help inform clinical practice changes and review of *Criteria* dosing recommendations in ITP.

*No conflict of interest to disclose*

P004

## The Use of Intravenous Immunoglobulin (IVIg) in Renal Transplantation – The Victorian Experience

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### Introduction

IVIg is a precious blood product made from human plasma. IVIg is used in renal transplantation (tx) to (1) overcome sensitisation to HLA antigens, (2) support ABO-incompatible tx, and (3) manage rejection. These have been considered “emerging” indications and included in the national ‘Criteria for clinical use of intravenous immunoglobulin in Australia’.

### Aim

Review IVIg use in renal tx in Victoria, from March 2007 - March 2009, including growth in use, to inform treatment decisions and planning.

### Method

Data extracted from the ARCBS Supply Tracking and Reporting System (STARS) for March 2007-08 were compared to March 2008-09 (after introduction of the Criteria) and analysed to determine total grams used, average age/ weight/ dose/ treatment episodes, and available clinical & laboratory information.

### Results

103 patients in 2007-08 and 138 in 2008-09 received IVIg for renal tx indications at 6 co-ordinating centres (some infusions given at regional centres), an increase of 25% (some patients treated more than once). Average dose increased from 54g to 112g (increase 52%). Average weight (74kg) remained constant. The average number of treatments/patient increased from 1.9 to 2.26 (16%). Average age was ~46 years, approx 60% male. Total IVIg used in renal tx was 21,268g in 2007-08, and 33,290g in 2008-09 (increase 36%). Overall outcomes have been promising. A template IVIg request/review form is being revised with treating centres to capture more comprehensive clinical review data.

### Conclusion

IVIg use in renal transplantation in Victoria has increased by 34% between 2007 and 2009, due to increased clinical experience and number of complex transplants undertaken. The impact of this growth should be considered in future IVIg demand planning. Close clinical liaison and communication is critical to managing patient needs. Documents are being revised to be more user friendly and comprehensive.

*No conflict of interest to disclose*

A239

## P005

## Update on Provision of Low Titre Anti-A and Anti-B Group O Apheresis Platelets

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### Aim and Background

In December 2008, ARCBS commenced national testing of group O apheresis platelets for high titre anti-A and anti-B. The absence of high anti-A and anti-B titres has been used to identify components with a lower risk of causing clinically-significant haemolysis due to anti-A or anti-B if transfused to a non-group O recipient. We review progress of provision of components which can preferentially be considered for use in circumstances where ABO-identical apheresis platelets are unavailable.

### Method and Results

Since testing commenced, in SA 1203 units and 164 Tasmanian units have been tested. ARCBS red cell phenotyping laboratories perform this manual testing, with significant additional workload. As these components are often distributed as part of the overall supply of group O platelets held at major hospitals, transfusion services have ready availability of these products without having to specifically request them. This has been welcomed by our end users.

MONTH	SA			TAS		
	TOTAL TESTED	LOW ANTI-A/B (NEG FOR HIGH TITRE)	%	TOTAL TESTED	LOW ANTI-A/B (NEG FOR HIGH TITRE)	%
DEC	162	87	53.7	22	10	45.5
JAN	193	120	62.2	35	16	45.7
FEB	188	116	61.7	15	8	53.3
MARCH	162	103	63.6	29	14	48.3
APRIL	159	106	66.6	16	9	56.3
MAY	174	118	67.9	22	13	59.1
JUNE	165	115	69.7	25	17	68
TOTAL	1203	765	63.6	164	87	53

### Conclusion

Availability of low-titre group O apheresis platelets is supported by routine testing at ARCBS SA laboratories (and nationally) and gives greater flexibility where ABO-identical platelets are not available.

*No conflict of interest to disclose*

**P006****Preoperative Haematological Abnormalities Relating to Perioperative Transfusion Requirements****Annmarie Bosco**, Susan Maccallum*South Eastern Area Laboratory Services, Prince of Wales Hospital, Randwick, NSW***Aim**

To determine the rates of preoperative haematology testing and abnormalities within a local surgical population, and their relationship to rates of perioperative transfusion.

**Method**

A retrospective analysis was conducted on a cohort of local surgical cases excluding minor cases which would not have routine preoperative testing. Preoperative haematology parameters and perioperative transfusion profiles were obtained where available. Pearson's chi-square test was performed to determine parameters that were significantly associated with transfusion.

**Results**

548 surgical cases were performed at our institution in the month of September 2008. 333/548 (61 %) of all procedures had at least one haematology parameter tested preoperatively. There were no transfusions in the cases that did not have preoperative blood tests. Within available results, 88/330 (27%) cases were anaemic, 27/328 (8%) of cases were thrombocytopenic and 42/183 (23%) cases had at least one abnormality of PT, INR or APTT. Only 14 out of 88 anaemic patients (15.9%) had iron studies performed and 3 (21.4%) were iron deficient. Overall, 57/548 (10%) cases received a transfusion, 19/57 (33%) of these were anaemic. 10/38 (26%) of anaemic females were transfused and 9/50 (22%) of anaemic males were transfused. Preoperative anaemia was significantly associated with increased allogeneic transfusions in the female population ( $p = 0.043$ ) but not in the male population ( $p = 0.772$ ). Thrombocytopenia ( $p = 0.06$ ) or coagulation abnormalities ( $p = 0.272$ ) were not significantly associated with transfusion.

**Conclusion**

Preoperative anaemia is associated with an increased risk of perioperative allogeneic blood transfusions, especially in the female population. Few patients have their anaemia investigated with tests such as iron studies. This reinforces the need for thorough preoperative haematological assessment.

*No conflict of interest to disclose*

**A241**

P007

## Prothrombinex Usage Canberra Hospital

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### Aims

To determine if Prothrombinex (Prothrombin complex concentrate, CSL) use within a tertiary hospital is aligned to clinical practice guidelines and identify potential for practice improvement.

### Method

Forty consecutive patients issued Prothrombinex were identified from the transfusion database at a 500 bed tertiary hospital between 01/01/2007 and 20/04/2009. Clinical records were examined for diagnoses, clinical indications, concurrent therapies and responses to therapy. Findings were compared to warfarin reversal guidelines. Patients with Haemophilia B were excluded.

### Results

Of the 40 Prothrombinex issues, 7 were excluded: 5 due to insufficient clinical documentation and 2 where the Prothrombinex was not administered and returned to stock. (n=33). In 30 cases Prothrombinex was used for warfarin reversal, in 2 cases for coagulopathy of liver disease and one for massive transfusion. The majority of episodes (28) aligned with guidelines, although five did not. Reasons for Prothrombinex use outside recommendations included coagulopathy of liver disease (2), massive transfusion (1) and near-normal INR results (2). Consultation with a haematologist or haematology registrar ensured alignment to guidelines, although consultation was not always undertaken prior to other interventions for warfarin reversal. Where haematology consultation did not occur (n=16) 37% did not align with guidelines. Fresh frozen plasma (FFP) usage ranged between 1 and 6 units. More than 2 units of FFP were given during 15 episodes, indicating that Prothrombinex is being used in addition to, rather than in place of, large volume plasma replacement.

### Conclusions and Recommendations

Prothrombinex was used for inappropriate indications, including coagulopathy unrelated to warfarin and for trivial elevations in INR. Transfusion laboratory scientists should recommend consultation with haematologist if patient is not on warfarin, rather than issue Prothrombinex and for warfarin reversal where excessive FFP has been requested.

*No conflict of interest to disclose*

**P008****Potential Effectiveness of Blood Transfusion Demand Control Strategies****Philip Crispin**<sup>1</sup>, Maria Burgess<sup>2</sup>, Therese Crispin<sup>3</sup>

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**Aim**

To determine the potential reduction in blood utilization during blood supply limitation in the ACT with different contingency plan strategies.

**Method**

The ACT Haemovigilance and ACT Pathology transfusion databases were reviewed from March to September 2003. All transfusion episodes were prioritised in accordance with the Australian National Blood Supply Contingency Plan. The number of red cell transfusions related to elective surgery was determined. The strategies were compared for their potential to reduce red cell transfusion demand.

**Results**

There were 894 transfusion episodes, accounting for 2008 units of red cells, in the haemovigilance database. A further 14 episodes of massive transfusion were identified from the pathology database. This accounted for an estimated 70% of all red cell transfusions in the ACT. After correcting for the number of red cells transfused at each hospital, red cells were prioritised as category 1 in 59%, 2 in 27% and 3 in 13%. The remainder had insufficient data for classification. Transfusion for elective surgery accounted for 14.7% of red cells used, with 9.0% rated category 3 under the contingency plan.

There were 17.3% of red cells transfused for inappropriate indications, when reviewed against NHMRC Guidelines. After excluding inappropriate transfusions, cancelling elective surgery could potentially save a further 5.5% and 4.3% of blood utilisation for category 3 and 2 patients respectively. Significant differences were found between hospitals.

**Conclusion**

Targeting inappropriate transfusions by vetting prior to issue not only re-directs blood away from those unlikely to benefit, but may also conserve more blood than cancelling or postponing elective surgery during times of supply limitation. Contingency planning needs to accommodate the variable case-mix in hospitals, and may be better coordinated at a jurisdictional level.

*No conflicts of interest to disclose*

**A243**



P009

## Anti-In(b) Highlights Australia's New Repertoire of Challenging Red Cell Polymorphisms

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### Objectives

This case of a clinically significant red cell antibody to a high incidence antigen new to South Australia emphasises the importance of developing strategies for supply of newly appearing blood types. With increasing ethnic diversity in Australia it emphasises the need for increased surveillance of rare polymorphisms and the development of recommendations for management.

### Background

A newly arrived 29 year old Indian migrant, slightly anaemic in early pregnancy, presented at the Women's and Children's Hospital early 2009. Routine screening detected an antibody strongly reactive with all red cell panel members using Diamed and tube methods. On referral Anti-In(b) was detected by Melbourne Red Cross Reference Laboratory.

The Indian (In) blood group system consists of low incidence In(a) and high incidence In(b). The frequency of In(b) antigen within the Australian Caucasian population is approximately 99%. Hence Anti-In(b) is rare in Australia. The literature indicates that Anti-In(b) rarely causes severe HDN. However, because of reports of it causing haemolytic transfusion reactions, this case was closely monitored.

### Results and Discussion

South Australia's first case of Anti-In(b) was found on routine antenatal screening. Confirming the unavailability of emergency blood Australia-wide suitable for this patient the laboratory found that of 12 random red cell units matched, all were incompatible (score 3). Hence the case presented significant clinical risk. To mitigate this risk the treating obstetricians devised emergency strategies to treat significant perinatal anaemia.

Key issues for discussion emerge:

- need for clear clinical strategy to manage pregnancies with rare red cell antibodies;
- difficulty of sourcing compatible antigen negative blood for the mother in the case of maternal or, less likely, neonatal complications / haemolysis;
- expansion of the repertoire of stored rare blood types at ARCBS;
- responsibility of manufacturers to provide access by reference laboratories to red cells representative of Australia's current population.

*There is no conflict of interest to declare*

P010

**Rhesus and Kell Phenotype Matched Units for Young Women****Emily Hinds**, Gina Aitken*Transfusion Medicine, Royal Hobart Hospital, Tasmania, Australia***Introduction**

Whilst performing a previous study on Duffy typing of Refugee and Humanitarian Arrival Clinic (RAHAC) patients it was noted that a large number had a Rhesus (Rh) phenotype of cDe. In order to minimise the formation of antibodies it would be preferable to give these patients Rh phenotype matched units. There are difficulties encountered in selectively phenotyping this refugee population. It is also ethically questionable whether one section of the community should receive a service that is not offered to all.

**Aim**

To determine the feasibility of issuing Rh and Kell phenotype specific units to women of childbearing age.

**Method**

The Rh and Kell (K) phenotype of all potentially transfused women of childbearing age (under 50) was determined over a 6 month period. These were determined using the Ortho Biovue® System. For the month of June, the Rh and K phenotypes of our red blood cell (RBC) stock were recorded as stated on each unit. K positive RBCs were excluded from the investigation. The Rh and K phenotypes of the patients were compared to the phenotypes of the RBCs in stock to determine the probability that phenotype matched RBCs could be issued.

**Results**

Of the Rh(D) negative blood groups all patients were true negatives, as were at least 94% of RBCs in stock. Of the O Positive patients 5 of 56 (9%) had a cDe phenotype, however only 1 of 50 (2%) of O Positive RBCs in stock on any given day were a match. For the remaining Rh(D) positive blood groups the frequency of patient phenotypes matched that of the RBC phenotypes.

**Conclusion**

It would be feasible to issue Rh and K phenotype matched RBCs with minimal effort. However there may be logistical difficulties with implementation that include compliance issues depending on the laboratory information system (LIS) used.

*No conflict of interest to disclose*

P012

**Pathogen Reduction Technology: An Alternative to Leukodepletion?****Lacey Johnson<sup>1</sup>**, Kelly Winter<sup>1</sup>, Ray Goodrich<sup>2</sup>, Denese Marks<sup>1</sup><sup>1</sup>*Australian Red Cross Blood Service, Sydney, NSW, Australia*<sup>2</sup>*CaridianBCT Biotechnologies LLC, Lakewood, CO, USA***Background**

Blood products are leukodepleted and/or gamma irradiated prior to transfusion to reduce the incidence of severe leukocyte-mediated immune responses in the recipient. Pathogen reduction technologies (PRT) such as the Mirasol® system produce irreparable nucleic acid modifications following exposure to UV light in the presence of riboflavin, and thus have the ability to inactivate contaminating leukocytes through DNA damage in addition to a pathogen reduction function.

**Aim**

This study aimed to evaluate the ability of Mirasol Generation III, which can accommodate platelet additive solutions, to inactivate leukocytes.

**Method**

PBMC and granulocyte populations were isolated from the disposable cassette of a plateletpheresis set, followed by Mirasol illumination in plasma/SSP+. The viability and function of PRT treated cells were compared to untreated cells. The assays used include immunophenotyping, T cell activation by PMA, proliferation in response to mitogens, cytokine production, phagocytosis, oxidative (respiratory burst) and chemotactic activity.

**Results**

The results indicate that Mirasol treatment does not significantly alter the distribution of leukocyte sub-populations. However, Mirasol treatment does greatly reduce leukocyte viability and the ability of lymphocytes to become activated and proliferate in response to standard stimuli, such as CD3/CD28 and PHA-M. Further, granulocyte migration also appears to be reduced by Mirasol treatment.

**Conclusion**

Mirasol PRT technology is capable of functionally inactivating leukocytes in platelet additive solution. Therefore, this technology may offer an alternative to current leukodepletion for reducing immunologic consequences of platelet transfusions.

*This research was, in part, supported by Caridian BCT. This abstract was reviewed by a company representative prior to submission.*

**P013**

## **Safe Handling of Blood Products in Theatre...A Single Institution Retrospective Audit**

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### **Aim**

Appropriate storage and handling of blood products is essential for safe transfusion practice. National guidelines are stringently adhered to in the hospital blood bank, but upon leaving blood products may reach temperatures outside specification prior to patient infusion. We retrospectively audited the compliance of blood storage and the correct tracking of blood units in the theatre fridge at our institution.

### **Method**

Dandenong Hospital is an acute care tertiary hospital that supports traumas and major surgeries. A blood product fridge is located in theatre and a comprehensive documentation system is in place to ensure safe storage and traceability of blood components. The records of units issued to the Operating Theatre from 16<sup>th</sup> January 2009 to 28<sup>th</sup> March 2009 were audited to establish compliance to national guidelines.

### **Results**

Of the 151 units issued over this time period, the audit trail was incomplete in 111 cases. Multiple documentation errors occurred including –

- non entry of the products into the theatre record book
- discrepancy in the time that the blood was issued from the laboratory and entered in the theatre record book
- missing data on removal from the theatre fridge
- missing data for returns to the theatre fridge

In some cases despite an unrecorded time interval of over 30 minutes, unused blood was still returned to the Blood Bank stock. An incomplete audit trail was seen with 31 donor units which were returned to stock and subsequently transfused to other patients. There were no reported adverse events.

### **Conclusion**

Despite having a documentation system that ensures full traceability, there was very poor compliance by the theatre staff. Our audit demonstrates that ongoing education for theatre staff, including the risks of bacterial contamination in non-storage compliant units, is crucial to ensure guideline compliance.

*No conflict of interest to disclose*

P014

## The ARCBS STARS Database: Improving the Collection and Reporting of Data on the Use of Intravenous Immunoglobulin in Australia

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### Background and Aim

ARCBS developed and introduced a national intranet web-based database (Supply Tracking Analysis Reporting System, STARS) to collect data on the clinical use of intravenous immunoglobulin (IVIg) in Australia. Aims included replacement of state-based Microsoft Access™ databases, ability to follow 'nomad' patients, inclusion of rules to ensure issue of appropriate IVIg product, consistent and comprehensive data collection and reporting.

### Method and Results

Following a normalisation of required data the database was set up using Oracle™. Process flow was mapped and input/output screens were designed and built using HTML, AJAX and Java. The system operates on a client/server basis and rules are run on the client. The password protected database sits securely behind the ARCBS firewall. Data collected include patient demographics, clinical history, order and usage.

Reporting is available using a versatile report matrix that automatically constructs SQL (query) statements. STARS provides data for reporting to ARCBS, the National Blood Authority and state IVIg user groups. Reports currently available include use by disease, product, average dose, population, sex and age.

As an example, analysis of STARS data reveals the sex and age cohort differences in the amount of IVIg issued for haematological conditions. Females aged 20 to 39 years have a higher volume of IVIg issued than men in the same range, while this is reversed in the older age range (45 to 84 years). Further analysis reveals reasons for the difference, which include support for indications in pregnancy, and reflect weight-based differences for the older cohorts, reflecting IVIg dosing according to patient weight.

### Conclusion

STARS has facilitated the collection of nationally consistent data and improved the ability to report IVIg use to end user groups and governments. Further analysis of IVIg data will continue to provide information that can shape future use of this valuable resource.

*No conflict of interest to disclose*

P015

## Expiry Rates in Context: Comparing Red Cell Losses in Four States Using the Electronic Returns Information Capture (ERIC) System

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### Aim

To determine whether transfusion laboratory red cell expiry is influenced by the number of red cell units issued and distance from ARCBS distribution point.

### Background

The ARCBS Electronic Returns Information Capture (ERIC) system collects data on component loss in transfusion laboratories and has been widely used for more than two years. Four states (South Australia, Tasmania, Queensland and Victoria) have >95% participation.

### Method

Data were analysed for July 2008 to June 2009 from 135 participating laboratories from the above four states were analysed.

Laboratories were placed into cohorts according to the time to deliver from ARCBS (<60; >60 <120; >120 minutes) and number of red cells received small (<200); medium (201 to 400); large (>400) units/month. Expiry, as a percentage of red cell issues, was calculated for each cohort.

### Results

The overall expiry rate was 4.4%. Expiry in small laboratories was 11.4%, medium 4.7% and large 2.9%. Small, distant (>120 minutes) laboratories had the highest expiry rate of 12.6%. The lowest expiry (2.8%) was at large, laboratories close to ARCBS depots. Group O Rh(D) negative red cells expired at the greatest rate (22.1%) in small distant laboratories.

### Conclusion

Analysis of expiry rates by distance and volume shows that distance from ARCBS and the number of red cells issued is associated with expiry rate. Group O negative expiry rates appear to reflect the increasing requirement for multiple small inventory holdings, especially but not limited to, outer metropolitan, regional and remote areas. ERIC reports are provided for institutional review. These data will contribute to better understanding of reasons for red cell expiry and inform discussion on expiry reduction strategies.

*No conflict of interest to disclose*

**P016****Clinical Appropriateness of Fresh Frozen Plasma Transfusion in Critically Ill Patients in a Liver Transplant Tertiary Medical Centre****Teresa Leung**, Cameron Knott, Slav Curcic, Carole Smith*Department of Haematology and Department of Intensive Care Medicine, Austin Health, Heidelberg, Victoria, 3084***Aim**

To assess clinical appropriateness of fresh frozen plasma (FFP) transfusion within an intensive care unit (ICU) of a liver transplant centre. Review of patient demographics, frequency, volume transfused, and an independent review of prescribed clinical indications by two experts were included.

**Method**

A retrospective audit of all episodes of FFP transfusions within ICU between August and October 2007 was performed based on ICU admission records and transfusion records from the laboratory information system, followed by reviewing individual inpatient clinical records. Appropriateness of transfusion was assessed by a consultant haematologist and transfusion nurse independently, based on National Health and Medical Research Council/ Australian and New Zealand Society for Blood Transfusion (NHMRC/ASBT) Clinical Practice Guidelines and documented clinical circumstances.

**Results**

33 patients with a total of 68 transfusion episodes were recorded within the designated period. The majority of patients were either post cardiac surgery (n= 13) or have chronic liver disease (n=6). The median volume of FFP transfused per patient was 600mls (2 units), with a standard deviation of 1879mls (7 units), likely reflecting the proactive approach taken for treating liver disease patients with coagulopathy. For each transfusion episode, the median amount transfused was 8.8ml/kg., which is lower than the recommended dosage of 10-15ml/kg by Australian Red Cross Blood Service (ARCBS). Independent assessment of clinical appropriateness reveals 60% of FFP transfusions are congruent with guidelines and clinically appropriate; 24% are incongruent with guidelines but clinically appropriate. Of the 16% incongruent and inappropriate transfusions, over 50% involved post cardiac surgery patients.

**Conclusions**

The study demonstrates that critically ill patients with liver disease and coagulopathy often require large volume FFP transfusions, which mostly has been appropriately prescribed. It also highlights the controversy of prophylactic FFP transfusions post cardiac surgery, and the tendency to underestimate the amount of FFP required for each transfusion. Ongoing education will help to modify future ordering practice.

*No conflict of interest to disclose*

P017

## **Bloodhound Revisited. A Transfusion Service Sub-analysis of Red Cell Usage Patterns**

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### **Aim**

To inform contingency planning for red cell shortage or demand surge with information from use in the private sector, we studied inventory management and clinical indication for red cell transfusion in a large private pathology provider in Victoria.

### **Method**

Retrospective data collection with tracking of all interim movement and final destination of tagged red cell units from the ARCBS Bloodhound audit issued to Melbourne Pathology Service (MPS) in 2007.

### **Results**

Tagged red cell units (424, 8.2% of all Bloodhound units) were sequentially allocated to a total of 551 patients. The majority (73%) had 1 allocation, but almost 7% had 3 or more allocations. Seven units expired without allocation and 29 units were discarded due to cold chain interruption. Mean duration within the inventory (accessible) or reserved for a patient (inaccessible) was calculated. Mean cumulative duration in inventory was 10 days. Mean individual allocation time was 4.6 days. The largest clinical areas of use were haemato-oncology (41%) and orthopaedics (18.9%). Red cells were required urgently (within 24 hours) 71.5% of the time in haemato-oncology but only 29.5% of the time for orthopaedics. Probability of transfusion was high (94.7%) in haemato-oncology, but low (31.4%) in orthopaedics. Probability of transfusion in orthopaedics was correlated with the haemoglobin at the time of crossmatch. Potentially deferrable units accounted for 20.5% (113/551) compared to 10% overall in Bloodhound.

### **Conclusion**

The documented pattern of movement and use of red cells reflects the demographic and geographic profile of the institutions serviced by MPS. Mean allocation time significantly exceeds crossmatch validity due to off site storage regardless of the likelihood of transfusion. This information could be used to model the impact of practice change in the private sector on blood availability and assist contingency planning for blood shortages.

*No conflict of interest to disclose*

A251



P018

## Providing On-line Transfusion Education to Nurses and Doctors: Experience and Learnings

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2. BloodSafe Collaborative, Australian Red Cross Blood Service, Adelaide

3. Blood, Organ and Tissue Programs, SA Department of Health

### Introduction

In late 2007 BloodSafe released an on-line education program for general nursing and medical professionals, to improve the safety and quality of clinical transfusion practice in Australia and to assist hospitals with accreditation requirements for transfusion training and credentialing.

### Uptake

This resource has been widely accepted with 24,485 users registered to June 30, 2009 from all states and territories of Australia (Table 1). A significant number of organisations including health services, health regions, universities and other organisations have promoted or made completion of this mandatory. User testing and unsolicited feedback has been extremely positive particularly around the instructional design, use of video and interactivity, and case studies.

### Learnings

Problems encountered have included completion certificates not being received by users due to classification by internet service providers as spam, email addresses not provided to all staff by hospitals and a perception that personal email addresses cannot be used, and typographic errors by users during the registration process. Solutions and support have been/are being developed to address these challenges.

### Conclusion and Future Directions

Submissions are currently underway to provide long term funding and strategic direction. This will provide staffing for support and further development including additional content, promotional strategy and tools, and a comprehensive evaluation of the effectiveness of this tool.

**Table 1: Registrations by Jurisdiction**

South Australia	6,541
NSW	7,569
Queensland	5,747
Victoria	2,008
Western Australia	1,219
ACT	684
Tasmania	672
Northern Territory	45
<b>Total Australia</b>	24,485
<b>Total International</b>	500

*No conflict of interest to disclose*

A252

P019

## Comparison of Antenatal Antibody Titres by Automated Analyser and Manual Tube Technique

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### Aim

Antenatal testing is essential to minimise the incidence and severity of Haemolytic Disease of the Newborn (HDN) by identifying females with clinically significant alloantibodies to red cell antigens. Titration is used to determine this. It involves a series of doubling dilutions of plasma, whereby the volume of each dilution is tested against selected red cell suspensions. This monitors the antibody level, thereby providing clinicians with appropriate information for the management of their patients. Titration by tube (NICE) has been the recommended technique by ANZSBT. However, with the introduction of automated column agglutination techniques this may provide more standardised results.

### Methods

Master dilutions ranging from 1- 2048 were prepared using 1mL of 5% bovine albumin and 1mL of patient plasma for the Autovue. For the NICE method, aliquots from the master dilution were transferred to test tubes. Both methods were then tested against test cells for the antibody under investigation.

### Results

The end points from the Autovue were slightly elevated by 2 or 3 serial dilutions compared to those of the tube method. Of the 16 anti-D titres processed on the Autovue 7 (44%) were > 32 (above clinical range), whereas when tested by tube 9 (56%) were < 32. Other antibodies (anti-CD, anti-Ec, anti-c, anti-s, anti-S, anti-Fya, anti-E, anti-Jka, anti-M and anti-K) were also tested of which 21% were > 32.

### Discussion

The NICE method set out in the ANZSBT guidelines interprets the endpoint of the reaction as being a score of 5, whereas the endpoint for automated titres is a grade 1. When assigning numerical values to reactions a difference in antibody level between the two methods can be seen.

### Conclusion

The automated titre is effective in producing standardised results because it is not predisposed to bias and results are easily reproduced.

*No conflict of interest to disclose*

**P021****Evaluation and Further Management of Acquired Type of Thrombotic Thrombocytopenic Purpura Which Has Responded to Plasma Exchange Poorly****Hansa Ramanayake***National Blood Center, Sri Lanka***Background**

Observations of the presenting features and clinical course of TTP suggested a pentad of clinical features for diagnosis and now only thrombocytopenia and microangiopathic hemolytic anemia are sufficient criteria to establish a clinical diagnosis and begin treatment with Plasma Exchange. A severe deficiency of ADAMTS13 (a disintegrin-like and metalloprotease with thrombospondin type 1 repeats) less than 5% of normal activity, may be specific for TTP. ADAMTS13 deficiency caused by an autoantibody provides a possible explanation for the effectiveness of plasma exchange and a role for ADAMTS13 activity measurements to guide treatment decisions has been suggested. However, the sensitivity of severe ADAMTS13 deficiency in patients with idiopathic / sporadic or acquired TTP can be clinically suspected as they respond poorly to plasma exchange with persistent high LDH level and very low platelet count and acute relapses during plasma exchange.

**Aims**

To evaluate and further follow up and management of TTP patients with poorly responds to Plasma exchange.

**Method**

A total number of Two patients (who had responded poorly and had longest TPE cycles) out of ten patients with provisional diagnosis of TTP were selected and referred to Specialized unit in London University Hospital to detect the ADAMTS 13 activity levels and further management.

**Results**

Total No of two patients responded poorly and had 24 and 40 Plasma Exchange cycles respectively. ADAMTS 13 activity of these two patients were less than 5 % and those two patients were started with Rituximab 375 mg/m<sup>2</sup> slow iv infusion quarter weekly for 4 weeks until the ADAMTS 13 activity reaches more than 80 % .

**Conclusion**

A severe ADAMTS13 deficiency can be clinically suspected as they respond poorly to plasma exchange and a role for ADAMTS13 activity measurements to guide the treatment decisions has been suggested and long term follow up for complete remission is essential.

*No conflict of interest to disclose*

**P022****Evaluation of Positive Direct Antiglobulin Test in Patients with Auto-Immune Hemolytic Anaemia and Provision of Transfusion Support****Hansa Ramanayake***National Blood Center, Sri Lanka***Background**

Auto-Immune Hemolytic Anemia (AIHA) are characterized by decreased red cell survival and presence of auto antibodies directed against red cell antigens. The Direct Antiglobulin Test (DAT) with other serological investigations carried out in blood bank will help to determine the type of haemolysis. And this study has been conducted to evaluate of positive DAT and to select blood product for transfusion support of patients with AIHA.

**Study Design and Methods**

Total number of 99 consecutive patients diagnosed as AIHA with a referral to reference immune haematology Laboratory In National Blood Center for serological investigation with DAT and for red cell products were retrospectively analyzed.

**Results**

86 adult Patients and 13 pediatric patients were included in this study. Warm Auto-immune Hemolytic Anemia (WAIHA) was the most common type auto immune hemolytic anaemia with both C3d and IgG specificities. Cold Agglutinin Syndrome (CAS) is the second common type and only C3d specificity could be found. Paroxysmal cold Hemoglobin Urea (PCH) which was found only in pediatric population in this study consisted only C3d specificity in positive DAT. Mixed type of AIHA and drug induced AIHA could be the minor categories of this study.

**Discussion**

The DAT will be helpful to determine the type of AIHA and to select blood products for transfusion support and to avoid unnecessary transfusions.

*No conflict of interest to disclose*

**P023**

## **Implementation of a Novel Statewide Ordering and Receipting Blood System (ORBS)**

**Geoff Simon**

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### **Background**

ORBS is a web based ordering and receipting system that was designed, developed and implemented by the Queensland Blood Management Program (QBMP) in conjunction with key stakeholders including the Australian Red Cross Blood Service, National Blood Authority, and private and public pathology organisations. It facilitates electronic ordering and receipting of blood and blood products in both public and private sectors, and has replaced manual fax based systems.

### **Method**

ORBS was implemented across the private and public sectors in Queensland over a thirteen week period from 1 September to 3 December 2008. Training and on-site 'go-live' support was provided by two staff members of the QBMP.

### **Result**

ORBS is now operational in sixty-six laboratories spanning five organisations. In addition to process enhancements, the system has led to quality improvements in the distribution process and provides valuable data for understanding and managing the blood supply. Work is underway to further enhance the system through the development of interfaces to laboratory and other computer systems.

### **Conclusion**

Lessons learned during implementation of the novel State-wide "Ordering and receipting Blood System" system will be outlined. Results of a 'User Satisfaction Survey' conducted six months post-implementation will be presented. Data sets and information now available to support the management of the blood supply, including 'real life' examples of interest to transfusion laboratory personnel and users of transfusion services, will support discussion on the merits of the system.

*No conflict of interest to disclose*

**P025**

### **Autologous Fibrin Glue – Successful TGA Licensure**

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Autologous fibrin glue also known as autologous fibrin or tissue sealant has been provided to patients by the Haematology Department of the SA Pathology – Royal Adelaide Hospital (formally the IMVS) since 1989. Nearly 2000 patients have been treated with product manufactured by this department.

Provision of this product includes autologous blood collection and subsequent manufacture and storage prior to dispatch to surgical theatre. This product consists of two components which when mixed form an adhesive coagulum that assists surgical procedures.

Initial manufacturing methods were based on those described by Siedentop KH et al utilising ammonium sulphate protein precipitation for the isolation of fibrinogen and pharmaceutical bovine-source thrombin. To achieve regulatory compliance, we currently utilise an in-house developed method based upon Cohn's protein fractionation for fibrinogen isolation and a proprietary method developed by our department for isolation of autologous thrombin. None of these steps involve use or incorporation of any animal products or reagents thereby rendering the product completely autologous.

Our method for the manufacture of autologous fibrin glue has been developed under cGMP principles and as such all related steps and procedures are compliant to the current Code of GMP – Human Blood and Tissues. This procedure has also undergone successful TGA audit and as such our facility is licensed to manufacture and supply this product nationally. To our knowledge this is the first facility world-wide that has successfully passed regulatory licensure for the manufacture of autologous fibrin glue.

This paper will detail the experiences leading up to successful licensure.

*No conflict of interest to disclose*

**A257**

P026

## Transfusion Data Collection in Australia – A National Approach

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### Introduction

In Australia, there is no national, systematic collection of information around the clinical use of fresh blood components.

The Blood Measures Project is a collaborative between the National Blood Authority (NBA) and the Australian Red Cross Blood Service (ARCBS). It aims to develop a national set of standard data definitions and parameters, which would allow results from independent studies/audits/projects to be compared meaningfully.

### Methods

Phase One involved desktop research, with a review of the range of indicators of blood and blood product use in Australia and internationally. An extensive literature review was undertaken, along with review of Australian and international Clinical Practice Guidelines in transfusion.

A National Working Group was formed, consisting of clinical experts, transfusion scientists, transfusion nurses, epidemiologists, government representatives and other relevant stakeholders. The *Blood Measures Guide* was then developed through a series of workshops and consultations.

### Results

The *Blood Measures Guide* consists of six chapters which describe data measures and definitions, recommended in relation to demographic and pre-transfusion patient information, the fresh blood components transfused (red blood cells, platelets, fresh frozen plasma and cryoprecipitate), and the outcomes of transfusion. Primary measures are a menu of data elements ideally collected in all studies of blood component usage, whilst the supplementary measures could be also be collected, in addition to the primary measures, where more information is needed.

A consultative draft *Blood Measures Guide* was then placed on the NBA website for public use and comment and is currently available. The *Guide* has been designed to be an easy reference for clinicians, transfusion practitioners, auditors and researchers. It is anticipated that the set of standard measures within the *Guide* will be used in audits, quality assurance activities, clinical registries, research projects, clinical trials, and surveys of usage and practice.

### Conclusion

The Blood Measures Project facilitates the collection of consistent and standardised data on the clinical usage of fresh blood components. The *Guide* is the set of these nationally endorsed measures and data definitions, arising from the Project. This *Guide* can now serve as a resource for those conducting investigations, trials and reviews of transfusion medicine practice. It is anticipated that, following an initial period of use, review and comment by the clinical and scientific community, the *Guide* will be revised and a final version published.

### Acknowledgements

We would to acknowledge the members of the National Working group and thank them for their significant time and efforts.

*No conflict of interest to disclose*

**P027****A Case of Drug Induced Haemolytic Anaemia with Blood Grouping Problem****Shaba Vakalia, David Roxby***Blood Transfusion Service, Flinders Medical Centre, Bedford Park, South Australia***Introduction**

Drug-induced haemolytic anaemia (DIHA) is an acquired form of haemolytic anaemia due to the interaction of certain drugs with the immune system resulting in antibodies directed against red cells. We describe a case of a 62-year old male with a history of gout, autoimmune haemolytic anaemia and a blood group anomaly.

**Case Report**

On presentation he was found to have severe anaemia, dark urine, jaundice, lumbar and abdominal pain with no history of transfusion. Three days prior to admission the patient commenced Diclofenac (Voltaren) 50mg. Presenting laboratory features included haemoglobin 32g/L (N: 130-175g/L), CRP 143mg/L (N: < 6 mg/L), lactate dehydrogenase 2259U/L (N: 115-200 U/L) and bilirubin 140umol/L (NV < 20 umol/L). A crossmatch for four units of red blood cells was requested. Using column agglutination and tube technique the patient's forward cell group was AB Positive. However strong agglutination was present in the reverse group against A1 and B cells. The indirect antiglobulin antibody screen (IAT) and direct antiglobulin test (DAT) were positive with anti-IgG/anti-C3d. Four O negative red cells were transfused. Antibody investigation and elution studies showed the presence of a non-specific autoagglutinin. Therapeutic plasma exchange was performed on three consecutive days. Following the second plasma exchange the group and screen was repeated and the patient's blood group was confirmed as AB Positive. The IAT and DAT remained positive. DIHA to Diclofenac was suspected. The presence of antibodies to Diclofenac was confirmed using alloabsorbed plasma. Diclofenac was discontinued and the patient's condition improved. In a sample collected 6 months after discharge, no drug related antibodies or other red cell related autoimmune antibodies were detected by IAT. The DAT was also negative.

**Discussion**

Although the clinical and the serological findings were variable and suggested idiopathic auto immune haemolytic anaemia, as a part of the differential diagnosis DIHA should be considered when investigating haemolytic anaemia. The above case report demonstrates the possible side effects of this drug and emphasizes the need for an increased awareness of drug induced haemolytic anaemia and complications of drug therapy.

*No conflict of interest to disclose***A259**



**P028****Comparison of Antibody-Absorbing Columns in Relation to Incompatible Kidney Transplants****Ofira Waldispuhl**, Susan Finch*PathWest Laboratory Medicine, Royal Perth Hospital, Western Australia***Background**

Shortage of cadaveric organs, especially renal transplants is a global problem. One solution to the increased discrepancy between the number of end-stage renal disease patients on waiting lists and the number of available deceased donor kidneys is to expand the donor pool. This can be achieved by expanding the criteria for accepting living donors and by overcoming the immunological barriers of ABO incompatibility. Blood groups antigens are expressed on the endothelium of solid organs and transplantation across a blood barrier can result in hyperacute or acute antibody-mediated rejection. Humoral rejection seems to correlate closely with pre-transplant antibody titre. This calls for exact measurements of ABO antibodies in recipient's serum and is critical for a successful ABO incompatible kidney transplant.

**Aim**

To study the effectiveness of two different antibody-absorbing columns, the Glycosorb® column, which specifically depletes Anti-A or Anti-B immunoglobulins, and the Evaflux™ plasma filter, which depletes a range of immunoglobulins based on their molecular weight. A titre of 8 or less immediately pre-transplant was required for the transplant to proceed.

**Method**

Isohaemagglutinin titre levels were determined by serological tube technique using the National Immunohaematology Continuing Education (NICE) method. Revercells™ (CSL) were suspended in PBS as a source of blood group antigens. Tests were incubated for 30 minutes at 37° C and then converted to the anti-human globulin (AHG) test phase.

**Result**

Both filters effectively reduced the anti-A titre. While only five patients have been studied to date, the limited data indicates that there may be a difference in the efficacy of the filters. The patient's ability to replenish depleted antibody also appeared to be dependent on the type of filter used.

**Conclusion**

The Glycosorb® filter appeared to be more efficient at reducing antibody titre compared to the Evaflux™ filter, however the Evaflux™ filter appeared to be more effective at limiting antibody replenishment post filtration.

*No conflict of interest to disclose*

P029

## Identification of Proteins that Accumulate in the Supernatant of Platelet Concentrates During Storage

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Platelet transfusion has been implicated in adverse reactions. During platelet storage, soluble factors such as plasma proteins and bioactive molecules released from platelets are likely to play a critical role in transfusion associated adverse reactions.

### Aim

The purpose of this study was to identify the soluble proteins that accumulate in the supernatant of platelet concentrates (PCs) during storage by using a comprehensive proteomics approach.

### Method

Prestorage leucocyte depleted pooled buffy-coat PCs in SSP+ platelet additive solution (PAS, MacoPharma) were prepared and stored in accordance to standard blood bank procedures. Platelet samples were collected at days 1, 3, 5, and 7 days of storage. Proteins in the supernatant of PCs were identified by two dimensional (2D) gel electrophoresis and cytokine antibody microarrays. Cytokines and bioactive molecules were quantitated by ELISAs.

### Results

A number of proteins appeared to accumulate in the PC supernatant over storage as assessed by 2D gels. Interestingly, the proteins that accumulated over storage in PCs in SSP+ PAS were different to those identified in our previous study of PCs in T-sol PAS. Microarray analysis suggested that the platelet derived proteins BDNF, ENA-78, GRO, PDGF, and EGF accumulated in the supernatant of PCs over storage and this was verified by ELISA. BDNF, ENA-78, GRO and EGF showed significant accumulation between days 1 and 5 of PC storage and PDGF by day 7 ( $p < 0.05$ ). In addition, the levels of RANTES and sCD40L (two proteins reportedly associated with adverse transfusion reactions) showed significant accumulation over storage. Encouragingly, the concentrations of these proteins were significantly lower in PCs in SSP+ than PCs in the previous generation PAS, T-sol.

### Conclusion

Further investigations is required to ascertain the biological and clinical significant of the accumulation of these bioactive proteins during storage of PCs. These results provide an expanded view of storage associated changes and may lead to a greater understanding of the factors that contribute to adverse transfusion reactions.

*No conflict of interest to disclose*

A261

## P030

## Six Patients with Acquired Haemophilia A Successfully Treated with Rituximab

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### Introduction

To describe six patients with acquired haemophilia A treated successfully with Rituximab in South Australia.

### Method

Clinical information was provided by the treating physicians, and both treatment modalities and responses were correlated with laboratory results.

### Results

Six patients (5 females, 1 male) with median age 62.5 years (range 24-84) presented with prolonged median APTT 83.5s (range 62 to 99). Four patients had inhibitor titre between 10-12 BU/ml; another two patients 16.5 and 460 BU/ml; the median baseline FVIII was 0.04 iu/ml (range <0.01 to 0.18). All patients presented with bleeding, predominantly mucocutaneous bleed, one patient had muscle bleed which was initially diagnosed as deep venous thrombosis. One patient had recurrent retroperitoneal bleed. All bleeds were treated with recombinant VIIa, maximum cost was \$752,652 incurred for retroperitoneal bleeding.

	Type of Bleed	Etiology	Rx for AH
46F	retroperitonealX2	Myasthenia	pred+ivlg+ Rituximab +azathioprine
24F	muscle,	post-partum	Pred, Rituximab
78M	mucocutaneous	lymphoma	Rituximab combination chemo
45F	DVT, muscle	idiopathic	pred, ivlg, Rituximab
84F	haematuria,haemathrosis	idiopathic	pred,cyclo,Rituximab
83F	mucocutaneous, muscle	idiopathic	Pred, Azathio, ivlg, cyclo,Rituximab

Rituximab was used as second/third line agent after immunosuppressive and cytotoxic agents in four patients with recurrent bleeding/relapse, and intolerant of steroid; three also had immunoglobulin as an adjuvant treatment. One patient had Rituximab up-front because of underlying lymphoproliferative disease. All six patients achieved complete remission following Rituximab therapy.

### Conclusion

Monoclonal anti-CD20 antibody (Rituximab) treatment is used in lymphoma and autoimmune disorder such as Rheumatoid Arthritis. Acquired haemophilia A is a rare acquired bleeding disorder with auto-antibodies to FVIII, and Rituximab is currently being used for refractory/relapsed cases, rather than up-front because of its cost, however it would be cost-effective for antibody eradication in patients with high titre inhibitor or major bleed on presentation given the significantly higher cost of bypassing agent such as recombinant FVIIa to treat life-threatening bleeding.

*No conflict of interest declared*

P031

## Analysis of Blood Product Management and Outcome in Patients Having Massive Blood Transfusion in a Tertiary Hospital - A Retrospective Study

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### Aim

Massive blood transfusion (MBT) is arbitrarily defined as the replacement of more than one blood volume within a 24 hour period. A retrospective study was performed at Liverpool Hospital, NSW, to determine the clinical aspects, transfusion practices and mortality in patients who had MBT from October 2007 to March 2009.

### Methods

Patients who were transfused  $\geq 10$  packed red cells in a 24-hour period were included in the study. Demographics, clinical details, lab parameters and number and type of blood products given during the 24 hours of MBT were collected on all the patients. Standard methods were used for statistical analyses.

### Results

There were 72 MBT episodes, one third of which were associated with cardiac surgery. Based on the underlying conditions for MBT, three groups were identified: - surgery: 52%, spontaneous bleeds: 28% and major trauma: 20%. The number of blood components transfused and recombinant FVIIa used in the three groups were not significantly different, but a higher platelet/PRBC transfusion ratio was observed in surgical patients. The overall mortality was 30.5% and was lowest in surgical (24%) and highest in trauma patients (50%). A significantly higher number of PRBCs (P value: 0.0004). and FFPs transfusions (P value: 0.026) with lower platelet/PRBC ratio was seen in patients who died (P value: 0.018). The FFP/PRBC ratio was not significantly different between the patients who survived and died (P value: 0.33). Mortality was highest in the group of patients who received more than 30 units of packed cells in 24 hours. The total number of packed red cells used in these 72 patients in 24 hr period was 7.5% of the total PRBCs used in the hospital in the 18 month period. Total FFP units, platelets, cryoprecipitates used were 16%, 8% and 37% of the total and overall the total blood products used in these patients were approximately 11 % of the blood products used in the 18 month period in Liverpool hospital.

### Conclusion

Patients with MBT used approximately 11% of the total hospital blood products in the same period thereby imposing a considerable strain on the blood resources. Prospective studies with a larger number of patients are required to determine the optimal blood product ratios and efficacy of rVIIa in patients with massive blood loss.

*No conflict of interest to disclose*

A263

**P032**

## **Thromboprophylaxis, Just Do It. The Peter Mac Experience**

**Kate Burbury<sup>1</sup>**, Verna Wallroth<sup>1</sup>, Michelle Cassumbhoy<sup>1</sup>, Neil Came<sup>1,2</sup>, Sue Kirsas<sup>1</sup>, David Westerman<sup>1,2</sup>

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### **Introduction**

Venous thromboembolism (VTE) is the commonest cause of preventable deaths in hospitalized patients. It is also a major complication of cancer, occurring in 4%-20% of patients and is one of the leading causes of death in cancer patients. Despite an insurmountable body of scientific literature over 50 years supporting thromboprophylaxis, it remains grossly underutilised. Studies have shown that multiple strategies are required to increase the rates of thromboprophylaxis assessment and prescribing.

### **Aim**

To assess and improve thromboprophylaxis rates in medical and surgical inpatients at the Peter MacCallum Cancer Centre.

### **Method**

We employed a multi-interventional approach comprising:

- Audit and feedback thromboprophylaxis rates in hospitalized patients pre implementation of mandatory VTE risk assessment on all inpatients.
- Introduce a simple hospital thromboprophylaxis guideline
- Introduce a mandatory thromboprophylaxis tool requiring assessment and/or prescription on all inpatient hospital drug charts, including rewritten charts.
- Develop & distribute a thromboprophylaxis education booklet
- Raise awareness through a hospital education campaign including a grand round
- Follow up audit to assess efficacy of interventions

### **Results**

Our preintervention cohort comprised 212 patients, 17 receiving therapeutic anticoagulation were excluded. 117 patients were male. 81 (38%) were surgical patients. All tumour streams were represented with the exclusion of haematological malignancies. 91/195 evaluable (47%) had thromboprophylaxis prescribed, 52% in surgical patients and 37 % in medical patients. LMWH was the treatment prescribed in 91% of cases, unfractionated heparin in 9%. 19/104 receiving no pharmacological thromboprophylaxis were prescribed TED stockings. The post intervention audit is currently in progress with preliminary results showing significant improvement in thromboprophylaxis prescription rate in the surgical, and a modest increase in medical patients.

### **Conclusion**

Thromboprophylaxis rates were congruent with recent literature and were suboptimal for both medical and surgical inpatients. Multiple and mandatory tools or systems are required for tackling this pivotal issue.

*No conflicts of interest to declare*

P033

## Use of Factor VIII Inhibitor Bypassing Agents in Bleeds Due to High Titre FVIII Inhibitors. Which Agent to Use? Three Cases Illustrating Treatment Decisions

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### Aims

In high titre FVIII inhibitors the decision to use either FVIII bypassing agents rFVIIa (NovoSeven: Novo Nordisk) and aPCC (FEIBA: Baxter) depends on the severity of bleed and response to initial therapy. We conducted a retrospective analysis of patients presenting with bleeding and Factor VIII inhibitors to assess the efficacy of recombinant Factor VIII and FEIBA.

### Methods/Results

Patient 1: 54 year old male bled after gum biopsy and developed a deltoid haematoma due to a FVIII inhibitor. There was a minor response to 4 doses of rFVIIa. Immediate improvement in the gum bleed and shoulder haematoma occurred after aPCC. Six months later he developed a haematoma in the left ankle (concurrent with a rise in FVIII inhibitor) which resolved after a single dose of rFVIIa 270mcg/kg. Two days later the haematoma progressed and responded to aPCC.

Patient 2: 59 year old male with mild haemophilia A presented with a tense haematoma in the right upper arm causing ulnar neuropathy. Factor VIII 26% and inhibitor screen was negative. He was treated with rFVIII responding over 7 days when inhibitors were detected (66 BU/ml): rFVIII was ceased and aPCC was administered. This was associated with further improvement in the haematoma and neuropathy.

Patient 3: 41 yr male with mild haemophilia A required surgical decompression for a spontaneous intracranial bleed under cover of recombinant Factor VIII. Further surgery was required after 6 months. Four weeks later he presented with haematuria: FVIII inhibitor 0.9 BU/ml rising to 14 BU/ml. Two doses of rFVIIa did not reduce the haematuria which settled after a single dose of aPCC.

### Conclusion

Each bleed is unique and requires individual management. Treatment decisions are required every 6-12 hours in the first 24 hours then daily depending on response which may require changing agents. Routine laboratory monitoring is not available for inhibitor bypass therapy although thrombin generation and thromboelastography may assess efficacy. Therefore regular clinical evaluation is critical for effective and economical use of expensive therapies.

*MFL received travel sponsorship from Novo Nordisk*

A265

**P034**

## Retrieval Rate of Radiologically Inserted Filters

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### Aims

To look at the retrieval rate of intravenous filters inserted following various indications.

### Methods

Retrospective review of filter insertions done at a single centre between 2005-2009.

### Results

Out of the 73 patients who had filter inserted, a major reason for filter insertion (in 46 cases) was because of contraindications to therapeutic anticoagulation. Out of these 46 patients: filters were successfully removed in 14 cases, filter removal was not attempted in 28 cases, in only one case filter removal was attempted unsuccessfully and outcome of three cases were unknown. Retrieval rate in this patient population was 32.55% (excluding the unknown). Filter insertion was carried out due to contraindications to prophylactic anticoagulation in seven patients; out of these five had it removed successfully. In three patients, filter was indicated because of failure of anticoagulation and none of them had it removed. The reason for filter insertion in six patients was chronic thromboembolic pulmonary hypertension and insertion was carried out to prevent further clots and none of these were removed. Five out of seven patients who had prophylactic placement of filter had it removed. Reasons for not attempting filter removal were: persisting indication (13), lack of follow up (9), patients who died within one month of insertion (7) and who died more than one month of insertion (8). The overall retrieval rate was 37.87%. There was no immediate complication related to filter insertion apart from failure to engage caval side wall in one case. Long term complications included IVC thrombus in two cases. Even after filter insertion, two patients were found to have new pulmonary embolism filter insertion.

### Conclusions

The overall retrieval rate in our cohort of patients was 37.87%. Reasons for not attempting retrieval include persisting indication and lack of follow up. Overall filter insertion appears to be a safe procedure.

*No conflict of interest to disclose*

**P035****Cyclical Thrombocytopenia and Neutropenia Associated with Rebound Thrombocytosis – A Case Report****David Connor**<sup>1</sup>, Joanne Joseph<sup>1,2</sup><sup>1</sup>*Department of Haematology, St Vincents Hospital, Sydney, NSW, Australia*<sup>2</sup>*University of New South Wales, Sydney, NSW, Australia*

Cyclical thrombocytopenia is an extremely rare condition characterised by periodic fluctuations in the platelet count. In some cases, rebound thrombocytosis may also occur. Here, we present a case of cyclical thrombocytopenia and neutropenia associated with rebound thrombocytosis in a 33 year old female originally considered to have immune thrombocytopenic purpura (ITP), but who failed to respond to typical ITP treatments including splenectomy.

Full blood count, serum luteinising and follicle stimulating hormones (LH, FSH), oestradiol, progesterone and C-reactive protein (CRP) levels were assayed 2-3 times per week for a total of 2 cycles. Reticulated platelets were also measured using flow cytometric analysis of thiazole orange staining of platelets. Serum samples were collected for performing thrombopoietin assays at a future date. The patient was not on any therapy designed to increase the platelet count during this period. Platelet count varied spontaneously from 8 to  $1,249 \times 10^9/L$  throughout a single cycle and cycle length was approximately 28 days duration. The patients platelet count was  $< 20 \times 10^9/L$  for a total of around 10 days. Each increase in platelet count was accompanied by an increase in the reticulated platelet count. The patient's neutrophil count also appeared to cycle, however the peak neutrophil count preceded the peak in the platelet count. The fluctuation in the neutrophil count was closely matched to serum CRP levels. Oestradiol levels also appeared to increase prior to the peak in platelet count.

The cyclical thrombocytopenia and neutropenia in this patient appear to be related to phases of the menstrual cycle. This may have potential therapeutic implications for this condition.

*No conflict of interest to disclose*



P036

## Venous Thromboembolism During Autologous Stem Cell Transplantation for Haematological Malignancies

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### Aim

To establish the risk of VTE during autologous stem cell transplant.

### Method

A retrospective review of all patients undergoing autologous stem cell transplant for haematological malignancies at our institution was undertaken to determine the incidence of radiologically confirmed symptomatic VTE and the presence of concurrent risk factors.

### Results

One hundred eighty four patients had follow up for at least one month following discharge and were included in the analysis. There were two deaths within the follow-up period (1 graft failure, 1 progressive disease). Non-Hodgkin lymphoma (NHL) and myeloma were the most common indications for transplant, with BEAM and melphalan, respectively the most common conditioning regimens. Twelve patients had a history of prior VTE, of whom three had pharmacological prophylaxis, which was withheld during severe thrombocytopenia in two cases.

A total of four (2.2%) patients had confirmed VTE. None had had a prior event. Three events were related to central venous catheters. Only one patient (0.5%) had a VTE not associated with a venous catheter, developing an above knee deep vein thrombosis after discharge. Two of 9 patients with peripherally inserted central catheters (PICC) developed catheter associated thrombosis, which was the only significant risk factor. No patients in this cohort had veno-occlusive disease of the liver.

### Conclusion

Autologous stem cell transplant with BEAM or melphalan has a low risk of venous thromboembolism. The use of PICC lines may carry a higher risk of thrombosis.

*No conflicts of interest to disclose*

P037

### **Patients' Risk of Recurrent Venous Thromboembolism (VTE) is Associated with Hypercoagulable Overall Haemostatic Potential (OHP) Assay Results**

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The ability to stratify patients according to risk of recurrent VTE would improve the risk-benefit ratio for use of long term anticoagulation in individual patients. The use of global coagulation assays, such as the OHP, for this purpose, may also provide insights into the mechanisms of hypercoagulability. We aimed to determine whether OHP assay results either during, or on completion of, warfarin therapy, were predictive of recurrent VTE. We conducted a prospective cohort follow up study of 134 consecutive VTE patients, recruited at Royal North Shore hospital from July 2005 to May 2008. OHP assays, D-dimer and coagulation factors were performed in all patients whilst on warfarin. In a subgroup of 40 patients, assays were also performed one month after warfarin cessation. Median duration of follow up was 18 months. 53% (71/134) patients were male with mean age 48.6 years (range 19-85). 80% had proximal DVT or PE, with 80% events spontaneous. 61% patients had a thrombophilia. 56% (75/134) patients ceased warfarin during the study period. 11% (8/75) had a recurrent VTE. Risk of VTE recurrence was associated with a positive D-dimer (OR 1.43, CI 0.19-10.6), hypercoagulable OHP (OR 1.64 CI 0.3-9.0), elevated fibrin generation (OR 4.8, CI 0.26-90) and reduced fibrinolysis (OR 2.30 CI 0.17-30).

Even whilst patients remain anticoagulated, hypercoagulable OHP assay results may predict risk of recurrent VTE, if warfarin is subsequently ceased. Incorporation of OHP results into a clinical prediction rule for stratification of VTE recurrence risk, may assist in decisions regarding duration of anticoagulation in individual patients, and should be further investigated.

*No conflict of interest to disclose*

P038

## The Pharmacogenetic Basis of Clopidogrel Resistance: CYP2C19 Genotype in a Suspected Myocardial Event Population

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### Aim

Clopidogrel, a widely used anti-platelet agent for primary and secondary prevention of arterial thrombosis, is administered as a pro-drug. Clopidogrel is metabolised to its active metabolite by the hepatic cytochrome P450 2C19 (CYP2C19) enzyme. Recent studies demonstrate that single nucleotide polymorphisms (SNP's) in the CYP2C19 gene result in significantly reduced production of the active metabolite of clopidogrel (Hulot et al, *Blood*. 2006). Additional studies demonstrate that patients with CYP2C19 gene SNP's, including CYP2C19\*2, \*3, \*4 and \*5, have reduced production of the active metabolite of clopidogrel and increased coronary, cerebrovascular, and coronary stent thrombosis (Simon et al and Mega et al, *N Eng J Med*. 2009, Collet et al, *Lancet* 2009). We were interested in determining the CYP2C19\*2 allelic frequency in a population of patients presenting for assessment of a suspected myocardial event as these patients may be receiving or considered for anti-platelet therapy.

### Method

Non-identifiable patient samples referred to a pathology practice for troponin levels (n=99) were analysed. DNA was extracted from whole blood using the Roche Diagnostics Magnapure and CYP2C19 was genotyped using the Sequenom Massarray technology. CYP2C19 gene alleles studied were: CYP2C19: \*2, \*3 & \*17.

### Result

34% of patient samples were heterozygous for CYP2C19\*2 whilst 3% were homozygotes for CYP2C19\*2. In a sub-group of samples with an elevated troponin level (>0.05µg/L) (n=18) 55% were heterozygous for CYP2C19\*2 with no homozygotes detected.

### Conclusion

Our results highlight the significant prevalence of the CYP2C19\*2 deficient allele in patients assessed for a suspected myocardial event. Interestingly, consistent with a recent report regarding stent thrombosis (Giusti et al *Am J Cardiol* 2009), a high CYP2C19\*2 prevalence was noted in patients with confirmed cardiac damage. As cardiac patients may be receiving or considered for clopidogrel treatment determination of CYP2C19\*2 genotype may be of benefit in assessing the potential efficacy of therapy.

### Conflict of Interest Statement

*This research was supported by Gribbles Pathology, Melbourne, Victoria, Australia. The company provided genotyping results for this study.*

P039

## Genetic Screening for FVIII mutations in Haemophilia A Patients at Fremantle Hospital

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### Aim

To screen all Haemophilia A patients attending the clinic at Fremantle Hospital in order to characterise the FVIII mutation responsible for the Haemophilia, and correlate this with the patient's phenotype

### Method

To date 16 patients have had samples referred for genetic analysis. Initial screening for the Intron 22 inversion was undertaken at Pathwest, Princess Margaret Hospital via DNA extraction from peripheral blood leucocytes followed by PCR and southern blotting methodology.

Those found to be negative for the rearrangement were subsequently referred to the Institute of Medical and Veterinary Science for further investigations. PCR and direct sequencing of the DNA was then used to screen the entire coding region and splice junctions of the FVIII gene.

Further data analysis was undertaken, by screening of patient records and laboratory results.

### Results

Out of 13 results received, 5 (38%) of patients were positive for the intron 22 rearrangement that is associated with severe Haemophilia A. 4 patients (31%) were found to have missense mutations in exons 1 (2), 7, 8 and correlate to mild disease. There was one instance of inhibitor formation in a patient having a mutation in exon 7. Of the remaining 4 patients, one possessed a nonsense mutation in exon 14 presenting as mild disease and the other 3 related patients were shown to have a frameshift mutation in exon 26. Clinically, a moderate to severe bleeding syndrome has resulted in these patients. 3 mutations that are described have not been previously reported on the HAMSTeRs database for FVIII mutations.

### Conclusion

Isolation and characterisation of the FVIII gene defect in haemophilia A patients, although expensive, may be important for genetic counselling of affected patients and their family members. Further evaluation may be indicated to determine whether specific genotypes may be associated with certain phenotypes, including propensity to inhibitor formation.

*No conflict of interest to declare*

A271

P040

## Aspirin for Primary Prevention of Cardiovascular Disease: A Meta-Analysis of Randomized Controlled Trials

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### Background

Aspirin reduces the risk of myocardial infarction (MI), stroke and death in patients with clinically manifest vascular disease but the benefits are less certain when aspirin is used for primary prevention of cardiovascular events

### Aim

To determine the benefits and harm of aspirin compared with no aspirin in primary prevention of cardiovascular disease.

### Method

Meta-analysis of randomized controlled trials of aspirin for primary prevention was conducted. Eligible studies were identified using MEDLINE, EMBASE, Cochrane library and CINAHL databases; review of bibliographies of relevant publications and a related article search. Outcomes of interest were: all cause mortality, cardiovascular mortality, the composite of MI, stroke or cardiovascular death, and bleeding complications. 2 reviewers independently extracted study information and data. Data were pooled from individual trials using the DerSimonian-Laird random-effects model and relative risks (RR) with 95% confidence intervals (CI) were computed.

### Results

8 studies enrolling a total of 96,726 subjects were included. Aspirin reduced all-cause mortality (RR 0.94; 95%CI 0.88-1.00), the composite of MI, stroke or cardiovascular death (RR 0.87; 95%CI 0.82-0.93), and MI (RR 0.8; 95%CI 0.66-0.98) but did not significantly reduce cardiovascular mortality (RR 0.94; 95%CI 0.82-1.08) or stroke (RR 0.93; 95%CI 0.81-1.07). Aspirin increased the risk of major bleeding (RR; 1.69 95%CI 1.38-2.08), gastrointestinal bleeding (RR 1.38; 95%CI 1.16-1.65) and hemorrhagic stroke (RR 1.36; 95%CI 1.01-1.84).

### Conclusion

Aspirin therapy in subjects with no prior history of cardiovascular disease reduces the risk of cardiovascular events, MI and overall mortality but at the expense of increased bleeding.

*No conflict of interest to disclose*

**P041**

## **Laboratory Monitoring of Oral Anticoagulation in Lupus Anticoagulant Patients**

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### **Aim**

International normalised ratios (INR) may be affected by the presence of a lupus anticoagulant (LA), making it difficult to monitor these patients when on oral anticoagulant (OAC) therapy. It has been reported that Factor X activity may be a preferred method for monitoring, as different thromboplastins have variable LA sensitivity. We evaluated our test systems to assess LA interference with INR reagents.

### **Method**

Samples from OAC patients with LA (n=9) and without LA (n=8) were collected from our INR clinic. INRs were performed with two different thromboplastins viz. ThromborelS and HemosIL Recombiplastin as well as Factor X (chromogenic), LA screening, B2 Glycoprotein antibody (B2GP) and Cardiolipin Antibodies (ACA). A subgroup of LA positive patients were tested with the CoaguChek XS instrument.

### **Results**

Result variation was evident in both the LA positive and LA negative groups when comparing the two thromboplastins. The difference in the LA positive group ranged from 0.0 – 1.1 INR units whereas the LA negative group ranged from -0.3 – 0.6 INR units. If therapeutic FX levels of 20-40% were applied, all LA positive patients were in range. In this group, Thromborel S INR results of 2/9 patients were out of range compared to 4/9 with Recombiplastin. In the LA negative group, 3/8 Thromborel S results and 3/8 Recombiplastin results were out of range. Two patients demonstrating significant differences between thromboplastins had markedly elevated B2GP and ACA with inaccurate results using the CoaguChek XS.

### **Conclusion**

Significant discrepancies existed within the three INR test systems. We suggest laboratories be aware of their thromboplastin sensitivity to LA, especially in the Point of Care setting, or investigate an alternative approach.

*No conflict of interest to disclose*

**A273**

## P042

### Audit of the HemosIL D-Dimer HS Assay with Radiological Diagnosis of Venous Thrombo-Embolism in Patients Presenting to an Emergency Department in a Tertiary Teaching Hospital

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*Haematology, Pathology Queensland<sup>1</sup>, Vascular Medicine<sup>2</sup> and Emergency Department<sup>3</sup>, Princess Alexandra Hospital, Brisbane, Queensland, Australia.*

#### Aim

To evaluate the utility of an automated D Dimer assay for exclusion of VTE in patients presenting to our Emergency Department with a low- or intermediate pre-test probability of VTE.

#### Methods

In October 2008, the automated latex immunoassay HemosIL D-Dimer HS replaced the qualitative Simplify D-Dimer assay in our laboratory. A threshold of 0.23mg/mL is reported to provide 100% negative predictive value for VTE in low or intermediate pre-test risk patients. Our laboratory information system identified all D-Dimer tests ordered from our Emergency Department between October 2008 and June 2009. Requests for patients with non-VTE provisional diagnoses were excluded. For patients with possible VTE, a search of the Radiology Database identified venous duplex ultrasound, CT pulmonary angiogram or ventilation perfusion lung scans, and the results were noted. The Emergency Department database identified all patients with the discharge diagnosis of DVT or PE during this period, including those without a D-Dimer test, enabling calculation of the incidence of VTE in this population. Results for patients with both a D-Dimer test and radiological imaging performed were analysed to determine the sensitivity, specificity and negative predictive value for the HemosIL D Dimer HS assay.

#### Results

435 patients were considered to have VTE included in their differential diagnosis. Of these, 314 D-Dimer tests were ordered. An emergency discharge diagnosis of VTE was made for 129 patients, 121 of these did not have a D-Dimer test performed. Of the 314 patients with a D-Dimer test, 113 had confirmatory imaging performed. 7 patients demonstrated venous thrombosis (2 positive USS, 4 positive CTPA and 1 positive on both). The sensitivity, specificity and negative predictive value of the HemosIL D-Dimer HS assay for the diagnosis of VTE was 100%, 28.3% and 100% respectively. Statistical analysis demonstrated a significant p value ( $p=0.05$ ) for comparison of D-Dimer values according to the presence or absence of thrombosis.

#### Conclusion

A threshold HemosIL D-Dimer HS assay level of 0.23mg/L provided a 100% negative-predictive value for exclusion of VTE in patients with a low or intermediate pre-test probability in our institution. Correlation with pre-test clinical risk assessment and D-Dimer level is essential to aid the diagnostic process in patients with probable venous thrombo-embolism. The diagnostic algorithm is being correctly applied with patients with a high pre-test probability proceeding directly to radiological imaging without a D-Dimer test being performed.

*No conflict of interest to disclose*

P043

## A Method to Determine If an Association Exists Between Stroke and Autoantibodies Directed Against Folate Receptor

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3 Stroke Unit, Royal Perth Hospital. Perth, Western Australia. Australia.

### Aim

A correlation between antiphospholipid autoantibodies and venous thromboembolism has been well documented, although debate continues as to whether a link exists with vascular thrombotic risk. Autoantibodies directed against folate receptor alpha (FR $\alpha$ ) are reported to be a risk factor for pregnancy complications including sub-fertility and neural-tube defect. We aimed to investigate if FR $\alpha$  autoantibodies are a risk factor for stroke.

### Method

Chinese Hamster Ovary (CHO) cells stably transfected with FR $\alpha$  and non-transfected, FR $\alpha$  negative, CHO cells were plated out on 96-well plates and fixed with glutaraldehyde. After blocking with fetal calf serum and bovine serum albumin, patient sera was added to FR+ and FR- wells in duplicate. A horse radish peroxidase labelled, antihuman IgG/IgM/IgA secondary antibody was added, followed by the addition of a colourimetric substrate that was quantitated using standard spectrophotometric techniques. 100 first ever stroke patients and 100 age/sex/postcode matched controls were investigated using the method and quartile analysis performed.

### Result

There was no significant statistical difference between the two groups, although the 75<sup>th</sup> quartile of the stroke cohort was greater than that of the control group.

### Conclusion

The results suggest that autoantibodies directed against the alpha form of folate receptor are not a direct risk factor for stroke. However, the greater representation of strongly sero-positive samples detected in the stroke cohort may warrant further investigation. The cell-based ELISA method we have developed may have applications in the assessment of other pathologies where FR $\alpha$  autoantibodies may be involved in the development or progression of the disease.

*No conflict of interest to disclose*

A275



**P044****Evaluation of a Clinical Pathway to Enable Patient Self-Monitoring of Anticoagulation**

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University of Tasmania, Hobart, Tasmania, Australia*

**Aim**

Previous studies suggest that self-monitoring (PSM) of the international normalised ratio (INR) may improve the outcomes of oral anticoagulation therapy through increasing the time spent within the target range (TTR), and improving both consumer satisfaction and participation in healthcare. The purpose of this study was to develop, implement and evaluate a pathway to enable people taking warfarin to monitor their own therapy in the community setting.

**Method**

A structured training program was developed to facilitate the transition of consumers from usual care to PSM using the existing Home Medicines Review (HMR) model. Consumers were recruited through their community pharmacies and, in collaboration with their general practitioners, received intensive one-on-one warfarin education and training in using the CoaguChek XS point of care INR monitor by a trained HMR accredited pharmacist. PSM was undertaken for six months. Outcome measures included TTR, quality of life, warfarin knowledge, and consumer satisfaction.

**Result**

Twenty-eight patients with a minimum six-month history of anticoagulation treatment were recruited from Tasmania and New South Wales. Sixteen (57.1%) were male and 64.3% required anticoagulation for atrial fibrillation. At baseline, the mean TTR was 64.8%. The mean baseline warfarin knowledge score was 72.4% using a validated warfarin knowledge questionnaire. Qualitative feedback from consumers and general practitioners has indicated a high level of satisfaction with both the training program and PSM. Qualitative and quantitative results after six months of PSM will be reported.

**Conclusion**

Using the proposed model, trained pharmacists successfully identified and trained suitable consumers to undertake PSM. Initial qualitative feedback has been positive. Future investigation of both qualitative and quantitative data will aim to provide objective data to support these positive findings. This shared model could be used to identify suitable candidates for PSM and provide Australians with access to appropriate training and support.

*No conflict of interest to disclose*

**P045****An Unusual Case of Rapid Development of an Acquired Factor V Inhibitor****Sarah Kamel<sup>1</sup>**, Ellen Maxwell<sup>1</sup>, Kate Burbury<sup>2</sup>, Max Wolf<sup>2</sup>, Robyn Coleman<sup>3</sup><sup>1</sup> *Melbourne Pathology, Collingwood, Victoria, Australia*<sup>2</sup> *Cabrini Hospital, Malvern, Victoria, Australia*<sup>3</sup> *Sullivan Nicolaides Pathology, Taringa, Queensland, Australia***Introduction**

We present a case of a spontaneous Factor V (FV) inhibitor complicating cholangitis, in the absence of bovine thrombin exposure.

An 85 year old male admitted with fever, abdominal pain and deranged liver function secondary to cholangitis demonstrated isolated mild prolongation of prothrombin time (PT 17secs: RR 10-14 secs). Abnormal coagulation results progressed, peaking 3 weeks from admission (PT 59 secs, APTT 110 secs: RR 23-35 secs). Daily therapy with vitamin K was unsuccessful. Normal plasma failed to correct the abnormalities, suggesting the presence of an inhibitor. Factor assays demonstrated a FV level of 0.04 U/ml (RR 0.50-2.00) with inhibitor strength of 5.9 Bethesda units. Endoscopic Retrograde Cholangiopancreatography performed under fresh frozen plasma and prior to completion of investigations, was not complicated by bleeding. The inhibitor remains detectable but clinically silent 8 weeks after initial detection.

**Conclusion**

Most acquired FV inhibitors result from exposure to bovine thrombin in fibrin glue. This patient had no recognised invasive vascular procedures and the aetiology of his FV inhibitor remains unclear. A contribution from sepsis and antibiotic exposure is presumed. Aetiology, natural history and therapy of FV inhibitors are discussed.

*No conflicts of interest to disclose*

P046

## Utility of the HemosIL™ D-Dimer Assay as a Screening Tool in Outpatients Presenting with a Suspected Deep Venous Thrombosis

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### Background

An elevated plasma D-Dimer level is a sensitive but non-specific marker of thrombosis. D-Dimer has been advocated as a screening test for excluding deep venous thrombosis (DVT) among patients presenting with suspected DVT, thereby reducing unnecessary radiological investigations and anticoagulation.

### Aim

To perform a single centre retrospective analysis of the sensitivity and specificity of an elevated D-Dimer among outpatients presenting with suspected proximal DVT.

### Methods

Using a data extraction algorithm, consecutive outpatients with proximal DVT confirmed on ultrasonography (US) were identified. An age- and sex-matched control group was identified using outpatients who presented with suspected DVT but had the diagnosis excluded by US. US and D-Dimer testing were performed within 72 hours of presentation. Clinical pre-test probability was not performed. D-Dimer measurements were performed using HemosIL™ D-Dimer HS kits on the ACL TOP™ automated coagulation analyser. A positive result was defined as  $\geq 0.2\text{mg/L}$ . The impact of alterations to the reference range was also evaluated. Statistical analysis was performed using MedCalc software.

### Results

Eighty-seven patients with proximal DVT and controls were identified in both groups. The D-Dimer was positive in all but one patient with a proximal DVT. Using statistical analysis the calculated sensitivity and specificity for a positive D-Dimer defined as a level of  $\geq 0.2\text{mg/L}$  were 98.85% (95%CI: 93.8%-100%) and 27.59% (95%CI: 18.5%-38.2%), respectively with a negative predictive value (NPV) of 95.8% (95%CI: 78.9%-99.9%). ROC curve analysis demonstrated that augmenting the cut-off to  $\geq 0.22\text{mg/L}$  resulted in a modest increase in specificity 31.3% (95%CI: 21.5%-42%) with only a small reduction in sensitivity 97.7% (95%CI 91.9%-99.7%) and NPV 92.9% (95%CI 76.5%-99.1%).

### Conclusion

This study demonstrates the HemosIL™ D-Dimer assay has a high NPV at a cut-off of  $\geq 0.2\text{mg/L}$  amongst patients presenting with suspected DVT. Changing the cut-off to  $\geq 0.22\text{mg/L}$  may improve specificity and reduce unnecessary US, with only a slight reduction in sensitivity. The cost: benefit ratio of increasing the cut-off level to  $0.22\text{mg/L}$  requires validation in a prospective study.

No conflict of interest to disclose

A278

P047

## Effect of a Single Indian Meal on Platelet Function and Coagulation

**Brad McEwen**<sup>1,2</sup>, Marie-Christine Morel-Kopp<sup>1,2</sup>, Geoffrey Tofler<sup>3</sup>, Christopher Ward<sup>1,2</sup>

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### Aim

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in Australia and New Zealand. Increased platelet aggregation and hypercoagulation play significant roles in the aetiology of cardiovascular disease. Garlic, ginger, onion, and tomatoes have independently been shown to modify platelet aggregation. The aim of this study was to demonstrate a combination of these dietary components in a meal in modifying platelet aggregation and coagulation.

### Methods

Twenty healthy volunteers were recruited with informed consent and tested pre- and post-Indian meal. Investigations included platelet aggregometry, flow cytometry, platelet function analyzer (PFA-100), overall haemostasis potential (OHP), thrombin generation (CAT), and thrombelastography (TEG). Statistical analyses were performed using the Wilcoxon method.

### Results

Changes in platelet aggregation were varied from subject to subject; reduced platelet aggregation was observed in response to low dose adrenaline, arachidonic acid, and U46619 (TXA(2) mimetic) while aggregation increased in response to high dose platelet agonists ADP, adrenaline, arachidonic acid, and collagen. Flow cytometry showed reductions in platelet-monocyte ( $P=0.022$ ) and platelet-granulocyte aggregates ( $P=0.025$ ). No significant difference in platelet function was observed using the PFA-100 assay. We observed reduced thrombus formation as determined by OHP, TEG and CAT. This was evident as reduced fibrin generation (increased lagtime and decreased maximum slope, OHP), significantly decreased clot strength ( $P=0.048$ ) (reduced maximum amplitude, TEG), which is dependent on number and function of platelets and its interaction with fibrin, and delayed start of thrombin generation (CAT).

### Conclusion

The results of this pilot study suggest that the consumption of an Indian meal, containing garlic, ginger, onion, tomato and other spices, has a mild hypocoagulant effect. This effect is likely due to changes in platelet function and interactions with fibrin. Future studies are underway to investigate the benefits to cardiovascular health of these dietary components.

*No conflict of interest to disclose*

A279

P048

## Garlic and its Potential in Cardiovascular Disease: A Review

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### Aim

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in Australia, the United Kingdom and the United States. Increased platelet aggregation plays a significant role in the aetiology of CVD, and is complex involving multiple mechanisms with platelet hyperactivity being one of the most important factors responsible for CVD incidence. It is proposed that garlic modifies CVD risk by inhibiting platelet aggregation, increasing HDL-cholesterol and reducing triglycerides. This presentation aims to review the current literature of garlic and CVD and the potential mechanisms involved.

### Methods

A PubMed and EBSCO literature search was conducted using the search terms "garlic", "cardiovascular disease", "platelet aggregation" and "aggregometry" in human studies for publications dated from 1966 to October 2008.

### Results

The studies using in-vitro methods of garlic on platelet aggregation found significant inhibition of platelet aggregation while using the platelet agonists ADP, arachidonic acid, collagen and epinephrine. Intra-platelet calcium was also inhibited. In ex-vivo studies, garlic consumed by subjects found platelet aggregation induced by ADP, collagen and epinephrine was also inhibited. Triglycerides were found to be reduced, HDL-cholesterol increased and fibrinolytic activity also increased. Preliminary results in our research centre have also shown inhibition of platelet aggregation.

### Conclusion

Garlic has the potential to reduce cardiovascular risk via inhibiting platelet aggregation, reducing triglyceride levels, and by increasing fibrinolysis and HDL-cholesterol levels. Modifying these risk factors can have favourable effects on cardiovascular health and the regular consumption of garlic can be suggested in treatment protocols of patients with increased cardiovascular risk, such as those with diabetes.

*No conflict of interest to disclose*

P049

## An Interesting Case of HIT

**Nick Michalopoulos;** Jeanene Lam  
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74 year old female underwent MV replacement (day 1). Pre-op platelet count was 283.

Following CPB platelets dropped to 36. She returned to theatre due to bleeding. 280mg of heparin was administered on each occasion in theatre. During that time she was transfused platelets, FFP, cryoglobulin and novo-7. Transfusion of another bag of single donor platelets on day 2 resulted in a platelet rise to 93. On day 6 the platelet count dropped to 23 with no evidence of bleeding or sepsis. During day 7 - 11 she was transfused with 5 single donor units (total platelets transfused was 15) at which time the platelet count stabilized. She was discharged from hospital 25/03/2009 with a platelet count of 203.

HIT testing was performed using ELISA (GTi diagnostics IgG and IgGAM) and platelet aggregation methods.

On day 1 (post-op) the initial screening test performed using IgG ELISA was negative.

On day 6 a repeat HIT test was requested and was found to be positive by ELISA IgG and by platelet aggregation. Results will be tabled.

Supplementary (using IgGAM ELISA) and retrospective testing was performed on samples between day 1 and day 6 which showed a graduation in the OD which was clearly negative on day 1 and becoming a clearly positive OD by day 6.

PTP score (based on the 4T's) for day 1 and day 6 was 2 (low risk) and 5 (intermediate risk) respectively.

### Conclusion

HIT was confirmed on day 6.

This case raises several points:

1. Was the initial negative result due to: HITS not being present or simply serologically not detectable?
2. Despite the low risk clinically (as assessed by the 4T's), consideration of HITS testing should not be excluded.
3. HITS should still be considered as a differential diagnosis of thrombocytopenia <5 days of heparin exposure, despite the negative laboratory HITS (ELISA) test.

*No conflict of interest to disclose*

P050

## Comparison of Platelet Function and Reticulated Platelet Fraction in Three Groups of Thrombocytopenic Patients

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### Aim

Bleeding risk can be dependent on platelet numbers, function and maturation. This study aims to investigate the platelet function in three different low platelet groups: ITP, bone marrow recovery post-chemotherapy and in Myelodysplasia.

### Method

From 34 participants, 12 were selected as suitable for this study based on study criteria: 3 ITP, 2 Myelodysplasia and 7 post-chemotherapy. EDTA and 0.109M citrate blood samples were collected from each patient and tested within 3 hours.

1. Platelet count was determined on the CELL-DYN Sapphire and verified using Anti-human CD61 monoclonal antibody on the same analyzer.
2. Platelet function was performed on the DiaMed Impact-R measuring Surface Coverage (SC) indicating platelet adhesion, and average size of platelet aggregates (AS) indicating platelet aggregation. Reference range for SC and AS were established from 12 healthy subjects (5M, 7F).
3. Reticulated platelet fraction (representing the immature platelet population) was determined by anti-RNA and CD41 using a BD FACSCalibur flow cytometer. Gate settings were determined based on 3 normal samples for each assay. Results were expressed as reticulated platelet percentage (RP%) and absolute reticulated platelet numbers.

### Result

ITP – all patients had platelet counts above  $100 \times 10^9/L$  showing normal SC and AS.

Myelodysplasia – both patients had low platelet counts and low SC and AS.

Chemotherapy – 2 patients with normal platelet counts had normal SC and AS, 1 patient with normal platelet count had borderline SC but low AS, 4 patients with platelet counts below  $100 \times 10^9/L$  had low SC and low/normal AS. A relationship could not be demonstrated between platelet function and reticulated platelet fraction.

### Conclusion

This study suggested normal platelet function in ITP, reduced in Myelodysplasia and variable in post-Chemotherapy. No relationship could be shown between platelet maturation and function.

*No conflict of interest to disclose*

**P051**

## **A Single Institution Experience with Retrievable Inferior Vena Caval Filters**

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### **Introduction**

Retrievable inferior vena cava (IVC) filters are recommended in the presence of a short term absolute contraindication to anticoagulation (AC) in patients with a recent history of venous thromboembolism (VTE). However there is marked variation in clinical practice regarding filter insertion, with the procedure being associated with a potentially significant complication rate. We report recent experience with IVC filter insertion at the Royal Adelaide Hospital.

### **Methods**

A retrospective audit was conducted on all retrievable IVC filters inserted between January 2006 and September 2008.

### **Results**

123 retrievable IVC filters were inserted. 89/123 (72%) filters were inserted for prevention of PE in patients with recent venous thrombosis unable to receive therapeutic anticoagulation due to active bleeding in 61 pts (50%), and planned surgery in 31 pts (25%). 6 were inserted for recurrent VTE despite therapeutic anticoagulation. 25 (20%) were inserted for non evidence based indications including 24 for primary PE prophylaxis in trauma patients, and 1 for atrial fibrillation and bleeding on AC. Only 37/123 (30%) of filters were removed at a median time of 22 days (range 7-502 days). Of the 86 filters left insitu, after a median follow up of 116 days (range 0-846 days) 12 failed attempted removal, 6 remained permanently for medical reasons and 28 patients died with the filter in situ. There were 8 definite complications including 3 complete IVC occlusions; 1 IVC perforation; 2 IVC wall penetrations without perforation; 2 misplaced filters and 1 filter fracture. Possible IVC filter complications included VTE distal to the filter in 5 patients, 2 fatal PEs with filter in situ, 11 filters with in situ thrombus and 2 episodes of bacteraemia post insertion.

### **Conclusion**

Filters were often used in settings which did not strictly meet standard clinical indications. Retrieval rate of temporary IVC filters was low (30%). There was a small but significant complication rate.

*No conflicts of interest to disclose*

**A283**



**P052**

## **Is IVC filter insertion always safe? : Report of two cases**

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### **Introduction**

In patients with contraindications to anticoagulation or those with recurrent DVTs, despite anticoagulation, placement of an IVC filter may be indicated. Occasionally, penetration of the IVC wall by filter components occurs in 3-38% of the total cases depending on the type of IVC filter used. Among those symptomatic penetration is very rare occurring in approximately in 0.3%. Here we present two cases of IVC filter penetration in our centre.

### **Case-1**

A 32 years old female, developed extensive lower limb DVT following the hormonal therapy for hirsutism. She developed bilateral pulmonary embolism (PE) with extension of thrombus to distal IVC. Left iliac thrombectomy was performed and temporary IVC filter inserted through internal jugular vein (IJV). A CT venogram performed after 6 days showed an appropriately placed IVC filter. Six weeks after the insertion of the IVC filter she developed postprandial abdominal pain. CT venogram demonstrated extension of one limb of filter perforating the posterior wall of second part of duodenum. Filter was successfully removed with resolution of symptoms.

### **Case-2**

A 61 years old woman with a history of recurrent PE presented with chest pain while on therapeutic enoxaparin. She was treated with heparin and had an IVC filter placed under CT scan guidance. Fifteen minutes after insertion of the filter, she developed severe pain in central abdominal and back. A CT scan performed demonstrated struts beyond the IVC wall abutting the aorta. Filter was removed successfully with resolution of abdominal and back pain.

### **Discussion**

In these two patients the IVC filter used were 'recovery vena cava' filter and 'Celect' IVC filter. Kalva et al (2006) shown in a large retrospective study that a incidence of penetration of the 'recovery' IVC filter seen in 27.5% on a follow up abdominal CT scan at a mean of 80days. Though there are case reports of similar complication with 'Celect' IVC filter, the exact incidence has not been reported. The majority of the patients with IVC filter penetration remain asymptomatic while less than one percent of patients become symptomatic.

### **Conclusion**

These cases demonstrate that penetrations can cause symptoms that require IVC filter removal. Safety of IVC filters remain an important issue in the management of these patients.

*No conflicts of interest to disclose*

P053

## Big Bleeds in Little People: Paediatric Use of rFVIIa Reported to the Haemostasis Registry

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### Background

Recombinant activated factor VII (rFVIIa, NovoSeven) is approved for the treatment of spontaneous and surgical bleeding in patients with haemophilia A or B and with inhibitors. Over the past years rFVIIa has increasingly been used for indications outside the approved areas, particularly in trauma, cardiac surgery and other critical bleeding episodes. Use in these areas remains controversial.

### Methods

Monash University established the Haemostasis Registry in 2005 to monitor the use of rFVIIa throughout Australia and New Zealand. More than 80 hospitals contribute data to the Registry including all major users of rFVIIa in Australia and New Zealand. This study examines the cohort of paediatric cases reported to the registry.

### Results

Between Jan 2002 and May 2009, 224 cases of rFVIIa use in children aged 16 years and under have been reported to the Registry. The major indication was in bleeding following cardiac surgery (49%) with haematology/oncology (12%) and trauma (12%) formed the next largest groups. Just under one half of the cases (46%) were infants less than one year of age (28% <4 weeks of age) and primarily related to cardiac surgery. Most patients (68%) received a single dose of rFVIIa with a median (IQR) dose of 121 (96-182) mcg/kg. Neonates were more likely to receive higher doses [160 (98-207) mcg/kg] but this was not associated with a higher rate of thromboembolic adverse events.

### Conclusions

Within the paediatric population, rFVIIa is most often used in the setting of cardiac surgery with a wide range of doses in neonates. This study does not allow conclusions regarding efficacy, nor comparison of adverse events rates with non-treated patients. The desire to minimise exposure to blood transfusion may be contributing to the increase in paediatric off-label use of rFVIIa. It is therefore important that in the absence of clinical trial data, independent monitoring of all aspects of rFVIIa use is continued.

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A285

P054

## A Case of Life Threatening Thrombosis in a Neonate Who is a Compound Heterozygote for 3 Thrombophilia Gene Mutations

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### Clinical Data

A male neonate was admitted on Day 4 to NICU with failure to thrive, hyperbiliribinaemia and thrombocytopenia. ABO incompatibility and sepsis were ruled out. A diagnosis of right sided renal vein thrombosis was confirmed by ultrasound. Thrombus extended to inferior vena cava and also involved left portal vein leading to deranged liver function tests and cardiac dysfunction due to hypertension.

Investigations revealed heterozygosity for factor V leiden, prothrombin 20210 and antithrombin gene mutations.

The child was treated with enoxaparin and platelet transfusion. The dose of enoxaparin, which started at 0.8mg/kg BD, was increased gradually to 3.3 mg/kg due to the suboptimal anti Xa levels, consequent to low antithrombin(31%). Antithrombin concentrate was infused during his inpatient period. The patient was also supported for a short period with protein C concentrate infusion until clinically stable, despite his protein C level of 21% being at the lower end of the normal range for a neonate(21-65%). Repeat ultrasound at 6 months of age showed significant clot regression, and enoxaparin has been discontinued.

### Discussion

Neonatal life threatening thrombosis due to thrombophilia is rare and the diagnosis can be challenging. The reference ranges for various coagulation parameters in neonates are different to the adult population and this must be considered when interpreting paediatric coagulation results. The maintenance dose of enoxaparin is higher in neonates, when compared to adults. Therapeutic strategies for neonatal thrombosis are variable and range from supportive care alone to specific anticoagulation or even thrombolysis.

*No conflict of interest to disclose*

P055

## Prevention of Deep Venous Thrombosis (DVTs) in Patients Undergoing Hip or Knee Replacement Surgery with Low Molecular Weight Heparin Therapy (LMWH) or Outpatient Calf Compression Device (CCD) After a Short Course of LMWH: A Randomised Prospective Equivalent Study

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### Aim

To test whether a combination of 5 days of post-operative LMWH therapy and outpatient CCD is as effective in preventing DVTs as 2 weeks of LMWH therapy.

### Method

Patients undergoing hip or knee replacement surgery at St. George Private Hospital were randomly assigned to either 2 weeks of LMWH therapy (group A) or 5-7 days of inpatient LMWH and CCD for 2 weeks at home (group B).

Exclusion criteria:

- Age <18 or >85
- morbid obesity
- gross lower limb oedema, or skin ulcerations
- active malignancy
- recurrent venous thrombosis
- current anti-coagulation therapy
- severe mental disorders
- severe language difficulties

All patients were subjected to lower limb Doppler study before discharge (day 5 or 7) and 4 weeks post-operatively. All patients with DVTs or cardiorespiratory symptoms were subjected to lung scan examination. Patients were also assessed for increased pain, leg swelling, bruising, haematoma, and wound infections.

### Results

84 patients were enrolled for the study, with 3 patients withdrawn for non-compliance and 2 lost to follow-up. There were 42 female and 37 male patients with a mean age of 72 (range 52-84). 40 patients were enrolled to group A and 39 in group B. There were 3 cases of DVT in each group and one case of asymptomatic pulmonary emboli in group B. There were no significant differences between group A and B in minor haematoma (2 and 1 patient respectively), and minor infections (5 and 7 patients respectively). Leg swelling was more marked in group A (11 patients) compared with group B (1 patient).

### Conclusion

There was no increased incidence of DVT in patients receiving a shorter duration of LMWH and CCD compared with 2 weeks of LMWH therapy. Group B had significant leg swelling reduction, perhaps related to longer CCD use. There were no significant differences in terms of bleeding and minor infections.

This small study may have a significant impact on reducing the use of LMWH in patients undergoing orthopaedic surgery and hence overall costs of pharmaceutical expenditure without compromising patient care or increasing the risk of DVT.

*No conflict of interest to disclose*

A287

P056

## Outcomes of Paediatric Immune Thrombocytopenia in Queensland: A 5 Year Single Centre Experience

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### Aim

To review the clinical and haematological outcomes of children treated for ITP at Royal Children's Hospital, Brisbane, and to apply the recently published standardised terminology to this cohort.

### Summary

The last 5 years has seen renewed interest in the management of ITP both in children and adults, as a number of new treatment options have become available for use in this condition. Recently an attempt to standardise the terminology used in ITP was published on behalf of an International Working Group (IWG). We will present data obtained from a retrospective chart review for 90 children treated for ITP from November 2003 to November 2005, including symptoms and platelet count at presentation, responses to first-line therapy, range of second-line therapies utilised, and long-term haematological outcomes. The impact of the revised terminology on outcome definitions will be examined.

*No conflict of interest to declare*

P057

## Heparin Induced Thrombotic Thrombocytopenia Testing – A Comparative Study of 4 Commercial Kits

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### Background

Heparin Induced Thrombotic Thrombocytopenia (HITT) is a clinicopathologic syndrome caused by an antibody-mediated reaction to heparin-platelet factor 4 complexes. Our institution previously used the particle agglutination gel card method to screen for HITT, with confirmatory testing done by Carbon-14 serotonin release assay where clinically indicated. Supply problems with gel cards prompted us to evaluate other available test systems.

### Aim

To compare diagnostic accuracy of four commercially available HITT screening tests: STAGO ELISA, DiaMed Gel Card, GTi and Zymutest.

### Method

We performed a prospective observational study of 35 consecutive patients requiring HITT testing. All samples were initially tested using the STAGO ELISA method and then stored at minus 30 degrees for subsequent testing with all four kits. All testing was performed according to the manufacturer's specifications.

### Results

Of the 35 samples tested, 10 were positive by STAGO ELISA, 9 by DiaMed, 8 by GTi and 6 by Zymutest. Only the GTi test includes a confirmatory step which demonstrates heparin dependence, a feature of true HIT antibodies. Of the 8 samples positive by the GTi test, only 1 did not show heparin dependence - this sample was positive with the STAGO test. Using the STAGO ELISA method as the reference, the sensitivity and specificity of the other tests were determined, and are as follows: DiaMed sensitivity 60%, specificity 84%; GTi sensitivity 70%, specificity 96%; Zymutest sensitivity 70%, specificity 100%. The Zymutest showed the greatest correlation with STAGO ELISA results (100%), while the DiaMed method had the greatest rate of false positive (33%) and false negative (33%) results. All samples have been sent for C14 serotonin release assay- when available, these results will be used as the gold standard test for comparison of other methods and included in the final poster.

### Conclusion

This study highlights the difficulty in laboratory diagnosis of HITT, with high false-positive and -negative results with the currently used DiaMed test, and variable correlation between the test methods. Careful test selection and assessment of pretest probability of HITT will greatly increase diagnostic accuracy.

*No conflict of interest to disclose*

A289

**P058**

## **Therapeutic Response to FVIII: VWF Concentrate in Acquired von Willebrand Syndrome: A Case Report**

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### **Background**

Acquired von Willebrand Syndrome (AVWS) is a heterogeneous bleeding disorder occurs in association with a variety of underlying conditions. The clinical and laboratory findings are similar to congenital vWD and characterised by mucocutaneous bleeding and a decrease in Ristocetin cofactor activity (vWF:RCo) and Collagen binding activity (vWF:CBA). Accelerated removal of vWF rather than decreased synthesis is the underlying mechanism.

Though the focus of management is the treatment of underlying condition, additional measures to correct the haemostasis defect is essential during acute bleed or invasive procedures.

Management options include desmopressin, factor VIII:vWF concentrate and immunoglobulin. Although immunoglobulin's effect is longer lasting the onset is slower and may not sufficient as a single agent when urgent haemostasis support is needed. This necessitates the use of desmopressin or FVIII:vWF during such settings but their duration of action is greatly shortened due to accelerated clearance in this condition.

### **Case**

We report a case of acquired von Willebrand disease associated with Ig G monoclonal gammopathy of uncertain significance. VWF studies showed decreased vWF:RCo, vWF CBA, vWF antigen and RCo/Ag ratio confirming Type 2A von Willebrand syndrome. FVIII:vWF concentrate (biostate) was administered and assays were repeated at 0,1,2,4,6,24 hours. We observed that vWF antigen level and activity persisted only for 6 hours and 2 hours respectively confirming the accelerated clearance of infused products. The non parallel nature of the decay is consistent with preferential removal of high molecular weight multimers which are haemostatically more active. High dose immunoglobulin infusion resulted in more lasting effect.

### **Conclusion**

The duration of action of biostate was greatly reduced in acquired vWD and this is important to recognise during perioperative or acute bleed scenarios. The extent of this reduction may vary depending upon the underlying conditions. Further studies are needed to evaluate this.

*No conflict of interest to disclose*

P059

## **Congenital Factor X Deficiency presenting with Severe Bleeding in the Newborn Period**

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### **Aim**

Bleeding in the neonatal period is potentially life-threatening and can be a diagnostic challenge. We present a 3 day old female who presents with severe cutaneous bleeding.

### **Case**

The infant was the first child of unrelated Caucasian parents, with normal vaginal delivery at 37<sup>+5</sup> weeks. The mother had epilepsy treated with lamotrigine, a drug not considered to cause fetal coagulopathy. There was no family history of abnormal bleeding. IM vitamin K was administered at birth. Severe periumbilical bleeding and cutaneous bruising was noted on Day 3. There was also persistent bleeding from previous venepuncture sites and heel-pricks. Haemoglobin on Day 3 was low, 130 g/L, falling to 75 g/L within 12 hours. Initial coagulation profile showed a marked coagulopathy with PT >200 secs, APTT >150 secs, with full correction on mixing tests. Fibrinogen levels were normal. Bleeding stopped and coagulopathy was corrected with FFP but not with repeated vitamin K administration. Factor studies showed an isolated severe FX deficiency (<1 U/mL, <1%). Periumbilical bleeding recurred on Day 11, and oro-pharyngeal bleeding at 5 weeks resulted in Hb of 51 g/L; bleeding was successfully treated with Prothrombinex. Prophylaxis with twice weekly Prothrombinex via a Hickman catheter was commenced at 6 weeks of age. Prophylaxis will be changed to FX concentrate when available. FX levels were at the lower limit of normal in both parents. Mutation analysis is in progress.

### **Conclusion**

Congenital Factor X deficiency is a rare autosomal recessive bleeding disorder which can manifest with severe bleeding. It should be considered in infants with mucocutaneous bleeding which do not respond to vitamin K. Mixing tests and factor studies should yield the diagnosis. Treatment is with FX-containing products such as FFP, Prothrombinex, or FX concentrate. Future prenatal testing in this family may be considered with identification of a mutation.

*No conflict of interest to disclose*

A291



P060

## The Sensitivity of Thrombin Generation and an Assay for Procoagulant Phospholipids to Platelet-Derived Microparticles

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STA-Procoag-PPL (Stago, France) is an automated clotting test able to detect procoagulant phospholipids (PPL). This assay monitors the decrease in clotting time caused by the sample PPL in the presence of a fixed amount of FXa and calcium. We studied the influence of platelet derived microparticles (PMP) on both the PPL assay and thrombin generation (TG).

PMP were obtained by activation of a platelet concentrate with calcium ionophore. A PMP range from 500 to 20000MP/ $\mu$ l was prepared in normal platelet poor plasma (NPPP). This range covers the expected normal count of circa 655MP/ $\mu$ l for males and 1775MP/ $\mu$ l for females [1].

The MP count and size were assessed in all preparations before and after freezing using flow cytometry with Megamix beads (Biocytex, France). PPL was assessed on the STA-R and TG was determined using the Calibrated Automated Thrombogram (CAT) with the PRP-reagent (1pM tissue factor, no phospholipid) as trigger (reagents and instrument Stago, France).

PPL clotting times measured for normal MP counts are consistent with the normal range established for STA-Procoag-PPL (61-83 sec for normal plasma double centrifuged for 15' at 2500g and stored frozen at  $<-20^{\circ}\text{C}$ ). The clotting times correlate with log MP count ( $r=0.998$ ).

As expected, as the PMP count increases, the STA-Procoag-PPL clotting time decreases. The TG peak correlates positively ( $r=0.954$ ) while lagtime correlates negatively ( $r=0.943$ ) with the logarithm of the PMP load. Endogenous Thrombin Potential is not altered since the procoagulant surfaces accelerate TG but do not affect the global thrombin potential of a plasma sample.

The correlation of STA®-Procoag-PPL with C.A.T. suggests a strong relationship between both assays for estimating procoagulant potential level. These results deserve further investigation on microparticles derived from other cellular origins.

### Reference

1: Robert S et al, J Thromb Haemost 2008; 7: 190-7.

**Disclosure of interest:** All authors are Stago employees

P061

## Changes in Coagulation Observed in Early and Late Foetal Loss

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Many recurrent miscarriages in patients without any know causes are characterized by defective placentation and/or microthrombi in the placental vasculature. However, in 50% of cases the cause of foetal loss remains unknown. Because of the key role thrombomodulin (TM) and tissue factor (TF) in coagulation and embryonic development we performed a study using new specific activity assays for these 2 factors (both prototype assays) in samples collected from patients with early (n=30), late (n=32) pregnancy loss (all with no known causes for the loss) and normal pregnancy (n=35) We also measured procoagulant phospholipids (PPL, STA Procoag PPL, Stago, France) and free Tissue Factor Pathway Inhibitor (f-TFPI, Asserachrom free TFPI, Stago, France).

The plasma levels of TF, TM, PPL (PPL reflected by a shortening clotting time) were significantly higher in cases than in controls subjects. In addition the ratio TF/ f-TFPI were higher in patients than in controls. Patients with late pregnancy loss had a higher TF/f-TFPI ratio than patients with early pregnancy loss ( $p>0.001$ ). The combinations of these different parameters reveal an increase in procoagulant activity and suggest that endothelial damage or activation may be involved in the pathogenesis of these pregnancy losses. The use of these 2 new activity assays and PPL may help in assessing the prognosis of pregnancy loss.

**Table**

	Normal Pregnancy	Early Pregnancy Loss	Late Pregnancy Loss
TM (%)	96	148	133
TF (pM)	1.18	3.65	3.92
TF/f-TFPI ratio	0.20	0.31	0.41
PPL (sec)	42.6	35.46	38

**Disclosure of interest:** P VanDreden, B Woodhams and A Rousseau are full time employees of Diagnostica Stago.

**P062****Effect of White Cell Concentration on Autologous Stem Cell Leukapheresis Products Stored Overnight Prior to Cryopreservation**

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**Aim**

There is some uncertainty whether high white cell concentration in a leukapheresis stem cell product stored overnight prior to cryopreservation has a deleterious effect on the viability of stem cells. This study was performed to assess the effect of white cell concentration on the viability of autologous stem cell leukapheresis product stored overnight prior to cryopreservation.

**Method**

Peripheral blood stem cells were stored overnight at 4-6°C in the stem cell collection bag and processed next day. CD34 cell counts were performed on a fresh specimen representative of collection bag kept at room temperature within 24 hours. The effect of harvest white cell count (WCC) at various cut points was studied by comparison of viable CD34 cell counts (BMT network NSW method) on fresh leukapheresis product with viable CD34 cell counts on thawed samples. Analysis was performed using Stata 10 software.

**Results**

Thirty-one leukapheresis products were stored overnight at 4-6°C with median WCC of  $254.4 \times 10^9/L$  (range 50.4-708) and median storage duration of 19 hours (range 9-24). Regression analysis demonstrated a linear relationship between log of fresh CD34 cell count and log of thawed CD34 cell count. The log of cell concentration adjusted for the log of fresh CD34 cell count was borderline significant in predicting the thawed viable CD34 cell counts ( $t=1.91$ ,  $p=0.066$ ). ANCOVA analysis at white cell concentration cut points of  $>200 \times 10^9/L$  ( $n=21$ ),  $>250 \times 10^9/L$  ( $n=16$ ) and  $>300 \times 10^9/L$  ( $n=8$ ) demonstrated no significant effect of white cell concentration on thawed viable CD34 cell counts at lower WCC (WCC  $>200$   $p=0.36$ , WCC  $>250$   $p=0.36$ ) but was borderline for higher WCC  $> 300$  ( $p=0.056$ ).

**Conclusion**

Our results indicate that higher white cell concentration does not have a definite significant effect on the autologous stem cell leukapheresis products stored overnight up to 24 hours at 4-6°C. However further study with larger numbers is required to exclude this possibility.

*No conflict of interest to disclose*

**P063**

## **Outcomes and Management of Hodgkin Lymphoma Patients in Western Australia Following Autologous Stem Cell Transplantation**

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### **Aim**

To review patient outcomes and management of patients following autologous stem cell transplantation for Hodgkin Lymphoma in Western Australia.

### **Method**

Case review of patients in Western Australia treated with autologous transplantation for Hodgkin Lymphoma, and a literature review- Despite advances in the use of combination chemotherapy, radiation therapy, imaging techniques and patient care, many patients undergoing treatment for Hodgkin lymphoma have relapsed from their disease and required therapy with autologous transplantation. Subsequent relapses occur post autograft, and the optimal management at this stage is difficult and unclear. Salvage therapy is challenging, and options include further chemotherapy including novel agents, radiation therapy and allogeneic transplantation (utilising PET to confirm remission prior to non-myeloablative conditioning).

### **Result**

We will report follow up of patients in Western Australia treated with autologous stem cell transplantation, including the relapse rate, overall survival and late salvage therapies. Various treatments were given to those relapsing including aggressive chemotherapy, palliative chemotherapy and radiotherapy. Four patients progressed to allogeneic transplantation, and one was treated with Panobinostat.

### **Conclusion**

Autologous transplantation is successful in many patients in obtaining long-term disease control. Relapse post autograft for Hodgkin lymphoma is a challenging area, and treatment options include novel therapeutic agents and also allogeneic transplantation.

*No conflict of interest to disclose*

**P064****Successful Cord Blood Transplant in Philadelphia Positive Acute Lymphoblastic Leukaemia (ALL)****Rachelle Augustes**, Julian Cooney*Haematology Dept, Royal Perth Hospital, Perth, WA, Australia***Aim**

To review management options in Ph+ve ALL, including cord transplantation.

**Method**

Literature review and case report- Philadelphia positive (Ph+) ALL, when treated with chemotherapy alone has a five year overall survival estimated to be between 10-20%. Results with chemotherapy in combination with tyrosine kinase inhibitors have provided limited improvements. When available, matched sibling or matched unrelated donor haemopoietic stem cell transplantation (HSCT) may provide the best long term disease control. Partially matched donors and cord blood sources of stem cells are being increasingly used in haematology. There are limited case reports to guide optimal management.

**Result**

We report the case of a 26yo female was diagnosed in December 2007 with Philadelphia positive ALL. She underwent induction with 3 cycles of Hyper CVAD and Imatinib. A suitable cord stem cell source of  $3.14 \times 10^7$  TNC/kg ( $17 \times 10^6$  CD34) matched at 4 out of the 6 HLA loci was identified, with no other suitable donors found. In May 2008 she underwent a matched unrelated cord blood transplant, with TBI and Cyclophosphamide conditioning and she continued Imatinib. GVH prophylaxis was Cyclosporine and Mycophenolate. The patient developed early severe graft versus host disease affecting the skin liver and gut. This was treated with intravenous Methylprednisolone, Etanercept, Basiliximab and mesenchymal stem cells (per MSC trial) with good response.

59 days post-transplant, Variable Number of Tandem Repeats (VNTR) PCR revealed donor engraftment and ongoing remission. Cytogenetics confirmed engraftment using sex chromosomes on day 99. Post transplant she developed *Pseudomonas aeruginosa* mastoiditis and otitis media, which was successfully treated, and a later episode of HSV. She later weaned off all medications and is currently very well, returning to work and has ongoing molecular remission.

**Conclusion**

Thus, cord transplantation may be a worthwhile option to provide long-term disease control in Ph<sup>+</sup> ALL.

*No conflict of interest to disclose*

P065

## Transplantation of Cord Blood – Volume Determination and Filtration at the Time of Infusion

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Since 2004, the volume of thawed cord blood (CB) units from 3 national and 8 international CB banks was measured before clinical transplantation. The thawed CB unit volume was measured with a 60 or 30mL syringe at the bedside before infusion. Data from 39 CB units demonstrated that the thawed volume was a median of 91% (range 76 to 127%) of the stated volume provided by the CB bank. There were insufficient CB units to ascribe any consistent volume discrepancy with any particular CB bank.

Many transplant facilities prefer to filter cryopreserved stem cell products before infusion, however this has proved difficult if the CB volume is also to be measured. The recent introduction of the Pedi-Syringe™ filter, a 60mL syringe fitted with a 150µm in-line filter connected to a transfer bag spike, has allowed this process to be performed. A validation study of Pedi-Syringe™ filter, performed using CB units unsuitable for the Sydney CB Bank, was performed to determine nucleated and CD34 cell loss after filtration via a Pedi-Syringe. Six thawed CB units, ranging from 20 to 45 mL, yielded a post-filtration median nucleated cell recovery of 111% (range 96 – 144%). Viable CD34 analysis, performed on 3 of these CB units, indicated a median recovery of 109% (range 92 – 131%) compared to the pre-filtration data.

This study demonstrates that (i) the frozen CB volume stated by the issuing bank is not always accurate, and (ii) that filtration of thawed CB through a 150µm syringe filter provides a convenient way of measuring the CB volume at the time of infusion that is not associated with significant loss of nucleated or viable CD34+ cells.

*This research was supported by GenesisBPS who provided samples for the validation study of the Pedi-Syringe™ filter. The company had no role in analysing the data or preparing the abstract*

**P066****FLT-3 and NPM1 Mutation Screening Using Capillary Electrophoresis**

**Kate Harrison**, Michael Vo, Monika Kutyna, Pamela Dyson, Ian Lewis  
*SA Pathology-RAH, Adelaide, South Australia, Australia*

Screening for mutations in NPM1 and FLT3, have been shown to be beneficial in predicting outcome for patients with cytogenetically normal AML.

Studies have shown that FLT3 Internal Tandem Duplications (FLT3-ITD) mutant to wild type allelic ratio has prognostic benefit, as patients with a high percentage of mutant alleles have an increased relapse risk and decreased overall survival.

We have validated a protocol to detect this mutation using capillary electrophoresis and GeneScan analysis of PCR fragment length. The GeneScan electropherogram produces peaks with specific band sizes for each product detected and the allelic ratio can be determined from the area under the peaks for each PCR product.

Previously, the method used to detect this mutation was PCR with analysis of product bands by agarose gel electrophoresis. GeneScan analysis is a more sensitive method for detecting mutations (as low as 2% of total DNA). The method is less time consuming and allows the allelic ratio and length of the ITD to be determined with much greater accuracy than agarose gel analysis.

NPM1 mutations indicate a better prognosis in the absence of FLT3-ITD mutations. Our previous method of mutation detection involved sequencing the NPM1 gene and analysing the sequence for mutations. We have now validated a protocol using fragment length analysis with capillary electrophoresis and GeneScan software. One set of primers are used to screen for the most common NPM1 mutations. This method is faster than the multi step sequencing protocol, but still retains the same level of accuracy and sensitivity in detecting mutations.

*No conflict of interest to be disclosed*

**P067**

**Clinical Implication of Genetic Mutation Testing for FLT3 and NPM1 in AML Patients**

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A common mutation in patients with AML occurs in the *FLT3* and *NPM1* genes. AML patient mutations in *FLT3* are either Internal Tandem Duplication (ITD) seen in up to 30% or D835, characterized with a missense mutation, in approximately 7%. Approximately 25% have *NPM1* mutations.

Royal Adelaide Hospital AML patients were tested for FLT3 -ITD/ D835 at diagnosis (95) and at relapse (16). The diagnostic method for detection of the FLT3 - ITD and D835 mutations, is based on PCR amplification of the region of usual occurrence. Testing for the D835 mutation requires digestion of the PCR product with EcoRV while ITD mutations are determined by estimating the size difference of the PCR product on agarose gel. Mutations in NPM1 are detected via sequencing of the PCR product of exon 12.

111 AML patients were studied: all were tested for ITD yielding 34 positives (31%) and 104 patients for D835 with 8 positive (8%). 84 patients were tested for NPM1 mutations with 16 positives (19%). 12 patients were NPM1/ FLT3 positive and 4 were NPM1 positive/ FLT3 negative. 58 of 99 patients had a normal karyotype.

Identification of the above mutations is useful for determining appropriate therapeutic options in normal karyotype AML. NPM1 positive/ FLT3 negative are the most favorable prognostic factor, with a high complete remission (CR) rate. Generally FLT3 mutations are less favorable tending to respond better to allogeneic stem cell transplantation. In such patients transplantation should be considered in first remission because of the increased likelihood of relapse. These patients may be eligible for FLT3 inhibitor clinical trials. NPM1/FLT3 positive patients are considered to have an intermediate prognosis and CR after chemotherapy is likely to be achieved, although sibling transplants may be considered.

*No conflict of interest to disclose*



P068

**Does -80°C Storage of Haemopoietic Progenitor Cells-Apheresis Product Affect Haemopoietic Recovery?****A Mouminoglu<sup>1</sup>**, K Stokes<sup>1</sup>, P Gambell<sup>1</sup>, D Wall<sup>1,2</sup>, K Herbert<sup>1</sup>, HM Prince<sup>1,2</sup><sup>1</sup>*Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia,* <sup>2</sup>*University of Melbourne, Melbourne, Australia***Introduction**

Haemopoietic progenitor cells-apheresis (HPC-A) transplantation is widely performed to support high-dose chemotherapy for treatment of haematologic and solid tumours. In the autologous setting, HPC-A products are routinely cryopreserved and reinfused at a later date. Evidence of cross-contamination of Hepatitis B in stem cell product stored submerged in liquid nitrogen (LN<sub>2</sub>) led to the recommendation of using vapour-phase storage for HPC-A products (Tedder et al). The Centre for Blood Cell Therapies (CBCT) at Peter Mac quarantines HPC-A products derived from sero-positive patients in a -80°C mechanical freezer. This however, does not conform to the Therapeutic Goods Administration requirement of storing HPC-A product at or below -140°C as per the British Pharmacopeia.

**Methods**

In this retrospective study, we reviewed post-transplantation haemopoietic recovery data for patients collected from 2001 onwards, whose product was stored at -80°C prior to transplantation, in order to validate our recommendation that products stored at -80°C are infused within 6 months of collection. Haemopoietic recovery was measured as time taken for neutrophil count to reach  $0.5 \times 10^9/L$  and platelets to reach  $20 \times 10^9/L$  unsupported.

**Results**

To date, 15 patients have been transplanted using cryopreserved HPC-A product stored at -80°C for between 20 days and 50 months (median 75 days). Haemopoietic recovery was achieved in all patients. The time to haemopoietic recovery following transplantation of -80°C product was compared to the median annual data for infusion with products stored in vapour-phase LN<sub>2</sub>. Time to neutrophil recovery was unchanged at 10 days ( $p=0.3356$ ); time to platelet recovery was slightly delayed at 13 days ( $p=0.0015$ ) compared with 11 days.

**Conclusion**

Our study has shown that HPC-A stored at -80°C for up to 50 months can achieve haemopoietic recovery thus confirming that storage up to 6 months is appropriate. The potential for longer-term storage at -80°C warrants further investigation.

*No conflict of interest to disclose*

**P069****Implementing an Effective HPC-A Collection Efficiency Monitoring System for Each Cell Separator****Sushil Narayan***Stem Cell Transplantation Programme, PA Hospital, Brisbane*

An effective HPC-A collection efficiency (**CE**) monitoring system for Cell Separators (**CS**) is essential to minimize procedure related risks to patients and maximizes cost effectiveness. This monitoring system should form part of the CS/Collection process validation and is a quality management system requirement. Recognising the limitations of CE measurement, the benefit of on-going quarterly CE monitoring verifies that each CS in-use is performing satisfactorily and problems are detected much earlier.

Validation of HPC-A collection procedure as part of commissioning 2 CS showed that median CE differed significantly (actual difference of > 20% for a quarter,  $n = 25$ ,  $P$  value = 0.0273). Had the facility continued overall CE monitoring as done historically, the difference in CE between the 2 CS would not have been detected in a timely manner. As a result of CE monitoring, investigation and corrective action, the CS's preventive maintenance schedule has been updated by the supplier and there is now no difference in CE between the two CS under observation. The facility has implemented quarterly monitoring of CE for each CS as part of its KPI & Clinical Indicators programme.

We have also implemented a review process for  $CE < 35\%$  using a set review template. This has resulted in changes to HPC-A collection procedures such as CS setting changes by the operator when harvesting patients with high WBC. Improved CE monitoring has worked well for our facility and may offer other facilities an early warning system for lower CE and poor performing CS. It has enhanced communications between apheresis staff, processing lab and the supplier. Additional benefits include improving the skills of the apheresis staff in instrument adjustment to improve harvest quality and consistency,

*No conflict of interest to disclose*

**A301**

P070

## Safety and Efficacy of Autografts Containing Reduced Numbers (<2 x 10<sup>6</sup>/kg) of CD34<sup>+</sup> Blood Stem Cells: A Comparison Study

Nicole Wright, Susan Wright, Elizabeth Tegg and **Scott Ragg**  
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### Aim

Whilst a minimum infusion of 2 x 10<sup>6</sup>/kg CD34<sup>+</sup> blood stem cells for an autologous BMT is generally regarded as safe practice, there is a clinical tendency to require up to 5 x 10<sup>6</sup>/kg for an autograft on the supposition of enhanced patient safety. This study aimed to compare the efficacy and safety of autografts containing <2 X 10<sup>6</sup>/kg CD34<sup>+</sup> cells with autografts composed of higher CD34<sup>+</sup> doses.

### Methods

150 consecutive PBSC first autografts for haematological malignancies between 1998 and 2008 were divided into 3 groups based on the dosage of CD34<sup>+</sup> cells. Engraftment kinetics and survival curves were assessed using Log Rank (Mantel-Cox) test, with all other factors scrutinised using Students unpaired t-test.

### Results

	<2 x 10 <sup>6</sup> /kg	2 - 5 x 10 <sup>6</sup> /kg	>5 x 10 <sup>6</sup> /kg
<b>n</b>	29	101	20
<b>Median CD34<sup>+</sup>/kg</b>	1.57 * <sup>#</sup>	2.68 * <sup>^</sup>	6.81 * <sup>^#</sup>
<b>Engraftment</b>	29/29	101/101	20/20
<b>Days Neut &gt;0.5</b>	11 * <sup>#</sup>	10 *	10 <sup>#</sup>
<b>Days Neut &gt;1.0</b>	11 * <sup>#</sup>	11 *	10 <sup>#</sup>
<b>Days Plat &gt;20</b>	18 * <sup>#</sup>	13 *	11 <sup>#</sup>
<b>Alive @ Day + 30</b>	27/29	100/101	20/20
<b>Alive @ Day + 100</b>	23/29 *	96/101*	19/20
<b>No of Admitted Days</b>	13 <sup>#</sup>	9	6 <sup>#</sup>

\* <sup>^</sup> <sup>#</sup> significant difference (p < 0.05) between these groups

All patients achieved myeloid engraftment after reinfusion. Neutrophil engraftment with <2 x 10<sup>6</sup>/kg CD34<sup>+</sup> cells was delayed by up to one day compared to the higher doses, a statistically significant but clinically irrelevant finding. Platelet engraftment was delayed by up to a week in the low dose population, indicating that these patients may require additional blood product support. Importantly, transplant related mortality (Day +30) was not significantly different between the groups, however, the tendency of the low dose group to have more admitted days may be indicative of increased morbidity. Medical record review revealed the significant difference in Day +100 survival was due to progressive disease, rather than the autograft *per se*.

### Conclusions

Successful and safe engraftment occurs after infusion with low numbers (<2 x 10<sup>6</sup>/kg) of CD34<sup>+</sup> cells. Clinicians should anticipate slightly delayed neutrophil and platelet engraftment. There is no evidence to support the notion of high dose (>5 x 10<sup>6</sup>/kg) CD34<sup>+</sup> cells as enhancing patient recovery and safety when compared to infusions between 2-5 x 10<sup>6</sup>/kg.

*There is no conflict of interest to declare*

**P071**

**Allogeneic HSCT with Reduced Intensity Conditioning with Fludarabine and 2 Gy TBI Is Safe and Provides an Adequate Donor Engraftment in Patients with Acute Myeloid Leukaemia and Multiple Myeloma**

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Patients with AML older than 56 years have a very poor outcome. Most patients with multiple myeloma (MM) would die by about 5 – 7 years following diagnosis. Allogeneic HSCT offers a chance for cure, however is associated with significant toxicity. Success using fludarabine and 2 Gy TBI as a regimen in preparation for an allogeneic HSCT is reported. We report our experience in using this regimen in patients with AML (n=5) and MM (n=5).

**Patients**

All patients were conditioned with fludarabine 30 mg/m<sup>2</sup> from day -4 to -2 and 2 Gy TBI on day 0. Cyclosporin was commenced at 6.25 mg/kg PO BD from day -3 and mycophenolate mofetil at 15 mg/kg BD from day 0.

Five patients with AML (median age 60 years, M=4/F=1) in CR1 underwent allogeneic HSCT. Three received grafts from unrelated donors and two from siblings.

Five patients with MM (4 ISS stage III, 1 stage II, M=5/F=0, median age 51 years), after undergoing cytoreductive melphalan 200 mg/m<sup>2</sup> autologous stem cell transplantation underwent allogeneic stem cell transplantation as per above.

**Results**

All patients had >90% donor T cell chimerism by day +30. No day 30 or day 100 mortality was seen.

AML patients: No patients dropped platelet count below 20 x 10<sup>9</sup>/l and neutrophils below 0.2 x 10<sup>9</sup>/l or required platelet transfusion. No grade III-IV AGVHD was seen. At 6 months, two patients have relapsed.

MM patients: Two patients dropped neutrophils to <0.2 x 10<sup>9</sup>/l. No patient required platelet transfusions or developed grade III-IV AGVHD. One patient developed limited oral chronic GVHD. All except one are in ongoing CR1.

**Conclusion**

The Fludarabine/2 Gy TBI conditioned allogeneic HSCT is safe with no day 30 and day 100 mortality, requires minimal transfusion support, is associated with modest cytopenias and GVHD and the entire protocol can be delivered as an outpatient resulting in major pharmaco-economic benefit.

*No conflict of interest to disclose*

**A303**

**P072****Mesenchymal Stromal Cell Culture : Improvements in Methodology**

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Clinical studies have indicated that ex-vivo expanded mesenchymal stromal cells (MSC) may play a role in reducing the effects of graft versus host disease.

Alternative media and cell factories were tested using normal bone marrow with a view to improving methodology. DMEM- low media supplemented with L-glutamine (DME-L) and DMEM-low glucose with Glutamax (DME-G) were trialled to determine the more effective media. (Glutamax is a stabilised form of L-glutamine).

Cells were cultured in two T175 flasks: one containing DME-L and the second DME-G. Both culture medias were supplemented with 10% FCS and 1% penicillin/streptomycin.

Superior results were obtained for DME-G when compared to DME-L. DME-G cultured cells achieved confluency within 18 days while DME-L media failed to achieve confluency in 26 days.

Flow cytometric analysis showed very low levels of mesenchymal markers for the cells cultured in DME-L compared to DME-G. Low levels of haematological markers were observed for cells cultured in both media types.

Clinical scale culture of MSC requires multiple flasks, considerable incubator space and is very labour intensive. A Nunc two layer cell factory was tested in parallel with T175 single layer flasks.

The cell factory with a surface area of  $1264\text{cm}^2$ , was set up with  $47.9 \times 10^6$  mononuclear cells. 350 mls of DME-G + 10% FCS /1% penicillin /streptomycin was used.

A total of  $14.14 \times 10^6$  MSC were obtained from the first passage. After three passages, the potential number of MSC was  $84.31 \times 10^6$ , a potential expansion of 5.96 from the initial passage.

We conclude that the use of cell factories is viable in the culture of MSC for transplant.

*No conflict of interest to disclose*

**P073**

## Exploring the Long Term Impact of Haemopoietic Stem Cell Transplantation on Quality Of Life in Order to Better Inform Patient Care

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### Aim

The Alfred has established a Late Effects Clinic (LEC) to assist in the management of long term survivors of haemopoietic stem cell transplantation. This study aims to highlight which long term effects on quality of life are prevalent amongst our patients in order to better target resources to meet their needs.

### Method

The FACT-BMT quality of life questionnaire was administered to patients attending the LEC. This questionnaire has 50 questions that cover physical, social/family, emotional and functional domains as well as transplant specific symptoms.

### Results

Thirty eight surveys were evaluated. Twenty three respondents had received an allograft and 15 an autograft. The median age was 55 years with an average of 5 years since transplantation (range 2-10). Overall quality of life was good with a mean FACT-BMT score of 119 (possible score 0-148).

Variables (min-max possible score)	Mean scores (range)
Physical well being (0-28)	23.2 (14-28)
Social well being (0-28)	23.7 (12-28)
Emotional well being (0-24)	20.6 (14-24)
Functional well being (0-28)	21.1 (8-28)
BMT well being module (0-40)	30.8 (220-39)
Overall FACT-BMT	119 (77-148)

The most common physical problems included lack of energy (82%), fatigue (72%), difficulty sleeping (29%), frequent infections (28%), blurry eyesight (26%), shortness of breath (24%) and skin problems (24%). Cognition was also impaired with 44% having significant difficulty with memory and 40% difficulty concentrating. Social/family well being was high with most feeling close to and supported by family and friends. Sexual health concerns were common with half of respondents indicating low satisfaction with their sex life and twenty percent were worried about fertility. Staff at the LEC clinic have been specifically trained in sexual health to tackle this issue. Emotional well being was also maintained with low scores for anxiety/depression and high scores for hope and confidence in staff. Some respondents worried that their condition would worsen (47%). Despite some respondents experiencing significant difficulties, none expressed strong regret at having undergone transplantation.

### Conclusions

While overall quality of life is good amongst long term survivors of transplantation, there are areas that are impacted in the long term. Use of a quality of life tool can assist in highlighting areas of need to focus resource development and provision.

*No conflict of interest to disclose*

**A305**

**P074****Immune Reconstitution Post Haemopoietic Stem Cell Transplantation: A Comparison of Myeloablative and Reduced Intensity Conditioning Approaches**

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**Aim**

To examine in detail the kinetics of immune reconstitution post allogeneic stem cell transplantation and compare the effects of myeloablative (MAB) and reduced intensity conditioning (RIC) approaches.

**Method**

Peripheral blood was collected pre transplant, D14, D30, then monthly to 6 months and 3 monthly thereafter. Flow cytometry was used to phenotype T, B and NK cells in detail.

**Results**

Thirty five patients have been enrolled with median follow up of 150 days (range 30-360). Twelve patients received myeloablative conditioning (MAB), 12 reduced intensity conditioning with fludarabine/melphalan/Campath (RIC-FMC) and 11 very reduced intensity conditioning with 2GyTBI +/- fludarabine (RIC-2GyTBI).

Early post transplant, the absolute number of NK cells fell, however they formed the majority of peripheral blood lymphocytes for MAB and RIC-FMC patients. In contrast, RIC-2GyTBI patient NK cell numbers remained steady. B cells were markedly depleted and recovery delayed until at least 9-12months.

T cell numbers showed recovery by D90-120, with RIC-2GyTBI patients reconstituting earliest. CD8<sup>+</sup> subsets recovered before CD4<sup>+</sup> (D90-120 vs D270-D360) for all groups. RIC-2GyTBI patients had more CD4<sup>+</sup> cells than RIC-FMC or MAB patients and in particular CD4<sup>+</sup>CD45RA<sup>+</sup> cells (naïve phenotype) showed higher numbers and appear to normalise by 12 months. RIC-FMC patients in contrast remained profoundly CD4<sup>+</sup>CD45RA<sup>+</sup> cytopenic at 12 months.

RIC-FMC patients had the lowest T regulatory cells and most delayed recovery (D120). T regulatory cells were higher for the RIC-2GyTBI group and normalised earlier (D60). NKT cells are detectable but absolute numbers remained low at 12 months post transplant and longer follow up is warranted.

**Conclusions**

These data shed light on the complex nature of immune reconstitution post allogeneic stem cell transplantation. The conditioning received appears to have an impact on the kinetics of some lymphocyte subset reconstitution. Further studies into the impact on thymic function, chimerism and clinical outcomes are ongoing.

*No conflict of interest to disclose*

P075

## First Reported Case of Philadelphia Chromosome-Positive Blastic Plasmacytoid Dendritic Cell Leukaemia

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### Background

The Philadelphia chromosome t(9;22)(q34;q11.2) whilst being the defining cytogenetic feature of chronic myeloid leukaemia (CML), has also been well described as a cytogenetic abnormality associated with acute lymphoblastic leukaemia (ALL), mixed phenotype acute leukaemia, and less frequently, *de novo* acute myeloid leukaemia (AML).

### Aim

We describe the first recorded case of blastic plasmacytoid dendritic cell leukaemia with Philadelphia chromosome, as evidenced on FISH, RT-PCR and cytogenetic analysis.

### Case

A 29 year old male was referred to our institution after sustaining an atraumatic pathological fracture of the right pubic ramus, which was associated with an extensive soft-tissue mass with bony destruction, and regional lymphadenopathy. Lesion, lymph node and bone marrow examination demonstrated blastic plasmacytoid dendritic cell leukaemia, with characteristic morphological appearances, immunohistochemistry, and flow cytometric profile. Cytogenetic analysis revealed a complex karyotype: 16 of 20 dividing cells showed a hypotriploid cell line with 58 chromosomes, including trisomies 7, 10, 12, 13, 14, 15 and 21, tetrasomy 8 and 19, additional material attached to chromosome 1, a translocation of 9;22, resulting in the Philadelphia chromosome, and an extra copy of the der(22) from the t(9;22).

FISH studies using the BCR/ABL dual fusion probe showed two fusion signals in 60 of 100 interphase cells, and three fusion signals in 26 of 100 interphase cells, consistent with the extra copy of the Philadelphia chromosome detected cytogenetically. Molecular analysis by RT PCR confirmed the existence of the BCR-ABL1 transcript.

The patient commenced on induction chemotherapy, and whilst pancytopenic, developed rapidly fatal clostridial necrotising fasciitis.

### Conclusion

Blastic plasmacytoid dendritic cell leukaemia/lymphoma is an uncommon disease, representing less than 1% leukaemia/lymphoma, with an aggressive clinical course. Diagnosis relies on the demonstration of co-expression of CD4+, CD56+ and CD123+ in the absence of any specific myeloid, B-, T-lymphoid, or natural killer (NK) lineage markers. Although complex karyotypes are common, and several major recurrent chromosomal abnormalities have been recognised, this is the first case reported to have the Philadelphia chromosome associated with blastic plasmacytoid dendritic leukaemia.

*No conflict of interest to disclose*



**P076**

## **Autoimmune Lymphoproliferative Syndrome (ALPS): A Case Report and Review of the Literature**

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### **Aim**

We present a case of an adult diagnosed with Autoimmune Lymphoproliferative Syndrome Type 1a by genetic analysis, and review the literature with regards this rare, but possibly under-diagnosed disorder.

### **Case**

A 26yo male was referred to our institution for consideration of therapy for his refractory relapsing trilineage autoimmune cytopenias, most recently dominated by an immune thrombocytopenia-like picture. He had undergone treatment with glucocorticoids, intravenous immunoglobulin, azathioprine and splenectomy, with documented massive splenomegaly of 1800g in the absence of a clear histological diagnosis, and no abnormalities on prior bone marrow examination or lymph node biopsy.

A repeat bone marrow examination was performed, which revealed a significant double negative T-cell population (CD2+/3+/4-/5+/7+/8-/αβ+) comprising 20% lymphocytes (4% total cells) and multiple lymphoid follicles on trephine section. Suspicion of autoimmune lymphoproliferative syndrome led to genetic testing, which revealed a heterozygous c.139C>T, PQ47X mutation, consistent with a missense exon 2 mutation in the Fas gene, indicating a diagnosis of ALPS Type 1a.

### **Discussion**

ALPS is a relatively recently defined condition, resulting in benign lymphoproliferation, autoimmune phenomena, and an increased risk of lymphoma, resulting from mutations in Fas (ALPS Ia), Fas ligand (ALPS Ib), or Caspase 10 (ALPSII), and resulting in impaired apoptosis. A characteristic finding is an increased population of αβ+/CD4-/CD8- T-cells on flow cytometry. Previous studies have shown that a genotype-phenotype correlation exists with mutations in exons 6-9 being the most common, affecting the intracellular death domain, and representing the more severe end of the disease spectrum, while those in exons 1-5 are associated with a clinically milder form of the disease, and code for extracellular portions of the protein.

### **Conclusion**

This case demonstrates the variation in clinical presentation of ALPS, with adult presentation in the absence of lymphadenopathy, and delayed demonstration of a double negative T-cell population on flow cytometry. It highlights the importance of repeat testing, and a high clinical suspicion in the presence of suggestive features.

*No conflict of interest to disclose*

**A308**

P077

## Stem Cell Mobilization Using Out-patient ICE and Single Dose of Pegfilgrastim is Associated with Inferior Mobilization Outcomes

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### Aim

This pilot study was aimed at assessing the feasibility of mobilization using outpatient fractionated ifosfamide, carboplatin and etoposide (ICE) as salvage therapy along with a single dose of pegfilgrastim in patients with relapsed or refractory Hodgkin's and non-Hodgkin's lymphoma.

### Methods

Between 2005 and 2008, 10 patients (5 Hodgkin's and 5 diffuse large cell lymphoma) underwent salvage therapy with ICE and 9 with evidence of responsive disease received pegfilgrastim (6 mg SC) on Day 4 following the 3<sup>rd</sup> cycle of ICE towards stem cell mobilization. Stem cells were harvested once the peripheral blood CD34 dose was > 20/ul.

### Results

Six patients (66.6%) successfully mobilised at a median of 31 days (range: 22 – 36) from administration of pegfilgrastim while 3 had failure of mobilization. The median peripheral blood CD34 on the day of harvest was 31.5/ul (range: 22 -38) while a median dose of  $3.1 \times 10^6$  CD34/kg (range: 2.6 to 4.1) was harvested. Four of the six patients required 2 harvests to obtain an adequate cell dose. Compared with a retrospective cohort of patients mobilised with ICE chemotherapy and G-CSF (5 -10 ug/kg/day), mobilization results were clearly inferior (Table 1). Of the 3 patients with mobilization failure, 2 were mobilised with AMD3100 and 1 with Cyclophosphamide /G-CSF. All patients underwent autologous stem cell transplant with median neutrophil engraftment of 15 days (range: 11 – 21) and platelet engraftment of 19 days (range: 13-35). At a median follow up of 12 months (range: 6 -37), eight patients (80%) are alive.

### Conclusion

Stem cell mobilization using ICE chemotherapy and pegfilgrastim has inferior mobilization characteristics compared with ICE chemotherapy and daily filgrastim. In patients who had successful mobilization, neutrophil and platelet engraftment following autologous SCT was prompt and not delayed.

*No conflict of interest to declare*

Table1: Mobilization data of patients mobilized using different mobilization regimens

	ICE with Pegfilgrastim	ICE with G-CSF (5ug/kg)	ICE with G-CSF (10 ug/kg)
Mobilization success (%)	66.6%	100%	87.5%
Median time to harvest (days)	31 (22-36)	14 (10-20)	14 (12 – 18)
Median peripheral blood CD34 (/ul)	31.5 (22 – 38)	44 (17 – 427)	115 (25 – 1159)
Median number of harvests	2 (1-2)	1 (1-3)	1 (1)
Median cell dose (x $10^6$ CD34/Kg)	3.1 (2.6 – 4.1)	5.0 (2.3 – 27.2)	5.1 (2.3 – 32)

**P078**

## **Twenty years of Chronic Myeloid Leukaemia in Christchurch, New Zealand**

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### **Aim**

This study aims to examine the diagnosis, assessment and outcome of Chronic Myeloid Leukaemia (CML) in patients at Christchurch Hospital in the South Island of New Zealand, comparing local results with national and international outcomes and recommendations where available.

### **Methods**

Patients diagnosed with CML who had treatment at Christchurch Hospital over a 20 year period (01/01/1988 – 31/07/2008) were studied. Retrospective data collection was performed by reviewing the clinical record of each patient and by searching laboratory results from the computerized hospital information systems.

### **Results**

There were a total of 91 patients (age 13-85, median 49). 47 patients were treated with imatinib mesylate, and 30 patients received an allogeneic bone marrow transplant. 26 out of 29 patients diagnosed after November 2002 when imatinib was available for all CML patients in New Zealand, received imatinib in chronic phase. Only one out of 29 patients (diagnosed post- November 2002) received a bone marrow transplant. 4 year overall survival in all patients diagnosed after imatinib became available was 89% compared with 66% for all patients diagnosed prior to this time, ( $p=0.33$ ). 5 year survival for patients who received imatinib at any time after diagnosis was 87% compared with 42% for patients who never received imatinib at any stage ( $p=0.008$ ). The 5 year survival for patients treated with bone marrow transplant was 72% and for all patients who were never transplanted was 55% ( $p=0.04$ ). Regular follow up and molecular monitoring by methods such as quantitative-PCR (Q-PCR) for BCR/ABL was employed for early detection of treatment failure and disease progression.

### **Conclusion**

Treatment of CML with tyrosine kinase inhibitors has improved the survival and reduced the progression of disease to advanced phases and the use of treatment with bone marrow transplant.

*No conflict of interest to disclose*

**P079**

**A Cross-Sectional Study to Assess the Prevalence of Cardiovascular Risk Factors and Metabolic Syndrome in Adult Long-Term Survivors of Haemopoietic Stem Cell Transplantation (HSCT)**

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**Aim**

Long-term survivors of HSCT have late mortality from a variety of complications including cardiovascular events. This provides an opportunity for early intervention and risk factor modification to impact on long-term survivorship. We have evaluated the prevalence of metabolic syndrome, its components and other established risk factors for atherosclerotic complications in long-term survivors of HSCT attending a dedicated late effects clinic (LEC).

**Method**

Consecutive long-term survivors of HSCT (alive and disease free for  $\geq 2$  years) attending a dedicated LEC had weight, height, waist circumference, smoking habit, blood pressure, fasting blood glucose and lipid profile recorded. The metabolic syndrome was defined according to the International Diabetes Federation, by the presence of obesity and any two of raised triglycerides, blood pressure, fasting glucose or reduced HDL-cholesterol.

**Result**

Between May 2008 and June 2009, 48 patients (25 male, 23 female) were evaluated. Median age at transplantation was 48.5 years (range 17 – 63) and at first LEC attendance, 54 years (range 25 – 67). Median time from transplantation was 5.25 years (range 1.5 – 11.3). 18 patients received autologous and 30 patients allogeneic HSCT (50% non-myeloablative). Lymphoma and acute myeloid leukaemia were the dominant indications for HSCT, accounting for 66%. The overall prevalence of metabolic syndrome was 35% whilst an additional 29% had two or more of its components. Individual risk factors are shown below. Three patients had an established diagnosis of cardiovascular disease.

Risk factor	Autograft n=18	Allograft n=30	Total n=48
Elevated fasting glucose ( $\geq 5.7$ or drug treatment)	6(33%)	7(23%)	13(27%)
Hypertension ( $\geq 130$ mmHg systolic or drug treatment)	8(44%)	14(47%)	22(46%)
Dyslipidaemia (Cholesterol/HDL ratio $> 3.5$ )	14(48%)	19(63%)	33(69%)
Obesity (BMI $\geq 25$ )	11(61%)	16(53%)	27(56%)
Smoking	4(22%)	4(13%)	8(17%)

**Conclusion**

We conclude that cardiovascular risk factors occur frequently among survivors of both autologous and allogeneic HSCT. Cardiovascular problems typically manifest after a long latent period, and may therefore require this HSCT cohort to mature further before a complete picture emerges. These results should encourage systematic screening for cardiovascular risk factors in long-term survivors of HSCT and the institution of appropriate preventative treatment when identified.

*No conflict of interest to disclose*

P080

## Myeloproliferative Disorders with Coexisting BCR-ABL Translocation and JAK2V617F Mutation: Potential for Combination Therapy with Tyrosine Kinase Inhibitors and JAK Inhibitors

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### Aim

Chronic myeloid leukaemia (CML) is a clonal disease of hematopoietic progenitor cells that have acquired the BCR-ABL fusion gene. Imatinib mesylate (IM) provides a highly successful treatment approach in the majority of chronic phase (CP) - CML patients. Here we report uncommon case of CML-CP patient which might provide insight into the disease biology and potential role of combination therapy.

### Method

An 81 year old man presented with a history of lethargy and shortness of breath for 4-5 weeks. Clinical examination showed hepatosplenomegaly. Blood counts at presentation: Hb-112g/L, WBC-62.5x10<sup>9</sup>/L, platelets-232x10<sup>9</sup>/L, Eosinophils-0.63x10<sup>9</sup>/L, Basophils-1.88x10<sup>9</sup>/L. Bone marrow (BM) cytogenetic demonstrated-45,X,-Y,t(9;22)(q34;q11.2)[20] and RQ-PCR showed 36% BCR-ABL transcript, consistent with the diagnosis of CML. He achieved complete cytogenetic response (CCR) and major molecular response (MMR) following 6 months of IM therapy. However, a steadily increasing platelet count (717x10<sup>9</sup>/L at 6 months) was observed. All other blood parameters were normal, the likely causes of reactive thrombocytosis were excluded and there were no other features of disease progression. Repeat BM biopsy demonstrated a significant increase in megakaryocyte clusters and increased reticulin fibrosis, not present at the time of presentation. Importantly, further investigation identified JAKV617F mutant clone in 30% of cells. Retrospective analysis of presentation BM sample revealed 2% JAKV617F clone.

We postulate that this patient harboured two myeloproliferative disease (MPD) clones: one Ph<sup>+</sup>/JAK<sup>-</sup> and the other Ph<sup>-</sup>/JAK<sup>+</sup>. The rapidly proliferating Ph<sup>+</sup> clone was dominant at presentation resulting in clinical features of CML. Successful suppression of the Ph<sup>+</sup> clone with imatinib, resulted in relative expansion of the Ph<sup>-</sup>/JAK<sup>+</sup> clone with the concomitant clinical features of ET.

### Conclusions

This case demonstrates that two MPD clone can exist in a single patient and highlights the potential for combination therapy, using JAK inhibitors (currently in clinical trial), and BCR-ABL inhibitors in such cases.

*No conflict of interest to disclose*

A312

P081

## Correlating the Haematopoietic Cell Transplantation Co-Morbidity Index (HCT-CI) to Post Transplant Quality of Life (QOL) Assessments: A Single Institution Cross-sectional Review

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The HCT-CI is a newly developed index designed to assess the risk of NRM in patients undergoing allogeneic transplantation. Aims: we assessed whether the HCT-CI could also be used to predict QOL outcomes in patients surviving RIC-HCT. Methods: between 2001-08, 46 patients who had received a RIC-SCT at RNSH were assigned a pre-transplant HCT-CI score and FACT-BMT scores were used to assess QOL in 21 survivors post-transplant. Survival estimates were calculated to determine a correlation between HCT-CI and NRM, DFS and OS at 12months. Results: overall survival (OS) at 12mths was 77% with a mean OS of 26.1 months (95% Confidence interval, 19.1-33.0). There was no significant correlation between HCT-CI scores and TRM ( $p=0.693$ ) however there was trend to improved NRM at 12months in the lower risk groups (0-0%, 1-2 -5.1% & >3 -7.7%). In patients surviving >2yrs post transplant, higher pre-transplant HCT-CI scores predicted poorer long term survival. Overall, surviving patients achieved good QOL with high median FACT-BMT scores, however there was no correlation with HCT-CI scores ( $p=0.388$ ), or age ( $p=0.312$ ) and post-transplant QOL. QOL scores were strongly associated with patients perception of GVHD severity ( $p=0.044$ ). cGVHD was associated with improved OS ( $p=0.006$ ). Conclusions: prospective studies are required to determine the utility of the HCT-CI in predicting NRM. Further efforts are required to develop tools which accurately predict post RIC-SCT QOL outcomes.

*No conflict of interest to disclose*

P082

## Validation of the Australasian Guidelines for Antifungal Prophylaxis in Patients Undergoing Chemotherapy for the Treatment of Acute Myeloid Leukaemia

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### Aim

In order to determine if the rate of invasive fungal disease (IFD) is sufficiently high to warrant broad-spectrum antifungal coverage as recommended by recent Australasian consensus guidelines, we audited the incidence/characteristics of IFD during remission induction and subsequent chemotherapy in patients with acute myeloid leukaemia (AML) treated at our institution over a 4 year period. Our institution had not routinely employed broad-spectrum antifungal coverage to date.

### Methods

Case notes of patients diagnosed with AML receiving remission induction chemotherapy between 2004 and 2008 were reviewed. IFD was classified as possible, probable or proven using standard internationally recognised definitions. Chemotherapy cycles were classified as high-risk (induction/reinduction/high-dose cytarabine) or standard-risk (consolidation with standard dose cytarabine) of IFD.

### Results

76 cycles of chemotherapy (51 high risk, 25 standard risk) were included involving 34 patients. Primary fungal chemoprophylaxis was with fluconazole (51 cycles), posaconazole (2 cycles) or no prophylaxis (10 cycles). Secondary prophylaxis was used in 13 cycles. The overall number of chemotherapy cycles (high and standard risk) complicated by possible, probable and proven IFD was 5 (7.1%), 1 (1.4%) and 0 (0%) respectively. All cases occurred in high-risk cycles (risk for high-risk cycles: 13% possible, probable and proven IFD, 2% for probable and proven IFD). There was no IFD in the 25 standard risk cycles. Pharmacoeconomic analysis of posaconazole prophylaxis in this patient population is underway.

### Conclusion

We conclude that there was a significant (13%) incidence of potential IFD in high-risk cycles in patients not receiving broad-spectrum antifungal prophylaxis. However, the risk of invasive fungal disease in standard-risk cycles is negligible and does not warrant the use of broad-spectrum antifungal drugs. These results confirm the validity of the recent Australasian guidelines for fungal prophylaxis and allow for accurate assessment of future interventions (e.g. posaconazole prophylaxis for high-risk cycles) at our institution.

*No conflict of interest to disclose*

A314

P083

## BK Virus Specific Cytotoxic T Cells for Clinical Use as Immune Therapy in Haemopoietic Stem Cell Transplant Recipients

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### Introduction

BK virus (BKV) is a polyomavirus that is ubiquitous in humans, infecting over 85% of normal individuals. After initial infection it persists in a latent state in the urothelium from whence it can reactivate causing disease in immunocompromised patients. BKV is a cause of haemorrhagic cystitis after allogeneic SCT and is emerging as one of the major causes of graft loss after renal transplant. Current treatment is limited to reduction of immunosuppression as possible.

### Aim

To develop a method for production of a T cell product with BKV specificity for use in adoptive immunotherapy post haemopoietic stem cell transplantation.

### Method

Monocyte derived DC (DC) were generated from peripheral blood by adherence to plastic and exposure to IL-4 and GM-CSF. DC were exposed to mixes of overlapping peptides covering 5 BKV proteins (VP1, VP2, VP2 isoform 3, large T antigen (LTA) and small T antigen (sTA)) and matured with TNF. Mature DC were used to stimulate T cells from the same donor. Cells were cultured for 21 days with a second stimulation on day 7, and exposed to increasing doses of IL-2 thereafter. The cellular product was then analysed for phenotype and BKV specificity by examining cytokine production and cytotoxicity.

### Results

Cellular proliferation was seen in all of 7 normal donors with a mean increase of 7.7 fold in total cell number. All cellular products were >90% CD3 positive (Mean 97%, SEM .799) with CD4 and CD8 ratio varying significantly between individual donors. (CD4 range 9.7 to 93.4%, mean 72.9; CD8 range 3.9 to 77.0%, mean 21.5). Cytokine responses to stimulation with BKV peptides were able to be elicited in 6 of 7 donors. Multiple cytokines were produced by the responding cells: IFN- $\gamma$  (mean 29.9% of CD3 cells, range 4.5 to 78.8), TNF (mean 19.9%, range 2.7 to 63) and IL-2 (mean 12.8%, range 1.2 to 37.8). Cytokine responses were seen in both CD4 and CD8 cells and showed significant individual variation. VP1 and LTA specific cells dominated most cultures while a smaller percentage of the cells were specific for VP2, VP2 isoform 3 and sTA. Cultures exhibited cytotoxic activity with the lysis of BKV antigen coated target cells in a pattern that correlated with the presence of CD8 positive cytokine producing cells (up to 78.9% specific lysis at effector to target ratio of 20:1).

### Discussion

The clinical utility of this product remains to be determined. Potential uses include prophylaxis and therapy of reactivation of BK virus after haemopoietic and renal transplantation.

*No conflicts of interest to disclose*



**P084**

## **A Case Report of Spontaneous Differentiation of B-Chronic Lymphocytic Leukaemia Cells in Culture Medium into Plasma Cells**

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### **Aim**

We report a case of spontaneous differentiation of a cell culture of B-chronic lymphocytic leukaemia cells into immunoglobulin-secreting plasma cells.

### **Case**

A 74yo woman was diagnosed with B-chronic lymphocytic leukaemia on lymph node biopsy and bone marrow examination, with typical peripheral blood morphology, a lymphocytic infiltrate of CD5+, CD20+, CD23+, CD79a+ B-cells, a nodular mixed infiltrate of small lymphocytes on bone marrow trephine, and flow cytometric phenotype showing a kappa-restricted population of CD5+,CD19+,CD20+,CD23+ B-lymphocytes, consistent with CLL. The patient subsequently agreed to participate in a study of CLL stem cells.

### **Method**

Peripheral blood was collected in EDTA, and white cells were isolated by the ammonium chloride method. Two hundred and fifty million cells were cultured in 25 cm<sup>3</sup> flasks, in 5mL culture medium (RPMI media with 4mM L-glutamine, 1x Streptomycin/Penicillin and 1mM sodium pyruvate and 10% serum supreme). A half medium change was performed once a week with fresh culture medium, with weekly cell count and May-Grunwald-Giemsa staining and examination of cells.

### **Results**

Upon routine weekly review of the cell cultures on day 56, it was noted that the previously morphologically typical CLL cells had differentiated into Mott cells, with numerous cytoplasmic vacuoles located adjacent to the eccentrically-placed nucleus, within deeply basophilic cytoplasm. These cells were positive for kappa-light chain on immunohistochemical staining. Plasma protein electrophoresis (EPP) of the culture supernatant showed a band against kappa-light chain antiserum on the CLL culture. By isoelectric focusing over the pH range of 3-10 and immunofixation, kappa light chains were detected in the absence of intact kappa-IgM.

### **Conclusion**

Although reports of differentiation have been described in association with stimulation with various ligands, including exposure to CD4+ helper T-cells, phorbol ester (TPA), interferon- $\alpha$ 2, and interferon- $\gamma$ , this is the first reported case of B-CLL, to our knowledge, spontaneously differentiating into immunoglobulin-secreting plasma cells in culture medium.

*No conflict of interest to disclose*

**A316**

P085

## Reduced-Intensity Allogeneic Stem Cell Transplantation for Older Patients with AML

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### Aims

Treatment of older patients with acute myeloid leukaemia (AML) remains challenging, with poor outcomes due to both high relapse risk and excessive treatment-related toxicity. We undertook a study to determine if reduced-intensity conditioning (RIC) allogeneic transplantation represents a strategy to improve long-term disease free survival in this subgroup.

### Methods

We retrospectively reviewed all allogeneic stem cell transplants for AML in patients greater than 60 years of age, performed at the Royal Brisbane and Women's Hospital, between January 2000 and May 2007. All patients received fludarabine (25mg/m<sup>2</sup>/dose D-7 to D-3) and melphalan (120mg/m<sup>2</sup> D-2) as conditioning. Standard GVHD prophylaxis was cyclosporine and methotrexate. Survival analysis was performed using the Kaplan-Meier product-limit method. Follow-up of patients was censored at May 2008

### Results

22 patients underwent transplantation during the study period. Median age at transplant was 64.4 years (range 60.2 – 66.6). All patients received G-CSF-stimulated PBSC, except 1 who received unprimed BM. 13 (59%) had de novo AML, 5 (23%) had prior MDS, 2 (9%) had a prior MPD, whilst 1 patient had each of biphenotypic leukaemia and therapy-related AML. 12 (55%) were transplanted in CR1 (including 2 with delayed CR), 6 (27%) in CR2, 3 (14%) in MDS phase following CR1, and 1 with untreated 2<sup>nd</sup> relapse. 11 (50%) received grafts from sibling donors (9 in CR1), whilst 11 (50%) received unrelated donor grafts. 9/21 (48%) evaluable patients developed Grades II-IV acute GVHD (5 with Grades III-IV). Chronic GVHD occurred in 10/20 (50%) evaluable patients, of whom 9 had extensive involvement. At a median follow-up of 2.4 years, 2 year overall survival is 52%. 10 patients died during follow-up – 6 from relapsed disease (median time to relapse 0.27 years), 3 from acute GVHD, and 1 from idiopathic pneumonia.

### Conclusion

RIC allografting with fludarabine/melphalan conditioning is feasible in older patients. Relapsed disease, rather than regimen-related toxicity, is the main cause of mortality.

*No conflict of interest to disclose*

**P086**

## **Cytogenetics in Elderly Patients with Acute Myeloid Leukaemia in Western Australia**

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Between January 1991 and December 2005 over 900 patients were diagnosed with acute myeloid leukaemia in Western Australia. Clinical information was available on 825 patients, and a karyotype was available on 674 of these. Patients over 60 constituted 63% of all cases, and more than half had secondary AML. Dramatic differences were observed in the relative frequency of prognostic groups over the age of 60. Specifically there was a decline in favourable karyotypes to only 5%, increase in normal karyotypes (47%), and increase in unfavourable karyotypes (27%), especially those of a complex nature. The aging Australian population will lead to an increase in cytogenetic demand, and it is important to evaluate the impact this will have on service.

*No conflict of interest to disclose*

P087

## BEZ235, a Dual Pan Class I PI3 Kinase and mTOR Inhibitor Promotes Osteogenic Differentiation of Human Mesenchymal Cells Through Modulation of TGF- $\beta$ and BMP Signaling

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### Aim

Multiple myeloma (MM) is unique amongst haematological malignancies in its capacity to cause massive osteolytic lesions throughout the skeleton which often persists beyond disease remission. One approach to restore lost bone is to stimulate osteoblast differentiation and function. We have previously shown that imatinib mesylate stimulates bone formation in CML patients through inhibition of PDGF-induced PI3 kinase activation<sup>1</sup>. The aim of this study was to further investigate this pathway using a novel PI3 kinase/mTOR inhibitor, BEZ235 (Novartis).

### Methods

Bone explant cultures, comprising multipotent mesenchymal stromal cells (MSCs), were generated from human iliac crest bone biopsies. MSCs were cultured under osteoinductive conditions and the effect of BEZ235 on osteoblast differentiation and function was assessed using *in vitro* mineralisation cultures, quantitative RT-PCR and Western blotting. To assess anabolic effects, organotypic cultures of mouse cranial calvaria were cultivated in the presence of BEZ235, processed into paraffin, sectioned, stained and new bone growth quantitated.

### Results

BEZ235 inhibits PDGF-induced activation of Akt and p70S6K (IC<sub>50</sub> 10nM and 4nM respectively) in MSCs, suppresses cell proliferation but does not induce apoptosis. In osteogenic cultures, BEZ235 potently induces an osteogenic response characterised by an upregulation in genes involved in osteoblast differentiation and function (*BMP2*, *BMP6*, *TGFB1*, *INHBA*) and an increase in mineralised ECM *in vitro*. Consistent with upregulation of TGF $\beta$  superfamily members, BEZ235 induces activation of SMAD proteins. Furthermore, BEZ235 has an anabolic effect on bone in mouse calvarial organotypic cultures. Using a pharmacological approach, we demonstrate that BEZ235 mediates its pro-osteogenic effect through inhibition of mTOR rather than PI3 kinase.

### Conclusions

We demonstrate that inhibition of mTOR by BEZ235 promotes osteogenic differentiation and function in human MSCs. In light of our findings, further examination of the mTOR pathway in MSC differentiation may reveal pharmacological targets that could be used to stimulate osteogenesis and restore tumour degraded bone.

### References

1. Fitter S, Dewar AL, Kostakis P, et al. Long-term imatinib therapy promotes bone formation in CML patients. *Blood*. 2008;111:2538-2547.

No conflict of interest to disclose

**P088**

## **A Review of all Bone Marrow Aspiration Pathology between 2001 and 2008 in Papua New Guinea**

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### **Aim**

To conduct a comprehensive quantitative and qualitative review of all bone marrow aspirate (BMA) reports from 2001 to 2008 at Port Moresby General Hospital (PMGH).

### **Method**

1. Retrospective analysis of all bone marrow aspiration reports from tumour registry at PMGH
2. Data collected using a set proforma
3. Microsoft excel was used to analyse the data

### **Results**

595 bone marrow studies were done between 2001 and 2008, over half (67%) were from PMGH, the remainder were from peripheral provincial hospitals. Gender distribution was nearly equal however the paediatric population accounted for only 22% of the cases studied. The main indications for biopsy were persistent anaemia, pancytopenia, and blasts on peripheral blood film, bleeding tendencies, fever and splenomegaly. 10% of specimens were unsatisfactory for interpretation, most of which came from provincial hospitals. Demographic and clinical information was adequate, however in over 90% of reports the Full Blood Examination report was not included. The turn around time (TAT) range at PMGH was 5 to 7 working days and for provincially received BMA's 6 to 12 weeks.

The most common diagnosis was nutritional deficiency (incidence rate (IR) 0.34/100,000) followed by acute myeloblastic leukaemia (IR: 0.18/100,000), reactive marrows (IR: 0.15/100,000) and chronic myeloid leukaemia (IR: 0.10/100,000). Of the 595 BMA's studied 35.4% were haematological malignancies with AML comprising 41% followed by CML 24% then ALL 18%, the trend being no different in both adult and paediatric populations.

### **Discussion**

The way forward will be establishing a database for the BMA registry, a new reporting proforma for BMA and telepathology to improve TAT range significantly with training of peripheral staff using ICSH guidelines for BMA.

*No conflict of interest to disclose*

**A320**

P089

## Performance of Soluble Transferrin Receptor Assay in Assessment of Iron Stores Compared to the Gold Standard of Bone Marrow Aspirate Perl's Stain: One Institution's Experience

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### Aim

To assess the utility, sensitivity and specificity of soluble transferrin receptor index for detection of iron deficiency compared to bone marrow aspirate iron staining in our laboratory.

### Methods

All patients undergoing bone marrow aspirations for clinically necessary indications had concurrent full blood count, iron studies and soluble transferrin receptor assay performed on peripheral blood. 40 patients were included; the indications for bone marrow aspirate included haematological malignancy for staging or diagnosis (n=22), isolated anaemia (12), pancytopenia (4), or paraproteinaemia (3). Full blood count was performed using a CellDyn Sapphire analyser; haemoglobin was measured by spectrophotometry; mean cell volume was measured using focused flow impedance. Iron studies, CRP and soluble transferrin receptor assays were performed using an Integra 800 (Roche Diagnostics) by Ferrozine (Iron) and immunoturbidimetric method (all other analytes). Aspirates were stained for iron using manually using Perl's stain; grading of bone marrow iron stores was performed by Haematology Registrars (CC, TC) and a Consultant Haematologist (ML) blind to ferritin result using the scoring system described in Bain [1].

### Results

The sensitivity of the soluble transferrin receptor index for iron deficiency was 100% with a specificity of 83% and positive predictive value of 45%. 2/5 patients (40%) with iron deficiency (determined by marrow aspirate) had ferritin levels in the normal range and would have been missed by ferritin levels alone. The correlation coefficient between serum ferritin and quantitative assessment of iron stain was 0.59. Of the 6 patients who had false positive results (raised soluble transferrin receptor index\*\* and normal iron stores by marrow aspirate) had raised CRP (mean 49, range 11-110mg/L) and 4/6 (66%) had active haematological malignancy. The remaining 2 patients were diagnosed with anaemia of chronic disease (1) and transient, infection related neutropaenia.

### Conclusion

Soluble transferrin receptor assay has excellent sensitivity and reasonable specificity in the diagnosis of iron deficiency anaemia, including some patients in whom ferritin is normal. In patients who are not truly iron deficient, haematological malignancy is a common cause of raised soluble transferrin receptor index.

*No conflict of interest to declare*

### Reference

1. Bain BJ, Clark DM, Lampert IA, Wilkins BS. **Bone Marrow Pathology** 3<sup>rd</sup> Edition Blackwell Science 2000

\*\*Transferrin receptor Index = sTfR/log Ferritin

## P090

### **Case Report of a Patient Who Presented with Intestinal Lymphangiectasia and Clotting Factor Abnormalities Secondary to Waldentrom's Macroglobulinaemia**

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Lymphangiectasia secondary to acellular deposition of macroglobulin in the small intestine is a rare complication of Waldenstrom's macroglobulinaemia (WM). Patients often suffer from malabsorption and diarrhoea for years before a diagnosis is made. This case report describes a 51-year old male patient who presented with 3 weeks of diarrhoea and 3kg weight loss. Additionally he had abnormal clotting studies with prolongation of Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) but no bleeding diathesis. Mixing the patient's plasma with pooled normal plasma did not correct the PT and APTT, suggestive of the presence of an inhibitor. A lupus anticoagulant was not detected. WM was confirmed on bone marrow biopsy. Serum electrophoresis demonstrated an IgM kappa monoclonal gammopathy consistent with the diagnosis. Computerised tomography revealed no lymphadenopathy or hepatosplenomegaly but thickening of the duodenum. Biopsies of the proximal small bowel and terminal ileum showed dilation and expansion of the villous lymphatic spaces by proteinaceous material, which were negative for amyloidosis but stained positively for IgM and kappa light chains. Random biopsies from the colon were normal. The patient was treated with cyclophosphamide, vincristine and prednisolone (CVP), and after only 1 cycle, the abnormal clotting studies had normalised and his diarrhoeal symptoms subsided. He has now completed 3 cycles of CVP and has maintained a serum paraprotein of 5g/L (from 17g/L pre-treatment), as well as gaining 8kg in weight. Treatment of the underlying WM therefore appears to ameliorate symptoms from intestinal lymphangiectasia and also correct the abnormal clotting studies.

*No conflict of interest to disclose*

**P091****Aplastic Anaemia in a Patient with Essential Thrombocythemia: A Case Report****Briony Ann Cutts<sup>1</sup>**, Huyen Tran<sup>1,2</sup>, Susan Brown<sup>2</sup><sup>1</sup> *Department of Clinical Haematology, The Alfred Hospital, Melbourne, Victoria, Australia.*<sup>2</sup> *Department of Clinical Haematology, Monash Medical Centre, Melbourne, Victoria, Australia***Case Summary**

A 54 year old lady was diagnosed with JAK<sub>2</sub> positive essential thrombocythemia (ET) in 2006 in the USA following presentation with headaches and persistent thrombocytosis. The use of hydroxyurea 500mg daily normalised her platelet count. Although the initial platelet count was  $236 \times 10^9/L$  upon return to Australia two years later, she developed profound and prolonged pancytopenia complicated by febrile neutropenia and required transfusion support despite stopping hydroxyurea for 8 weeks. Bone marrow biopsy showed virtually absent normal haematopoiesis consistent with aplastic anaemia. There was no abnormal cytogenetics and PNH studies were negative. She received treatment for aplastic anaemia and became transfusion independent one month later. A repeat bone marrow biopsy showed restoration of normal trilineage haematopoiesis. We believe that the prolonged pancytopenia was not a complication of hydroxyurea and that this is likely the first case report of JAK<sub>2</sub> positive essential thrombocythemia with transformation to aplastic anaemia in the literature.

*No conflict of interest to disclose*



**P092**

## **KoRV ( Koala Retrovirus) Associated Acute Leukaemia/Lymphoma**

**Lyndall Dial<sup>1</sup>**, Kimmy Lim<sup>1</sup>, Fabian Lim<sup>1</sup>, Lydia Pitcher<sup>1</sup>, Shaun Hickey<sup>2</sup>, Brett Stone<sup>2</sup>

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The increased incidence of acute leukaemia and lymphoma in the Koala (*Phascolarctos cinereus*), has been recognized for some time..

In the past year, two cases of retroviral-associated acute lymphoblastic leukaemia/lymphosarcoma have been diagnosed in koalas tested by the veterinary laboratory in our private pathology practice. On haematology parameters and cellular morphology, the cases appear quite similar to human lymphoid malignancies.

With human haematological malignancies, the techniques of immunophenotyping and cytogenetic/molecular analysis are essential for a definitive diagnosis. However, there are no koala monoclonal antibodies available to allow immunophenotyping, and cytogenetic analysis is not performed. Differentiation between leukaemia and lymphosarcoma is often based on clinical grounds. Lymph node and organ enlargement typically precedes the leukaemic profile with lymphosarcoma. Autopsy samples showing infiltration of many organs and tissues with neoplastic lymphoid cells confirm the peripheral blood findings of lymphoid leukaemia/lymphosarcoma most likely associated with retrovirus infection.

Most of the Koalas in Queensland are infected with Koala Retrovirus. Lymphoid malignancy is a relatively common disease of both free-living and captive koalas.

Mortality surveys indicate that these diseases account for around 3 to 5% of deaths in free-living koalas in the survey areas of New South Wales and southern Queensland.

The Koala Retrovirus Project is an ongoing collaborative project between the University of Queensland and Australia Zoo, examining the role of the koala retrovirus (KoRV) in leukaemia, immune deficiency and related diseases.

The virus represents one of the key threatening processes affecting the long-term survival of koalas in Australia.

Studies of the germline koala retrovirus infection may help us understand incorporation of other retroviruses into the human genome which have occurred over tens of thousands of years. (274 words)

*No conflict of interest to disclose*

P093

**Never Too Old: Is This the Oldest Reported Patient with Infectious Mononucleosis ? Epstein-Barr Virus Infection and Immunosenescence in Extreme Longevity**

**Lyndall Dial**<sup>1</sup>, Sue Moreton<sup>1</sup>, Peter Davidson<sup>1</sup>, Lydia Pitcher<sup>1</sup>, Erin Simleit<sup>1</sup>, Kimmy Lim<sup>1</sup>, Fabian Lim<sup>1</sup>, Shane Sondergeld<sup>2</sup>

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Infectious mononucleosis (IM) due to Epstein Barr Virus (EBV) is typically a disease of children and young adults. There are few reports of primary EBV infection in geriatric patients, with evidence that most persons (90-97%) aged >60 years are seropositive. This case demonstrates that clinical presentation of IM in the elderly can be significantly different to that typically seen in younger patients (pharyngitis, lymphadenopathy and hepatosplenomegaly).

We report the diagnosis of acute EBV infection in a 100 year-old woman presenting with confusion and fever, who may be the oldest reported case of IM. Her haematology parameters, blood picture, biochemistry and serology were identical to those seen in classic cases of glandular fever in younger cases. It is not clear whether this represented a primary infection in this centenarian or a reactivation of a prior infection. IgM positive serology for EBV with negative IgG serology suggested a primary infection.

Endogenous reactivation of latent Epstein-Barr virus infection in institutionalized elderly patients has been reported. Decreased specific antibody production was associated with the high mortality seen in this age group . Control over EBV reactivation is mediated by cytotoxic T-lymphocytes and EBV-specific T cells in the elderly can have impaired effector function.

Increasing longevity and quality of life has resulted in patients of advanced age representing a higher proportion of hospital admissions. Whilst commonly a disease of younger patients, it is important not to dismiss the possibility of IM in geriatric patients. With increased automation in Haematology, important morphological clues to this infection (atypical lymphocytes) may be missed, particularly if there are no clinical prompts for a slide review. Early identification of EBV infection in these elderly patients can prevent unnecessary and invasive procedures. (282 words)

*No conflict of interest to disclose*

P094

## Infections During Treatment of Burkitt's Lymphoma in HIV-Infected Patients: A Tertiary Referral Centre Experience

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### Aims

To describe the frequency and type of infections and their related outcomes during treatment of Burkitt's lymphoma in Human Immunodeficiency Virus (HIV) infected patients.

### Methods

A retrospective review was conducted of all HIV-infected patients with Burkitt's lymphoma treated between January 2006 and June 2009. Data was collected from medical records and included demographics, HIV parameters, lymphoma management, and antimicrobial use. These variables were compared to the frequency and type of infections, response to antimicrobials, and overall survival.

### Results

Twelve patients (100% male; median age 44 years), were treated during the study period. Eight patients had morphological features of typical Burkitt's, while four patients had atypical/Burkitt's-like features. At lymphoma diagnosis, the median duration of HIV infection was 2.7 years (range: 0-7.2 years), with CD4 count of  $268 \times 10^9/l$  and HIV viral load of 10,000 copies/ml. Four patients were initially on combination antiretroviral therapy (cART). During chemotherapy, all patients received cART, and most received prophylactic anti-fungals (92%), antibiotics (83%), or antivirals (83%). Six patients (50%) were treated using CODOX-M/IVAC, while 6 received CHOP, with 3 patients receiving Rituximab in each group.

Four patients (33%) had one or more proven disseminated infections; including bacteremia with *Streptococci* (1), *Enterococci* (1), *Pseudomonas* (1), *Fusobacterium* (1); disseminated Mucormycosis (1); and viremia with Human Herpes Virus-6 (1). All infections occurred while patients were neutropenic and in those who received CODOX-M/IVAC chemotherapy. There was no relationship between infection and chemotherapy cycle number. There were six episodes of coagulase-negative *Staphylococci* isolated from blood cultures of variable significance. The majority (83%) of disseminated infections resolved. One patient died from disseminated Mucormycosis. Median follow up has been for 1.2 years (range: 0.2-3.2 years), with two deaths occurring (1 with infection, 1 with progressive lymphoma).

### Conclusions

A larger collaborative cohort would help further assess influences on infection rates, given the small numbers in this study. This could also include comparison with lower grade lymphomas in HIV-infected patients.

No conflict of interest to disclose

A326

P095

**Bone Marrow and Peripheral Blood Stem Cell Transplantation:  
An Audit of All Patient Outcomes at Liverpool Hospital 1995-2008**

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**Aim**

Bone marrow (BM) and Peripheral Blood Stem Cell (PBSC) transplantation has been used for many years for the treatment of haematological malignancies with the aim of cure, or prolonging remission, in otherwise invariably fatal diseases. A review of outcomes since transplantation commenced at Liverpool Hospital in 1995 was performed aimed at addressing issues that may impact on patient management and survival.

**Methods**

The entire cohort of patients who were identified as having a bone marrow or peripheral blood stem cell (PBSC) transplant at Liverpool Hospital between 1995 and 31<sup>st</sup> December 2008 were included in the analysis of clinical outcomes. Statistical analysis performed, including Kaplan-Meier curves, log-rank tests and Cox proportional hazard model development used Stata 10 software.

**Results**

271 patients were identified as having admission for a transplants from 1995-2008. Of these admissions 270 patients proceeded to have BM and/or PBSC infusions. The major diagnoses for transplantation were Non-Hodgkins Lymphoma (NHL) (114), Multiple Myeloma (MM) (99), Hodgkins Disease (HD) (17) and Acute Lymphoblastic Leukaemia (ALL) (10). Survival was similar for these diagnoses apart from HD which had a significantly better 5 year survival of >80%. Multivariate analysis found that sex, packed red cell transfusions, CD34 cells infused, admission length, year of transplant did not predict survival though platelet transfusions and age were predictors of survival. Median survival following first transplant for Multiple Myeloma was 7.6 years, with 10 year survival being 42% [95%CI (26%,58%)]. The type of heavy or light chain did not predict survival in patients with MM. Of interest in patient with MM there was no difference in median survival from first transplant for patients having 1, 2 or 3 transplants; however survival after each successive transplant decreased as shown below. Toxic deaths were found to be <2%.

**Survival after each successive transplant in Myeloma**

last transplant performed	patient numbers	median survival (years)	95% CI
1st	59	not reached	(3.3,-)
2nd	17	2	(1.4,5.6)
3rd	2	0.7	-

**Conclusion**

The results of this Audit demonstrate similar survival at Liverpool Hospital to those reported by the ABMTRR. Survival for patients with MM was unexpectedly good with a median of 7.6 years from first transplant with no effect of heavy or light chain in a multivariate Cox proportional Hazard model.

*No conflict of interest*

P096

## Neutrophil Engraftment after Peripheral Blood Stem Cell Transplant: Effect of G-CSF Commencing on Day +1 Compared to Day+5 Post Transplant

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<sup>2</sup> Department of Statistics, Macquarie University, Sydney, NSW, Australia

### Aim

There is doubt about the benefits of the use of G-CSF after Peripheral Blood Stem Cell Transplant (PBSCT). At Liverpool Hospital following a review of transplant engraftment data from 1995-2001, a change was made in the day on which G-CSF was commenced after PBSCT from Day +1 to Day +5. In order to assess the effects this change, a retrospective study was performed comparing the time to neutrophil engraftment for patients having G-CSF commencing on day +1 (1995 to 2001 - period 1), to the group having G-CSF commencing day +5 post PBSCT (2002-2008 - period 2).

### Methods

From 1995 until December 2008 there were 271 admissions for transplant at Liverpool Hospital. 219 patients were included in the analysis. Patients having bone marrow alone or bone marrow in addition to PBSC, chronic myeloid and acute myeloid leukaemia, and patients with solid tumours were excluded due to potential confounding that may occur because of different sources of stem cells, the infusion of potentially abnormal stem cells and use of non-myeloablative therapy, respectively. In addition to there being a change in the day on which G-CSF was commenced between the 2 time periods, there was an attempt to aim for higher stem cell collection between the time periods, with the aim of a minimum  $2.5 \times 10^6$  CD34+ cells/kg in the first period and  $4.0 \times 10^6$  CD34+ cells/kg in the second. Assessment was made of the days to engraftment between the time periods and between the number of CD34+ cells infused. Statistical analysis was performed using Stata 10 software.

### Results

There was a difference between the number of CD34+ cells infused between period one and two (median 3.5 period 1 versus  $4.38 \times 10^6$  CD34 cells/kg for period 2, rank sum  $p < 0.001$ ). However, despite the increase in the number of stem cells infused between period one and two, the time until PMN engraftment rose paradoxically, from 9.67 days (period 1) to 10.31 days (period 2) ( $p < 0.001$  rank sum). The difference between the periods in the time for PMN engraftment was of significance in the group having  $\geq 4 \times 10^6$  CD34+ cells/kg infused at the time of transplantation, ( $p < 0.001$ , t-test and rank sum), though not in the group infused  $< 4 \times 10^6$  CD34+ cells/kg.

### Conclusion

The change in the day on which G-CSF was commenced post PBSC transplant from D+1 to D+5 resulted in a prolongation of time to PMN engraftment in patients having optimal CD34+ cells infused, with no significant change in patients having less than  $4 \times 10^6$  CD34+ cells/kg infused. Speculation as to the reason for this difference in engraftment times could be that once a saturating number of stem cells are infused extra stimulation with a myeloid growth factor, i.e. G-CSF, is the only factor that can reduce the period of neutropenia.

*No conflict of interest to declare*

A328

**P097****Incidence of Bleomycin Lung Toxicity (BLT) in Hodgkin's Lymphoma (HL) Patients Receiving Treatment with Adriamycin, Bleomycin, Vinblastine, Dacarbazine (ABVD) and Pegfilgrastim**

**Sunelle Engelbrecht**, Jeremy Wellwood, Michelle Bryson, Tara Seshadri  
*Haematology Dept, Gold Coast Hospital, Southport, QLD, Australia*

**Aim**

Animal studies suggest that concurrent use of G-CSF may predispose to BLT; however, human data is conflicting. The aim of this study was to analyse the incidence of BLT and associated risk factors in patients with HL treated with ABVD and pegfilgrastim.

**Methods**

Retrospective, single centre study of consecutive HL patients treated with ABVD from 2005-2009. All patients received at least one dose of pegfilgrastim. Risk factors for BLT collected included age, creatinine, smoking/respiratory history, radiotherapy and G-CSF use (filgrastim and pegfilgrastim). Patients underwent routine respiratory function testing during treatment, regardless of symptoms. BLT was defined by symptoms accompanied by a reduction in DLCO.

**Results**

Study cohort comprised 20 patients: Median age 38 (range 16-78), 55% male, 75% nodular sclerosing histology. At diagnosis 60% had limited stage, 35% had B-symptoms, 10% had bulky disease. Median number of cycles of ABVD was 6 (range 2-8). 45% had neutropenia ( $<0.5 \times 10^9/L$ ) prior to commencing pegfilgrastim, 2 developed neutropenic sepsis. 95% of patients achieved complete remission at end of therapy, 2 have subsequently relapsed. Median follow-up was 21.5 months. BLT occurred in 6 patients (30%) and was fatal in one. 2/6 had CT changes. Median decrease in DLCO was 21% (range 12-46%). Median age for BLT patients was 69 (range 21-78) compared to 31.5 for those 14 without BLT. All patients had normal creatinine. Median number of bleomycin doses received prior to BLT development was 4 (3-5). No patient developing BLT had a respiratory history, none were currently smoking, 3 were ex-smokers and none received chest radiotherapy. One received corticosteroids for BLT. The remaining 5 had symptomatic improvement after bleomycin cessation.

**Conclusion**

The incidence of BLT in our cohort is concerning and appears more prevalent in the elderly. Further studies are required to delineate the role of pegfilgrastim on the development of BLT.

*No conflict of interest to disclose*

P098

## **A Case of Concurrent Hodgkin's Lymphoma and Multiple Myeloma Treated with ABVD (Adriamycin, Bleomycin, Vinblastine and Dacarbazine) Chemotherapy and Dexamethasone**

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### **Summary**

We present the first reported case of Multiple Myeloma occurring concurrently with Hodgkin's Lymphoma.

### **Case Report**

A 39 year old female presented with 2 months of weight loss, myalgia and supraclavicular and axillary lymphadenopathy. Initial bloods revealed a normocytic anaemia (Hb106g/L), hypoalbuminaemia and mildly deranged liver function. Renal function, corrected serum calcium and s-β2microglobulin were normal. Serum protein electrophoresis demonstrated a monoclonal Kappa IgG band (15g/L). CT showed left supraclavicular, axillary and mediastinal lymphadenopathy (6cm maximum diameter) with FDG-avidity on PET scanning. Whilst awaiting excisional lymph node biopsy, she had worsening weight loss, myalgia and liver function. The lymph node biopsy revealed nodular sclerosing Hodgkin's Lymphoma. Staging bone marrow biopsy showed no evidence of involvement with Hodgkin's Lymphoma but surprisingly revealed a kappa-restricted plasma cell population of 30%. CT skeletal survey was normal. She commenced ABVD with dexamethasone 40 mg (ABVDD) on the first four days of each cycle. Restaging after 3 cycles demonstrated resolution of axillary and supraclavicular lymphadenopathy with reduced mediastinal lymph nodes (14mm), which were mildly FDG-avid. Bleomycin was discontinued at this stage due to a reduction DLCO (18% from baseline). The monoclonal IgG band decreased to 4g/L and repeat bone marrow studies showed reduced plasma cell numbers of 10-15%. She also developed a left leg DVT, treated with enoxaparin. The patient has completed 4 cycles of A(B)VDD with plans to collect stem cells on recovery after cycle 5 of A(B)VDD prior to autograft after 6.

### **Discussion**

Myeloma has infrequently been reported concurrently with Non-Hodgkin Lymphoma and CLL. There are also case reports of serum and urinary monoclonal immunoglobulins in Hodgkin's Lymphoma. However, this is to our knowledge the first case of concurrent Hodgkin's Lymphoma and Multiple Myeloma. We also demonstrate the potential viability of treatment with ABVD and high dose dexamethasone prior to autologous transplant.

*No conflict of interest to disclose*

A330

P099

## Post-therapy Calcification Can Result in False Positive FDG PET Scans in Patients with Non-Hodgkin's Lymphoma

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### Introduction

We report 2 cases of post-therapy calcification in patients with lymphoma resulting in false positive FDG PET-CT scans, and highlight the implications of these findings for interpretation of post-therapy FDG PET imaging when these changes occur

### Case 1

A 19yr male with Stage 2B lymphoblastic lymphoma underwent chemotherapy with Hyper-CVAD. Staging FDG PET-CT performed prior to commencing therapy showed markedly increased FDG uptake in a large mediastinal mass. On restaging CT post cycles 2, 4 and 6 of therapy, a PR was achieved, with a persistent lymph node displaying progressive calcification within its outer rim present in the anterior mediastinum. Repeat FDG PET-CT post cycle 6 of therapy displayed persistent FDG uptake within the outer (calcified) rim of this node. The patient subsequently underwent autologous SCT followed by IFRT; repeat FDG PET-CT scans post SCT and post IFRT were unchanged in comparison to the post-chemotherapy scan, with ongoing FDG uptake corresponding to the area of calcification within the residual node. The patient remains in ongoing clinical remission 30mths post IFRT with no change in the appearance of the residual calcified node on multiple interval CT scans.

### Case 2

A 33yr old male was diagnosed with stage IVB DLBCL, including the presence of multiple splenic lesions on staging CT and FDG PET-CT scans. 3wks post completing CHOP-R chemotherapy, repeat CT demonstrated focal areas of calcification within many of the splenic lesions; these lesions also displayed persistent avidity on repeat FDG PET-CT imaging. Subsequent splenectomy revealed calcification within organizing areas of focal infarction, though no morphological evidence of residual DLBCL.

### Conclusion

FDG PET findings in the setting of post-therapy calcification should be interpreted with caution, as persistent FDG avid lesions in this subgroup of patients may represent false positive findings related to underlying tissue inflammation / reaction secondary to dystrophic calcification rather than refractory lymphoma *per se*.

*No conflict of interest to disclose*



## P100

### Imatinib-induced Bone Formation in CML Patients is Associated with a Decrease in Osteoclast Function and Numbers

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#### Aims

Imatinib mesylate (Novartis) is a rationally designed tyrosine kinase (TK) inhibitor that is a highly effective therapy for CML. In addition to inhibiting BCR-Abl, imatinib also inhibits PDGFR and c-FMS, TKs involved in bone homeostasis. In a previous retrospective study, we showed an increase in trabecular bone volume in CML patients treated with imatinib<sup>1</sup>. The aim of this current study was to perform a prospective analysis of bone indices in CML patients treated with imatinib to gain mechanistic insight.

#### Methods

At presentation, chronic phase CML patients were recruited to an addendum study of the ALLG TIDEL II imatinib CML trial (11 patients, data up to 12 months). At presentation, and at 3 monthly intervals, DEXA analysis, blood and a bone biopsy was taken. Serum biochemistry and markers of bone metabolism were measured. Bone biopsies were analysed by microCT, embedded in plastic and histomorphometric assessment was performed.

#### Results

Imatinib therapy resulted in a significant decrease in serum phosphate and total calcium and a concomitant increase in parathyroid hormone (PTH). Serum markers of osteoclast activity ( $\beta$ -xlaps) were significantly decreased whereas vitamin-D3, a marker of osteoblast function, was elevated. Micro-CT analysis of bone biopsies revealed a significant increase in bone volume, trabecular thickness and a decrease in the bone surface to bone volume ratio. DEXA analysis revealed no significant change in BMD at the sites examined. Histomorphometric assessment of bone biopsies revealed a significant decrease in osteoclast and osteoblast numbers.

#### Conclusions

Imatinib promotes trabecular bone volume and trabecular thickness in CML patients. While the mechanism(s) remain to be elucidated, the decrease in osteoclast function, as determined by serum  $\beta$ -xlaps, and the decrease in osteoclast number suggests that inhibition of c-Fms is important in this process. Our findings raise the possibility of using imatinib to treat diseases characterised by bone loss.

#### References

1. Fitter S, Dewar AL, Kostakis P, et al. Long-term imatinib therapy promotes bone formation in CML patients. *Blood*. 2008;111:2538-2547.

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**P101****Auto-immune Thrombocytopenia Post Autologous Bone Marrow Transplant for AML in a Patient with HIV and Treatment with Romiplostim****Shane Gangatharan**, Julian Cooney*Department of Haematology, Royal Perth Hospital, Perth, WA, Australia*

A 54 year old man presented with acute myeloid leukaemia with trisomy 8 cytogenetic abnormality. He had a significant history of HIV for more than 10 years, well controlled with highly active anti-retroviral treatment.

The disease was initially refractory to induction with standard dose idarubicin and cytarabine. Salvage chemotherapy with a fludarabine based regimen induced remission. High-dose cytarabine was used for subsequent consolidation, after which stem cells were collected. To reduce risk of relapse, the patient underwent autologous bone marrow transplant in first remission. Allogeneic bone marrow transplant was deemed high risk.

Neutrophils engrafted normally, however platelets failed to engraft. Repeat bone marrow biopsy confirmed remission and normal numbers of megakaryocytes. Alternate causes such as drugs or increasing HIV viral load were excluded and a further infusion of stem cells failed to improve counts. Peripheral destruction of platelets was suspected.

Post transplant, T and B-cell subsets had altered. CD4 T-cells were below  $200 \times 10^6/L$ , CD19 B-cells were markedly decreased while CD8 T-cells were increased. Thrombocytopenia was thought to be auto-immune though platelet antibody studies were negative.

Standard therapy for ITP was ineffective including high-dose prednisolone, intravenous immunoglobulin and rituximab. The thrombopoietin receptor agonist romiplostim was associated with minor improvement and delayed the need for splenectomy.

Rare cases of immune-mediated thrombocytopenia have been reported post-autologous stem cell transplant. This case is the first documented in an HIV patient and may reflect altered immune balance.

*No conflict of interest to disclose*

## P102

### **An Historical Review of Acute Leukaemia in Papua New Guinea, 1958-2007, in Preparation for the Launch of the Melanesian Acute Leukaemia Biology Project (MALBP) in 2009**

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#### **Introduction**

Current knowledge of acute myeloblastic (AML) and lymphoblastic (ALL) leukaemia in Papua New Guinea is limited. Epidemiological studies, since the advent of a tumour registry in 1958, have demonstrated both intriguing population differences, and confirmed a universally poor clinical outcome compared to globally available data. There is global mobilisation of will and resources through many campaigns to address this disparity, as envisioned by the Melanesian Acute Leukaemia Biology Project (MALBP).

#### **Aim**

To identify the known epidemiological, morphological and clinical features of acute leukaemia in Papua New Guinea

#### **Methodology**

A retrospective review of all relevant literature on acute leukaemia in Papua New Guinea was conducted through relevant searches on Pubmed and the University of Papua New Guinea medical library from 1958 to 2007.

#### **Results**

Since 1958 to 2007 there have been a total of 405 acute leukaemia diagnoses. Paediatric data is more comprehensive than adult, with at least 305 cases of paediatric leukaemia recorded. Of total cases that have been classified, AML represents 61% and ALL 26%. Most diagnoses are made by FAB morphological criteria. The overall incidence is variable, ranging from 0.8 to 0.37/100,000 population. There is a distinct male preponderance that is decreasing over the time of analysis. In paediatric leukaemia there is an absence of a childhood peak of ALL and a much higher incidence of AML. No immunophenotypic, cytogenetic or cytochemical data is available. One year survival data is between 0-7 percent; however few clinical studies document this.

#### **Conclusion**

Acute leukaemia in Papua New Guinea has novel epidemiological features with an unexplained causation and very poor survival. There has been no reported serological, virological, immunophenotypic or cytogenetic analysis of cases and the MALBP is proposed to investigate and define possible new mechanisms of leukaemogenesis to explain these phenomena and focus on improving survival.

*No conflict of interest to disclose*

P103

**Reduced Intensity Conditioning Regimens are Associated with a Better Survival Compared with Myeloablative Transplants in Patients over 50 Years with Acute Myeloid Leukemia**

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**Aim**

To compare the outcome of myeloablative (MAT) with reduced intensity (RIC) transplants in patients older than 50 years with acute myeloid leukemia (AML).

**Methods**

Cyclophosphamide (Cy) combined with Busulfan (Bu) or TBI was used for MAT while Fludarabine combined with either Melphalan (Mel), Bu or Cy was used for RIC transplants. Unrelated donors were used in 44% MAT and 45% RIC transplants. PBSC was the graft source in all RIC and 76% MAT transplants. T cell depletion (ATG or Alemtuzumab) was used in 14 MAT (52%) and 22 RIC transplants (55%).

**Results**

Between 1998 and 2009, we performed 27 MAT and 40 RIC transplants in patients over the age of 50 years. At a median follow up of 31 months (range: 1 – 87), the overall (OS) and leukemia free survival (LFS) for MAT are 29% and 22% respectively compared to 60% OS ( $p = 0.024$ ) and 50% LFS ( $p = 0.039$ ) with RIC transplants. There was no difference in percentage or time to neutrophil engraftment [MAT 85.1% engraftment, median time: 16.1 days (range: 12 – 23) versus RIC 95% engraftment; median time: 16.8 days (range: 10-41)]. Non-relapse mortality (NRM) at Day 100 and 1 year was 47.8% and 60.8% for MAT and 20% and 27.5% respectively for RIC transplants ( $p = 0.026$ ). Four MAT patients expired < 3 weeks due to sepsis prior to engraftment. One patient undergoing a RIC transplant had primary graft failure and 1 expired on Day 15 due to an intracranial bleed. Grade II-IV GVHD occurred in 48% of MAT and 29% of RIC transplants ( $p = 0.173$ ). Relapses occurred in 6 MAT and 9 RIC transplants.

**Conclusions**

RIC regimens are associated with reduced mortality, similar GVHD and relapse rates with better overall and leukemia free survival in patients over 50 years with acute myeloid leukemia.

*No conflict of interest to disclose*

## P104

### **Incidence and Outcome of Acute Graft Versus Host Disease (GVHD) Following Myeloablative and Reduced Intensity Conditioning Regimens**

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#### **Aim**

To compare the incidence and outcome of acute graft versus host disease (aGVHD) following myeloablative (MAC) and reduced intensity (RIC) transplants.

#### **Methods**

Between 1998 and 2009, 428 patients underwent MAC (n=273) or RIC (n = 155) transplants. Cyclophosphamide (Cy) combined with Busulfan (Bu) or TBI was used for MAC transplants while Fludarabine combined with Melphalan (Mel), Bu or Cy was used in RIC. Unrelated donors comprised 34.7% of MAC and 22.5% of RIC transplants. Peripheral blood stem cells (PBSC) were the graft source in 98% of RIC and 74% of MAC transplants. T cell depletion with ATG, Alemtuzumab or OKT3 was used in 73 MAC (26.7%) and 35 RIC transplants (22.5%).

#### **Results**

Acute GVHD occurred in 177 MAC (64.8%) and 89 RIC transplants (57.4%) [ $p = 0.146$ ] at median of 25 (range: 7-98) and 29 days (range: 8 – 98) respectively. Incidence of grade II-IV and III-IV aGVHD was 36.6% and 20.1% respectively for MAC and 43.8% and 23.2% respectively for RIC [ $p = 0.150$ ]. Steroid refractory GVHD was seen in 18.6% of MAC and 28.9% of RIC transplants ( $p = 0.08$ ). At median follow up of 44 months (3 – 120), 76 RIC (49%) and 141 MAC (51.6%) patients are alive. Deaths directly attributable to GVHD (either progression, infection or organ failure) occurred in 14 RIC (26.9%) and 42 MAC (42.8%). The major cause of mortality among RIC transplants was relapse (42%). The OS for patients with and without GVHD was 44.6% and 64.5% respectively for MAC and 41.5% and 63.6% for RIC transplants.

#### **Conclusions**

Acute GVHD is seen commonly following MAC and RIC transplants. GVHD is a major cause for mortality in MAC and is associated with a lower OS in both MAC and RIC transplants. Further efforts are required to reduce aGVHD in both MAC and RIC transplants.

*No conflict of interest to disclose*

**P105**

## **Striking Survival Outcomes Observed in Poor Risk AML Patients in CR1 Receiving Cyclosporin-only Graft vs Host Disease Prophylaxis After Matched Sibling Cy/TBI Conditioned Allogeneic Stem Cell Transplantation**

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### **Introduction**

Survival outcomes for AML patients with secondary AML, poor risk karyotype or refractory disease after induction chemotherapy are dismal. Post-transplant immunosuppression with cyclosporin and methotrexate after HLA-identical sibling stem cell transplantation for AML prevents graft-versus-host disease (GVHD) but counterproductively increases the risk of disease recurrence by suppressing the graft-versus-leukaemia effect. Previous reports prior to 2000 have suggested an improved leukaemia free survival by eliminating methotrexate from GVHD prophylaxis at the cost of increased acute graft-versus-host disease (40% vs 26%;  $p < 0.001$ ) and no overall survival benefit. We hypothesise that with modern transplantation approaches, cyclosporin-only GVHD prophylaxis may improve outcomes for high-risk AML patients in 1<sup>st</sup> remission (CR1).

### **Methods**

Since 2001, our institution has undertaken sibling allogeneic stem cell transplants using cyclosporin (6mg/kg/day) only for GVHD prophylaxis in 9 poor risk AML patients in CR1. These patients had secondary AML, poor risk karyotype or refractory disease after induction chemotherapy. A retrospective analysis of these patients was undertaken. All patients had 10/10 HLA-matched sibling donors and conditioning was with cyclophosphamide and total body irradiation (13.2Gy). Kaplan-Meier survival was plotted using GraphPad Prism.

### **Results**

After a median follow-up of 2.7 years for the 9 patients, overall survival was 89%, with the only reported death due to progressive disease. Relapse-free survival was similar, with only 2 of 9 patients relapsing. The incidence of acute GVHD was 44% (4/9). Chronic GVHD was identified in 5 of 9 patients. There were no deaths attributable to graft-versus-host disease. When compared to a group of 9 standard-risk AML patients, who were age and sex-matched and underwent allogeneic stem cell transplant at our institution during the same time period with cyclosporine and methotrexate (CyA/MTX) prophylaxis, there was no significant difference in overall survival at 3 years (89% vs 67%;  $p = 0.26$ ), nor relapse-free survival (78% vs 67%;  $p = 0.46$ ). The incidence of acute GVHD was higher in the cyclosporin group (4/9) vs the CyA/MTX group (1/9).

### **Conclusions**

In this small retrospective analysis at our institution, sibling-matched allogeneic stem cell transplantation with cyclosporin-only GVHD prophylaxis results in a better than expected overall and relapse-free survival for high-risk AML in CR1, with no increase in deaths attributable to GVHD. This approach, using modern transplantation approaches, should be examined in larger randomised clinical trials.

*No conflict of interest to disclose*

P106

## **HIV Associated Burkitt Lymphoma and Burkitt-like Lymphoma Is Associated with a Better Than Expected Prognosis, Justifying Intensive Chemotherapy with Curative Intent**

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### **Aim**

To investigate the survival of patients with HIV associated Burkitt Lymphoma and the chemotherapy and the complications associated with this.

### **Methods**

Retrospective study of all patients diagnosed and treated for HIV associated Burkitt Lymphoma at a single institution between 2003 and 2009. The data obtained included the survival following the diagnosis of Burkitt Lymphoma, the stage of the disease and response to chemotherapy, stratified by chemotherapy regimen, and the infectious complications associated with this.

### **Results**

There were 13 patients diagnosed with HIV associated Burkitt and Burkitt-like lymphoma between 2003 and 2009 at our institution. The survival rate was at a median follow-up of 1 year was 84%. All patients who were alive remained in complete remission. The median CD4 count at diagnosis was 272 cells/mL (range: 10-610 cells/ml). In 3 patients, Burkitt Lymphoma was the first AIDS defining illness. Most patients presented with advanced disease at diagnosis with 8/13 patients having stage IV disease. 13 patients received CHOP chemotherapy. 6/13 patients were treated with CODOX-M/IVAC chemotherapy. A total of 8/13 patients received Rituximab. All patients received HAART therapy for their HIV and appropriate antimicrobial prophylaxis throughout the duration of their chemotherapy. Of the 2 patients that died, one patient died from progressive disease after CHOP chemotherapy 8 months following diagnosis, the other patient died from disseminated fungal infection 4 months into chemotherapy with CODOX-M/IVAC treatment.

### **Conclusions**

In this small retrospective series at a single institution, HIV positive patients with Burkitt Lymphoma had a better than expected survival compared to published series, despite receiving varying chemotherapy regimens. This demonstrates that treatment with curative intent is justified in this immuno-suppressed population.

*There were no conflicts of interest identified*

A338

**P107****Positron Emission Tomography using 18F-FDG (PET) Negativity at End of Treatment Predicts Durability of Response to Therapy in Hodgkin Lymphoma (HL): Single Institution Experience in 30 Patients**

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**Aim**

To assess the use of PET at diagnosis and end of treatment to predict durability of response to ABVD chemotherapy.

**Methods**

30 patients were studied over 4 years. Initial treatment was ABVD in all patients. Patients either received 4 cycles of ABVD with involved field radiation or 6 cycles of ABVD. Residual borderline PET positivity in site of original disease was considered a positive result.

**Results**

Median patient age was 28 yrs (range 18-67), male to female ratio 1:1, median follow up 29 months (range 9-56) with 34 surviving at last follow up. Patients were 50% early stage (Stage I and IIa), 50% advanced stage (IIb, III and IV). In the 30 patients re-staged at the end of treatment with PET, 25 (83 %) were negative and 5 (17%) were positive. Of the 5 who were positive, 3 had additional therapy and 2 were monitored. Of the 25 PET negative patients, 22 (88%) remained in CR and 3 (12%) relapsed giving a Negative Predictive Value of 88%.

**Conclusion**

PET use in clinical practice can achieve a Negative Predictive Value similar to that demonstrated in the current literature. However, 12% of patients with PET negativity at the end of treatment still relapsed; indicating additional novel prognostic data (such as gene expression profiling) is required in this disease.

*No conflict of interest to disclose*



P108

## The Model OCT-1 Substrate MPP Does Not Provide a Commercially Available Surrogate for OCT-1 Activity Measurement

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### Aim

The intracellular uptake of imatinib (IM) is variable and related to the functional activity of the OCT-1 transporter (OCT-1 Activity:OA). The OA is predictive of response in IM treated CML patients, but relies on availability of radiolabelled 14-C IM. The aim of this study was to determine if the model substrate of OCT-1 Methyl-4-Phenylpyridium (MPP+) which is commercially available, was transported in the same way as imatinib, and could thus be used as in a surrogate assay for IM OA.

### Method

K562 (BCR-ABL +) and Hek293 (high OCT-1) cells were incubated for at least 2hrs at 37°C 100% humidity in the presence of a combination of 2µM C<sup>14</sup>IM and/or 160nM MPP+ and prazosin. Intracellular drug levels were obtained by scintillation counting.

### Results

MPP+ and IM intracellular levels were higher in Hek293 cells compared to K562, as would be expected. MPP+ and imatinib uptake were reduced in the presence of prazosin in K562 cells by up to 40% (24ng IM to 14ng and 36pg MPP+ to 29pg) providing evidence for both drugs being transported by OCT-1. The kinetics of MPP uptake varied between cell lines: In Hek293 cells MPP uptake was inhibited by prazosin at 5 minutes but not 2hrs. In K562 cells MPP+ uptake was inhibited at 2hrs. IM uptake was inhibited in both cell lines at 2 hours and at 5 mins. Coincubation with IM did not alter the amount of intracellular MPP+ at any timepoint in either cell line. Similarly the addition of MPP+ had no effect on the uptake of IM.

### Discussion

Prazosin, a known OCT-1 inhibitor, decreased the intracellular concentration of both compounds demonstrating that these drugs utilize OCT-1 as a transporter. Coincubation experiments indicate that while both substrates are transported by OCT-1 it is likely that they utilize a different part of the protein for transport, and thus MPP does not provide a good candidate substrate for IM in the OA assay.

*This research was supported by Novartis. The company had no role in analysing the data or preparing the abstract*

P109

# **Lack of Response to Imatinib in a Patient with Acute Transformation of a Myeloid Neoplasm with Eosinophilia and Rearrangement of PDGFRB**

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The diagnosis of chronic eosinophilia leukaemia associated with rearrangement of the PDGFRB (Platelet Derived Growth Factor Beta) gene is reported to have major therapeutic relevance because imatinib responsiveness has been demonstrated for the majority of fusion products.

We report the case of a 62 year old man who presented with bone pain, fevers and an abnormal full blood count and film (FBE). The blood findings included a leucocytosis with prominent eosinophilia, abnormal eosinophils and 5-10% circulating blasts. He had a background history of an abnormal FBE with eosinophilia for several years which had not previously been investigated.

A bone marrow examination was performed. The morphology suggested a chronic eosinophilic leukaemia with impending transformation to acute leukaemia, as a patchy but significant increase in blasts was seen in the trephine biopsy (10-20%). Cytogenetic analysis revealed a t(5;12)(q33;p13), and FISH studies were positive for ETV6 rearrangement in 61% of cells examined. The fusion gene derived from the rearrangement of PDGFRB and ETV6 encodes a constitutively activated receptor tyrosine kinase. Such genes are inhibited by imatinib mesylate, which has been reported to induce durable responses in patients with this rearrangement.

In our case, the patient had rapid deterioration in his clinical state following presentation, with the development of significant constitutional symptoms, increasing bone pain and rapidly progressive splenomegaly. He was treated with imatinib and acute myeloid leukaemia-style induction chemotherapy. Whilst therapy reduced the blast number to less than 5%, the myeloproliferative features of the marrow persisted and the t(5;12) was still detected on cytogenetic analysis. Following consolidation therapy the marrow was hypocellular and insufficient dividing cells were present to allow chromosome analysis. The patient continued on imatinib but unfortunately his disease relapsed after five months. He proceeded to an allogeneic stem cell transplant but despite this has not achieved morphological or cytogenetic remission.

*No conflict of interest to disclose*

**P110**

## **Analysis of Busulphan Blood Levels in Patients Receiving Oral Busulphan Pre-conditioning Therapy for Bone Marrow/Peripheral Blood Stem Cell Transplant**

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<sup>2</sup> *PathWest QEII Sir Charles Gairdner Hospital, Nedlands, Western Australia.*

### **Aim**

To assess the plasma busulphan levels in a cohort of patients undergoing haematopoietic stem cell transplant at RPH with busulphan pre-conditioning in order to gain insight into the current busulphan blood levels achieved and variability within and between patients' levels in a representative population of our service.

### **Method**

Measurement of busulphan plasma levels at 28 time points in each of 10 consecutive consenting patients undergoing allogeneic haematopoietic stem cell transplant at Royal Perth Hospital from January 2009 onward. Busulphan administered as an oral suspension of crushed tablets at 1mg/kg 6 hourly for 16 doses. Area under the Curve (AUC) calculations will be used to determine drug exposure in each subject and to assess intra- and inter-subject variability.

### **Result**

Notable inter-subject variability in plasma busulphan levels is emerging from analysis of preliminary results. Trough levels obtained range from 150-325µg/L whilst peak levels range from 810-1650µg/L.

### **Conclusion**

Busulphan has been described as having a relatively narrow therapeutic range and concerns have been raised regarding its potential for toxicity due to variable absorption and distribution of the drug and its metabolites. Some authors have suggested an increase in toxicity with AUC levels >1500µM.min and a threat to therapeutic outcome with AUC levels <900µM.min, whilst others refute this. The oral formulation of busulphan has been the mainstay for patients undergoing pre-transplant conditioning at RPH, however preliminary results of this small cohort analysis raise concerns regarding the drug's variable bioavailability between subjects.

*No conflict of interest to disclose*

P111

## Increased T Regulatory Cells and Decreased Th1 Pro-inflammatory Cytokines Correlate with Culture-positive Infection in Childhood Oncology Patients with Febrile Neutropenia

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<sup>4</sup>Child Health Research Institute, Women's and Children's Hospital, North Adelaide, South Australia, and CRC for Diagnostics

### Background

Paediatric oncology patients with febrile neutropenia are usually hospitalised and treated with empirical broad-spectrum antibiotic therapy to counter the risk of infection. However, there is currently no method available to rapidly identify bacteremia in these patients. T-helper-type-1 (Th1) cytokines are required for effective immune response to many pathogenic organisms and T regulatory cells are known suppressors of Th1 cells. We hypothesized that characterization of reduced intracellular Th1 cytokines and increased T regulatory cells (Tregs) may prove useful in identifying infection in childhood oncology patients with febrile neutropenia.

### Method

Intracellular Th 1 cytokines and Tregs were enumerated in peripheral blood from a group of childhood oncology patients with febrile neutropenia using multiparameter flow cytometry.

### Results

There was a significant increase in the percentage of CD25+CD127-CD8-CD3+ Tregs and a significant decrease in the percentage of T cells producing Th1 intracellular cytokines IFN $\gamma$ , IL-2 and TNF $\alpha$  in the blood of culture positive patients compared with culture negative patients.

### Conclusion

Enumeration of Tregs and intracellular Th1 cytokines may provide a sensitive, specific test for determining infection in childhood oncology patients before blood culture results become available.

Funded by a Women's and Children's Hospital Foundation Research Grant.

No conflict of interest to declare

**P112**

## **Meloxicam, a Cyclooxygenase-2 Inhibitor, Stimulates Haematopoiesis in Irradiated Mice**

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### **Aim**

Previous studies revealed haematopoiesis-stimulating effects of non-selective cyclooxygenase inhibitors (diclofenac, indomethacin) on radiation-suppressed mouse haematopoiesis. However, undesirable gastrointestinal side effects of these drugs were also observed. The aim of the present studies was to evaluate haematopoiesis-modulating effects of meloxicam, a selective cyclooxygenase-2 inhibitor, a non-steroidal anti-inflammatory drug having lower gastrointestinal toxicity.

### **Methods**

B10CBAF<sub>1</sub> male mice were whole-body irradiated with  $\gamma$ -rays. Meloxicam was administered intraperitoneally in doses of 20mg/kg, singly or repeatedly, before or after irradiation. In various time intervals, a complex analysis of haematopoiesis was performed. Peripheral blood cell counts and bone marrow cellularity were evaluated. Numbers of femoral bone marrow progenitor cells for granulocytes and macrophages (GM-CFC) and erythrocytes (BFU-E) (in vitro clonogenic assays), as well as for granulocytic and erythroid precursor cells (marrow smear differential counts), were determined. Concentrations of serum granulocyte colony-stimulating factor (G-CSF) were determined using an ELISA kit. For statistical comparisons of the differences between groups, the Mann-Whitney U test was used.

### **Results**

Stimulatory effects of meloxicam were observed in the parameters of peripheral blood granulocytes, bone marrow proliferative granulocytic cells, GM-CFC, and BFU-E. Meloxicam was also observed to enhance serum G-CSF levels.

### **Conclusions**

These results document the ability of meloxicam to stimulate haematopoiesis in irradiated mice. The findings suggest that meloxicam might find use in clinical practice in the treatment of myelosuppression of various etiology.

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**P113**

## **Pseudomonas aeruginosa osteomyelitis and Deep Tissue Abscess in Severe Congenital Neutropenia Treated with Short Course G-CSF: A Case Report and Review Literature**

**Sakara Hutspardol<sup>1</sup>**, Olarn Prommalikit<sup>2</sup>, Wannaporn Isvilanonda<sup>3</sup>

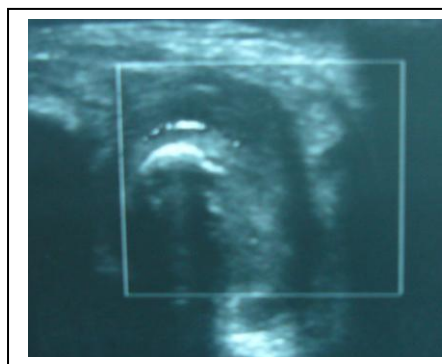
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### **Case Report**

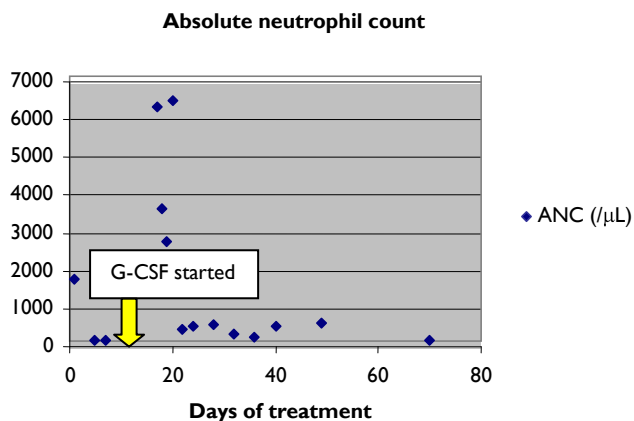
A 6-month-old male infant presented with fever and erythematous, edematous, and painful left elbow with decreased in range of motion. His full blood count revealed steadily low in absolute neutrophil count (ANC) with an average number of 183 / $\mu$ L. Bone marrow aspiration was determined maturation arrest of neutrophil precursors at promyelocyte and myelocyte stage. From these, severe congenital neutropenia (SCN) had been addressed. By x-ray and ultrasonographic findings, left proximal radius osteomyelitis and abscess formation of left elbow were demonstrated. *Pseudomonas aeruginosa* was identified from tissue samples culture after drainage and bony curettage. Intravenous cloxacillin, ceftazidime, and subcutaneous granulocyte colony stimulating factor (G-CSF) were commenced. Thus higher ANC at an average of 4,812 / $\mu$ L was obtained during 5 days of G-CSF treatment in order to increase initial ANC and enhance phagocytic function during an early treatment period. Acute osteomyelitis and abscess were well-controlled with 6-week-period antibiotics. After hospital discharge, ANC has been fallen right after G-CSF was stopped without any re-appearing infection. For this latter reason, no further G-CSF treatment was given to avoid future progression of SCN to myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML).

### **Discussion**

In patients with SCN, bacterial or fungal infections are reduced by treatment with G-CSF. Nonetheless, MDS and AML have been established after long-term G-CSF enrollment from previous reports. Short course G-CSF regimen was employed in this patient with a favorable result in serious infections as such pseudomonas osteomyelitis and deep tissue abscess treatment. Our hypothesis is the initially risen up in ANC might be sufficient to control infection in SCN.



**Figure 1.** Hypoechoic structure surrounding left ulnar head



**Figure 2.** ANC response after G-CSF therapy

P114

## Virtual Microscopy: The Role of Digital Images in a Haematology Morphology Quality Assurance Program

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### Aim

Virtual microscopy is the creation of digital images from glass slide material to enable distant viewing in a manner simulating light microscopy. Advantages for haematology external quality assurance (EQA) would be that cases with only a small volume of diagnostic material could be used; all participants receive identical material, and simpler survey preparation and distribution.

A virtual microscopy pilot survey using DVD's was encouraging so we aimed to evaluate online (web based) delivery of digital images for haematology EQA.

### Method

Three slides used in previous RCPA QAP haematology morphology surveys were scanned with an Aperio ScanScope OS virtual microscope. The scan area on each was 10x10mm. Digital SlideBox (SlidePath) enabled participants to view the images and submit responses online.

Participants were asked to submit morphological descriptions and diagnoses and complete a questionnaire about ease of website access, login and image quality.

### Results

112 (21%) laboratories completed the questionnaire including 20 (18%) that could not access the images for reasons including "no internet access", "slow download" and restrictive institutional policies. Otherwise there was an overall positive response with "satisfactory" or better ratings for image quality (86%) and ease of download (70%).

Correct diagnoses submitted for 2 of the 3 the cases resembled those from the previous glass slide surveys. In the third (myelofibrosis post-splenectomy) the scan was not sufficiently representative of myelofibrosis but hyposplenism were identified by the majority.

### Conclusions

This pilot study confirms that haematology surveys using virtual microscopy are feasible. However a significant group of participants had problems due to slow internet facilities so it cannot yet replace glass slides. Provided that digital images are representative of the whole slide, they will be an important adjunct for haematology morphology EQA

**Acknowledgement:** Quality Use of Pathology Program (QUPP) funding.

*No conflict of interest to disclose.*

A346

P115

## **A Retrospective Clinicopathological Study of Sporadic Burkitt Lymphoma and B-cell Lymphoma, with Features Intermediate Between Large B-cell Lymphoma and Burkitt Lymphoma: A Population Based Study of Successively Treated Patients from Auckland and Starship Hospitals**

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### **Background**

Recent studies have suggested that sporadic Burkitt lymphoma (BL) is potentially curable using rapidly cycling high intensity (RCHI) chemotherapy with CNS prophylaxis. In contrast, B cell lymphoma with features between BL and diffuse large B cell lymphoma (BL/DLBCL) has a less favourable prognosis.

### **Aim**

This retrospective study was conducted to compare the response rates of various era-specific treatments on patients with BL, BL/DLBCL and a control population of DLBC, and to identify prognostically important clinical and pathological features.

### **Methods**

Consecutive patients with aggressive B cell lymphoma (high Ki-67% +/- c-myc gene rearrangement) diagnosed in Auckland and Starship Hospitals between January 2001 and December 2006 were included. Morphology, immunophenotype and FISH studies were used to separate BL from BL/DLBCL and DLBCL. All patients were staged in a uniform manner and treated with era-specific regimens for that particular diagnosis. BL patients received rapidly cycling high intensity chemo with CNS prophylaxis. BL/DLBCL patients received either RCHI chemo or CHOP based chemotherapy and CNS prophylaxis. DLBC lymphoma patients were treated with CHOP based chemotherapy.

### **Results**

A total of 65 patients were eligible for the study. On review of the pathology, immunophenotype and molecular studies, 20 were classified as BL, 14 BL/DLBC and 33 DLBCL. The complete response (CR) rates were 94%, 54% and 53% respectively. With median follow up duration of 1162 days for living patients (range 67 to 2075 days), the overall survival rates (OS) are 67% for BL, 14% for BL/DLBCL and 45% for DLBCL.

### **Conclusion**

This retrospective study suggests a superior outcome for patients with Burkitt Lymphoma treated with high intensity rapidly cycling chemotherapy and CNS prophylaxis when compared to patients with BL / DLBCL or DLBCL. The outcome for patients with BL / DLBCL is particularly poor, and alternative therapeutic strategies are required for this group of patients.

*No conflict of interest to disclose*



P116

## Chronic Neutrophilic Leukemia (CNL) Presenting with a Chronic Granulomatous Disease (CGD) Like Phenotype

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CGD is a rare inherited primary immunodeficiency syndrome characterised by recurrent life-threatening infections and granulomata formation. CGD arises from defects in NADPH-oxidase function in phagocytic cells. NADPH-oxidase catalyses the formation of superoxide, a precursor for the production of reactive oxygen species (ROS) – the ‘oxidative burst’. The “oxidative burst” is critical in destruction of pathogens.

We report a case of an 84 year-old female who presented with *Escherichia coli* urosepsis on a background of chronic atonic bladder managed with a supra-pubic catheter. During the six months preceding admission she reported night sweats and weight loss. Her past history included osteoarthritis, hypothyroidism and depression. On presentation she had a marked leukocytosis ( $181 \times 10^9/L$ ) with a profound neutrophilia ( $122 \times 10^9/L$ ). Her haemoglobin was 101g/L and platelet count  $365 \times 10^9/L$ . The blood film showed no significant dysplasia, eosinophilia or basophilia. Clinical examination and imaging studies revealed massive splenomegaly with moderate hepatomegaly. Peripheral blood fluorescent in-situ hybridization failed to identify a BCR-ABL translocation. A bone marrow examination demonstrated a markedly hypercellular marrow with myeloid hyperplasia and minimal dysplasia, consistent with a myeloproliferative neoplasm – CNL; no cytogenetic abnormality was detected. Despite initial clinical improvement with intravenous antibiotics, she developed recurrent and relapsing infections including *Escherichia coli* and *Enterococcus faecalis* bacteraemia and *Candida albicans* fungaemia leading to her demise.

Given her history of infection despite profound neutrophilia, we performed flow-cytometric neutrophil function testing using the quantitative dihydrorhodamine 123 assay. Testing revealed normal phagocytic function, but ROS production was markedly reduced as seen in CGD.

We hypothesise the impaired neutrophil oxidative function was a manifestation of CNL. CGD results from mutations that cause loss or functional inactivation of one of the subunits of the NADPH-oxidase complex (gp91phox, p47phox, p22phox, and p67phox). Whether defects in these proteins could explain the patient’s clinical manifestation remains to be determined. As recurrent infections are relatively uncommon in myeloproliferative and myelodysplastic disorders in the absence of neutropenia, neutrophil function testing should be considered in all non-neutropenic patients with these disorders and recurrent infections.

*No conflict of interest to disclose*

A348

P117

## Study of the Rate of Viral and Bacterial Infections and the Effect of Immunoglobulin Therapy in Multiple Myeloma Patients Who Underwent Autologous Stem Cell Transplantation and Received New Immunomodulatory Drugs

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### Background

Multiple myeloma (MM) is associated with a high-risk of infection resulting in significant morbidity and mortality. To date, there are few data available regarding the prevalence of infection after autologous stem cell transplantation (ASCT) or in conjunction with newer generations of immunomodulatory therapy (thalidomide, lenalidomide, bortezomib) in MM.

### Rationale

Intravenous immunoglobulin (IVIg) has been used to reduce infection rates in the stable phase of MM, with limited data in other stages.

### Patients and Methods

We retrospectively analyzed 47 patients with MM from January 2007 to January 2009. All patients received thalidomide and steroid therapy. Nine patients received bortezomib and 11 lenalidomide subsequently to thalidomide, because of disease progression and 22 patients underwent ASCT. The median age was 64 years (range 37-86), with a female-to-male ratio of 18:29. The median residual-serum IgG level at time of infection was 3.2 g/L, IgA 0.3 g/L and IgM 0.2 g/L.

### Results

Most patients suffered from recurrent moderate to severe infections including the ASCT group. All patients except 3 received IVIg therapy with a significant decline of the rate of infection thereafter.

### Conclusion

Our analysis shows that IVIg therapy plays an important role in the supportive management during the active treatment of MM with ASCT as well as the new immunomodulatory agents. Accordingly, there was a significant benefit of IVIg therapy in MM patients with corresponding decrease in hospital admission for recurrent infections or use of IV antibiotic or antiviral therapy. Further studies to confirm our findings are warranted.

*No conflict of interest in relation to this research*

**Acknowledgments:** The authors acknowledge the Australia Red Cross Blood Service for supplying the immunoglobulin therapy according to the national guidelines for the patients involved in this study.

P118

## Early Implementation of Antifungal Therapy in the Treatment of Febrile Neutropenia is Associated With Favourable Outcome During Induction Chemotherapy for Acute Leukaemias

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### Rationale

Mortality related to induction chemotherapy during the treatment of acute leukaemias has been estimated at 5-20% and increases with age. Fungal infection remains one of the leading causes of morbidity and mortality and is considered to be one of the major obstacles in the successful management of acute leukaemias.

### Patients and Methods

We retrospectively analysed all patients treated at the Launceston General Hospital, Tasmania for acute leukaemias between July 2006 and December 2008 to assess the factors that influence outcome of induction therapy. Pretreatment factors that may influence outcome were analysed, such as patient age, performance status, disease risk-stratification, co-morbidities, ICU admission and complications of disease at presentation as well as the impact of antifungal therapy on outcome during induction chemotherapy.

There were 44 episodes of induction chemotherapy with a median age of 61 years (range 18-81), and 20 patients were over the age of 60 years. There were 29 patients with AML, 9 with ALL and 6 with relapsed acute leukaemias. At presentation, there were 29 episodes with ECOG scores <2, ten episodes with scores of 2, three episodes with scores of 3, and two episodes with scores of 4.

### Results

All patients who developed febrile neutropenia received broad-spectrum antibiotics, and most received empirical antifungal treatment with voriconazole (15 patients) or caspofungin (12 patients) if the fever did not resolve after 72 hours of antibiotic therapy. None of the patients succumbed during induction chemotherapy. Furthermore, the 120-day mortality rate after the induction therapy was 2.2% without any incidence of invasive fungal disease.

### Conclusion

Our analysis shows that early empirical treatment for fungal infection is associated with a favourable outcome of induction therapy for acute leukaemias. Further studies to confirm this finding are warranted.

*No conflict of interest in relation to this research*

A350

P119

## A Prospective Randomised-Controlled Trial Evaluating the Effect of Intravenous Versus Oral Iron on Haemoglobin and Ferritin Levels in the Management of Iron Deficiency Anaemia During Pregnancy

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### Background

Nutritional iron deficiency is the most common deficiency disorder in the world, affecting more than one billion people, with pregnant women at particular risk. There are no data available in Australia regarding prevalence of iron deficiency anaemia (IDA) during pregnancy. Debate continues regarding the optimal route of iron administration due to concerns with efficacy, tolerability and patient compliance.

### Methods

We prospectively screened 2654 pregnant women between March 2007 and November 2008 with a full blood count and iron studies. Amongst these women 461 (18%) had IDA, and 196 women were recruited to a prospective non-blinded randomised-controlled trial to determine whether a single intravenous iron polymaltose followed by oral iron is superior to daily oral ferrous sulphate for the management of IDA associated with pregnancy.

### Results

At recruitment, the mean haemoglobin was 109.3 g/L in the oral group and 107 g/L in the IV group (reference range (RR) 120-160g/L). The mean serum ferritin was 17.7 and 18 µg/L respectively (RR 30-460µg/L). At delivery the haemoglobin level was increased by a mean of 12 g/l versus 20 g/l respectively ( $p=0.002$ ). Mean serum ferritin was increased by only 18 µg/L on oral iron at delivery, while a significant large increase of 222 µg/L was seen 4 weeks after IV iron, and by 108 µg/L at delivery ( $p<0.001$ ). At delivery 79% of women on oral iron only and 4.5% of women treated with IV iron plus oral iron maintenance therapy had ferritin levels below 30µg/L. IV iron was well tolerated.

### Conclusion

Our data indicate that IDA is common during pregnancy in Tasmanian population. Intravenous iron polymaltose therapy is safe and superior to oral iron alone in this cohort of patients.

*No conflict of interest in relation to this research*

**Acknowledgement:** This research received a grant from the Clifford Craig Medical Research Trust, Launceston, Tasmania, Australia. The authors also acknowledge the enormous help of the staff of the Pharmacy Department at the Launceston General Hospital, Tasmania.

P120

## A Retrospective Analysis of the Effect of Filgrastim Compared to Pegfilgrastim on Neutrophil Recovery During the Treatment of Acute Leukaemias

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### Background

The rate of neutrophil recovery is crucial parameter for successful treatment of acute leukaemias. Traditionally, filgrastim, which is a short acting G-CSF, employed post-chemotherapy to shorten the neutropenia period, requires to be administered daily. While the recently developed pegfilgrastim is a long acting G-CSF that successfully support chemotherapy as a modified long-acting molecule by the addition of a polyethylene glycol and therefore has decreased renal clearance with a resultant increased serum half-life allowing for single injection dosing. The main mechanism of clearance is neutrophil-mediated endocytosis.

### Patients and Method

We retrospectively analysed the outcome of 103 episodes of chemotherapies in 33 patients who received induction and consolidation therapy for acute leukaemias with supporting G-CSF during the period from 2007 to 2009 at a single institution. The male to female ratio was 1.4: 1 and the median age was 56 years (Range 19–80). There were 24 patients were treated for AML and 9 for ALL/lymphoblastic lymphoma. G-CSF was commenced according body weight (filgrastin 5mcg/kg daily, pegfilgrastin 6mg, single dose) one day after completion of the chemotherapy. The time required for neutrophil recovery > 0.5 and > 1.0 were analysed for each patient for every treatment cycle. The study also incorporated other factors that may influence neutrophil recovery such as ECOG status of the patient, type of chemotherapy and the presence of febrile neutropenia or sepsis.

### Results

Patients who were treated with filgrastin (18 patients) had a neutrophil recovery >0.5/nl with a mean of 11 days compared to 12 days with pegfilgrastin, while neutrophil recovery >1.0/nl was observed on a mean of 12.5 days for both groups. There was no significant difference between the two groups with a p-value of 0.7 and 0.9 respectively. Further sub-analysis of induction and consolidation chemotherapies did not reveal a significant difference between the two cytokines, however it was noted that a prolonged neutropenia occurred during induction- compared to consolidation chemotherapy in both treatment groups.

### Conclusion

During the treatment of acute leukaemia, pegfilgrastim results in a comparable effect with filgrastin with decreased cost and less injections. Further studies to confirm these findings are warranted.

*No conflict of interest in relation to this retrospective analysis*

A352

**P121**

**Acute Lymphoblastic Leukaemia in Papua New Guinea – A Retrospective Study from 2001 to 2006**

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**Introduction**

ALL is the commonest malignancy in childhood with the majority of cases in the 2 to 10 age group (median 3.5). It is five times more frequent in childhood than AML. It is a rare leukaemia in adults, 0.7 to 1.8/100,000 annually. In adults, there is a peak at 15 to 24 years and a further peak in old age (2.3/100,000 >80 years).

**Aim**

The aim of this study was to determine the different types of acute lymphoblastic leukaemia cases in the country only on the basis of morphological identification and to see the prevalence in the provinces, the type of ALL diagnosed and the age group affected most.

**Method**

Data kept in the laboratory was analysed manually for the study period.

**Result**

A total of 30 patients were analysed from records in the laboratory and as for the other provincial hospitals around the country a total of 25 patients were diagnosed with ALL for the year 2001 to 2006, with the highest number (10) in 2005. These were followed by 6 cases in 2003, 5 in 2001, 4 in 2002 and 2 in 2006.

**Conclusion**

This retrospective study provides evidence of ALL cases in the country from 2001 to 2006 study period. The highest numbers of cases were from the Central Province in contrast to other provinces in this study. This may be due to the close proximity of services provided by the hospital for the patients to come and be treated for their illnesses; therefore the case incidences are higher as compared to the other provinces.

ALL L3 was the most common ALL identified, and the patients tend to be less than 20 years old.

*No conflict of interest to disclose*

**P122**

## **High Resolution Melt (HRM) Analysis as a Screening Tool to Identify Patients with BCR-ABL Point Mutations**

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### **Aim**

The emergence of point mutations in the BCR-ABL tyrosine kinase is a major cause of resistance to treatment with the first generation tyrosine kinase inhibitor (TKI) imatinib. A requirement exists for a rapid, sensitive and cost-effective screening technique with relative ease of use, to be available for patients who have increasing quantitative BCR-ABL transcript values as measured by PCR.

### **Method**

cDNA used for the quantitative BCR-ABL assay is placed in a PCR reaction using primers targeting the entire ABL kinase domain to amplify a long segment of the BCR-ABL transcript up to 1800 bp. 4 separate HRM reactions are then used to interrogate different regions along the BCR-ABL transcript that are known to contain point mutations using a real-time PCR technique involving HRM (High Resolution Melt) analysis.

### **Result**

Ten stored RNA samples from CML patients with known point mutations present in each of the four separate HRM reactions from the period 2004-2009 have been analysed. The HRM analysis was reproducibly able to detect point mutations at F359C, F486S, E459K, T315I and M244V of the BCR-ABL transcript. Sensitivity of the assay was examined by dilution of the mutant sample in wild type cDNA.

### **Conclusion**

High resolution melt curve analysis can be used as a screening tool to detect point mutations in the BCR-ABL transcript in routine patient samples. We plan to prospectively examine HRM analysis in CML patients who are on TKI with increasing BCR-ABL transcript values.

*No conflict of interest to declare*

P123

## **Cost-benefit Analysis of G-CSF Primary Prophylaxis in Reducing Hospital Admissions with Febrile Neutropenia in Patients Undergoing High Dose Chemotherapy for Non-Hodgkin's Lymphoma**

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### **Aim**

High dose chemotherapy for lymphoma is known to be associated with significant risk of febrile neutropenia (FN), especially in elderly patients. We have implemented standard guidelines for primary GCSF prophylaxis in our department, and the aim of the study is to determine the impact of this on the incidence of FN as well as cost-effectiveness.

### **Methods**

A cohort of 60 patients who underwent high dose chemotherapy for lymphoma from December 2006 to February 2009 was identified. Type of lymphoma, chemotherapy regimen, details on GCSF prophylaxis and hospital admissions due to FN as well as outcome of treatment were recorded. Primary GCSF prophylaxis was given for average of 7 doses. Cost-benefit analysis was carried out using actual cost of the hospital admissions and NNT (numbers needed to treat).

### **Result**

Overall, patients who received primary GCSF therapy had significantly less FN episodes compared to those who didn't [8%vs54%,  $p=0.0001$ , absolute risk reduction 44% (95% C.I. 22%, 60%) with NNT of 2.3 (95% C.I. 1.5, 4.5)]. In subgroup analysis, even patients aged  $\leq 65$  yrs appeared to receive benefit from primary GCSF prophylaxis in reduction of FN (4.8%vs 66.7%,  $p=0.0001$ ). In patients who underwent CHOP-like chemotherapy, the benefit of primary GCSF prophylaxis appeared to be greater compared to the cohort, with NNT of 1.9 (95% C.I. 1.3, 3.4). The average cost of hospital admission with FN was calculated to be \$6753.31. Using the NNT value obtained, primary GCSF prophylaxis appeared to be cost effective, leading to saving of \$1991.22 per patient.

### **Conclusion**

Our study shows that primary GCSF prophylaxis is highly effective in preventing FN in patients undergoing high dose chemotherapy. Furthermore, primary GCSF prophylaxis has been shown to be highly cost-effective. Considering other related costs such as patient morbidity/mortality associated with FN, primary GCSF prophylaxis is recommended in this patient group.

*No conflict of interest to disclose*



**P124**

## **Extramedullary Plasmacytoma With Local Amyloidosis Presenting as a Gastric Lesion: A Case Report**

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### **Background**

Localised deposits of AL-amyloid (amyloidoma) is usually associated with an underlying plasma cell dyscrasia and although rare, has been most commonly described in the nasal sinuses and respiratory tract. They frequently respond to local therapy only and rarely require systemic chemotherapy commonly used in primary (AL) amyloidosis. Gastric involvement by localised AL-amyloidosis is rare. We report a case of extramedullary gastric plasmacytoma with localised amyloidosis and review the existing literature.

### **Method**

A "Medline" search was performed using the key word "localised amyloidosis" and "plasmacytoma".

### **Results**

Less than 25 cases of localised AL-amyloidosis have been reported in the literature between 1993 to 2009. Of these, majority were successfully treated with local resection only. In our case report, we present a 41-year-old woman with six months of protracted dyspepsia. Upon gastroscopy, the stomach had evidence of superficial linear ulceration with a generalised thickened appearance. Histology of this area revealed sheets of monoclonal plasma cell infiltration with a heavy local amyloid deposition as demonstrated by polarised microscopy. Serum protein electrophoresis and serum free light chains were normal with a normal kappa:lamda ratio. Bone marrow biopsy showed no excess of plasma cells. There was no evidence of systemic amyloidosis. She is currently planned for involved field radiotherapy.

### **Conclusion**

Plasmacytoma involving the stomach wall with local amyloidosis is rare. Usually, malignant plasma cells preferentially home into the bone marrow due to the attraction of the chemokine CXCR4 on plasma cells to its receptor SDF-1a in the bone marrow. The mechanism for the distinct homing of plasma cells in extramedullary plasmacytoma and localised amyloidosis is unknown,. However in cases like this, cure can be potentiated by the expedient local treatment with radiotherapy or surgery.

*No conflict of interest to disclose*

**A356**

P125

### Extra-Nasal NK/ T Cell Lymphoma Masquerading as Renal Infarction

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#### Introduction

Extra-nasal NK/T cell lymphoma is a rare, aggressive non-hodgkins lymphoma. Definitive diagnosis is often delayed due to atypical presentations. We report a case of extra-nasal NK/T cell lymphoma masquerading as a renal infarct and renal carcinoma, which subsequently achieved complete remission post radical nephrectomy and involved field radiotherapy.

#### Case Report

A 72 year old Caucasian male with a previous abdominal aortic aneurysm repair presented with left flank pain. Initial abdominal CT demonstrated a left inferior pole renal infarct and haematoma with extensive intra-aortic thrombus within the aortic graft, suggestive of embolic event arising from a proximal aortic thrombus. Subsequent correlative ultrasonography revealed a typical vascular, enhancing left renal pole mass which was reported as consistent with a renal cell carcinoma. A left radical nephrectomy was performed; histology unexpectedly showing diffuse patchy lymphoid infiltrates with solid areas of large atypical lymphocytes which were CD3+, CD2+, CD7+ and CD56+, and CD20-. Granzyme showed granular positivity and perforin was negative. Ki67 was high (60%+) in the large cells areas. EBER-ISH was positive. These features were consistent with extra-nasal NK/T cell lymphoma. The patient had positive serum EBV IgG and negative IgM and a high  $\beta_2$ -microglobulin of 4.8mg/L. Further staging with CT and PET showed no evidence of FDG-avid disease elsewhere. The patient proceeded to involved field irradiation with 30 Gy without any chemotherapy and is currently in complete metabolic remission.

#### Conclusion

Extra-nasal presentation of nasal-type NK/T cell lymphoma is rare; definitive diagnosis of is often delayed due to atypical presentation. Early and correct diagnosis, especially in localised disease may offer a potential cure for this otherwise aggressive disease with particularly poor prognosis and poor response to treatment.

*No conflict of interest to disclose*

**P126**

## **Dengue Drama for the Tropics**

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Dengue fever is a disease of the tropics which is transmitted more commonly, by the mosquito *Aedes aegypti*. It is particularly prevalent in north Queensland with outbreaks occurring yearly. The recent outbreak of Dengue declared on 1 December 2008, was ratified by Queensland Health on the 21<sup>st</sup> May 2009 to be officially over. It has been confirmed to be the biggest outbreak in over 50 years, breaking the previous record of the type 2 outbreak in Charters Towers and Townsville in 1992/93 of over 900 dengue cases. Every outbreak requires preventative measures to be taken to confine the disease and prevent it from becoming endemic in north Queensland. This highlights the importance of recognizing and detecting Dengue fever. Dengue serology in collaboration with the full blood count plays an essential role in Dengue detection as well as disease progression and clinical management. We studied the full blood count of 65 cases with a positive Dengue serology for any noticeable trends. From the results collected, it was noted that approximately half of Dengue cases presents with a neutropenia (45%) or thrombocytopenia (48%) and with 30% showing a bicytopenia. Reactive lymphocytes were also a common finding, seen in 25% of cases. 4 cases (0.1%) presented with a neutrophilia. It is interesting to note that 20% of cases present with a normal full blood count and thus the importance to be aware that a normal full blood count does not rule out the possibility of Dengue fever.

*No conflict of interest to disclose*

P127

## HLA-G Expression and Relevance in Myeloma

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### Aim

HLA-G is a non-classical HLA class I molecule with immunomodulatory function implicated in tolerance of the fetus, allografts and tumours. To evaluate the role of HLA-G in protecting MM cells from the host's immune defences, expression of HLA-G in MM was investigated.

### Method

Bone marrow (n=50) and blood (n=38) samples were collected from patients with MM who attended our clinic for between January 2007 and June 2009. 25 patients were stage I, 14 stage II, and 6 stage III, according to the ISS staging system. Blood was also collected from 15 age-matched normals. Expression of HLA-G was determined by flow cytometry.

### Results

Expression of HLA-G on CD38++ cells in the bone marrow ranged between 0.2 to 96% with a mean of 21.3%. Overall survival was significantly worse for the 11/46 patients with HLA-G+ plasma cells not lost to follow-up (>5% CD38++ cells and expressing >12% HLA-G;  $\chi^2=12.4$ ;  $p<0.0004$ ). HLA-G was not significantly different between patients with stage I+II (mean 21.7%) and stage II/III+III disease (mean 18.8%) ( $p=0.77$ ). The percentage of HLA-G+ CD3+ T cells in the blood of controls ranged from 0.02 to 0.47% (mean 0.19%). Higher levels of HLA-G+ on CD3+ cells were demonstrated in blood from patients with MM (range 0.00 to 1.01%; mean 0.30%) although the difference in HLA-G expression between the two groups did not reach statistical significance. Overall, 26% of MM patients (10 of 38) demonstrated a concentration of HLA-G-expressing CD3+ T cells above the mean+2SD of controls. HLA-G expression was on both CD4 and CD8 cells but negligible on CD3- cells. Anti-CD3/CD28 beads and IL-2 stimulation failed to upregulate HLA-G expression on lymphocytes.

### Conclusion

HLA-G+ myeloma cells are associated with a significantly poorer prognosis. The functional significance of HLA-G+ lymphocytes in MM is intriguing and will require additional studies.

*No conflict of interest to disclose*

P128

## **Palliative Care and the Haemato-oncological patient: Can We Live Together? A Review of the Literature**

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### **Background**

Current evidence suggests that patients with haematological malignancies less frequently access palliative care services, and for those who do, this occurs at a later stage in their illness than their counterparts with solid malignancies. Patients with haematological malignancies are also more likely to die in an acute hospital following escalating interventions than those who die of solid tumours. This approach to care highlights a conception of palliative care as relevant only after most therapeutic treatments are exhausted, and has substantial implications for the quality of palliative care interventions possible. The aim of this review is to examine the interface between malignant haematology and palliative care and highlight aspects which may provide insights into current approaches to care.

### **Methods**

A literature review of electronic databases supplemented by hand searching.

### **Results**

The successful integration of palliative care into the care of patients diagnosed with haematological malignancies requires recognition by palliative care physicians of the particular issues encountered in care, namely, the difficulty in prognostication; the technical nature of treatments and their sequelae; the speed of change to a terminal event; the need for pathology testing and blood products even as death approaches; the possibility of catastrophic bleeding; and the close, and frequently long relationships between a patient and their haematologist. Meanwhile, haematologists should be aware of the benefits of palliative care for their patients and patients' families/carers earlier in the trajectory of an illness.

### **Conclusion**

This review summarises the current practice, barriers to referral, and areas requiring further investigation in the palliative care of patients with haematological malignancies. In doing so, it highlights to palliative care and haematology physicians how successful integration of their disciplines may improve the care of these patients and families, such as an approach which engages palliative care according to needs rather than prognosis. This may mean a model of palliative care input that is episodic, with times of high intensity when the need is high, but then be minimal during times of disease responsiveness.

*No conflict of interest to disclose*

A360

**P129**

**The Symptom Burden of Patients with Haematological Malignancy: A Cross-Sectional Observational Study**

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**Background**

A better interface between life prolonging and palliative approaches at all stages of cancer treatment is actively encouraged in order to achieve best possible quality of life for patients and their families. This has not always been achieved for patients with haematological malignancy. One possible explanation is that the intensity and burden of symptoms are not well understood in this diagnostic group. The purpose of this study is to determine the patterns of symptoms and level of distress in patients diagnosed with a haematological malignancy at different points during the illness trajectory.

**Methods**

100 consecutive patients with the diagnosis of a haematological malignancy, irrespective of stage of disease, attending outpatients, day-treatment unit or admitted as an inpatient at St Vincent's Hospital Melbourne completed the Memorial Symptom Assessment Scale - Short Form. Medical and demographic information was obtained from the medical record. Results were analysed using descriptive statistics.

**Results**

Preliminary results suggest that patients who are receiving treatment have a significantly higher physical symptom burden compared to patients who are not receiving treatment ( $p = 0.034$ ). In particular, pain was experienced in 38% of patients and lack of energy in 66% of patients irrespective of their stage of disease or treatment status. Psychological burden was also high across both treatment and non-treatment groups.

**Conclusions**

Patients currently receiving treatment for a haematological malignancy have a significant symptom burden irrespective of the stage of their illness. Knowledge of this pattern of symptoms suggests opportunities for palliative care input to improve symptom distress and quality of life in these patients.

*No conflict of interest to disclose*

**P130**

## **Review of Thrombocytopenia at Port Moresby General Hospital**

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### **Introduction**

Thrombocytopenia is commonly seen in patients with acute infections such as malaria, bacterial infections and acute leukaemia. Many cases of thrombocytopenia are self limiting, however in active bleeding, the management of these patients is challenging, given the availability of blood and blood products in a low resource setting.

### **Aim**

To document the incidence of thrombocytopenia in PMGH and to assess disease and clinical association, common clinical manifestations and the type of blood products used in their management. The outcomes of these patients were also assessed.

### **Methodology**

This was a prospective, non-randomised, and descriptive study. Phase 1, 2002-2003, was to review all patients with thrombocytopenia to obtain a general overview and then Phase 2, in 2006, was to assess their clinical manifestations, treatment and outcome. Patients were chosen by pre-defined selection criteria. Results were analysed using SPSS and Microsoft Excel programs.

### **Results**

Of all samples, 20% were found to be thrombocytopenic, and of these malaria was the most common cause in 16.4% and 33% respectively in phases 1 and 2. The age group most affected was the 21 to 50 year olds. In children acute leukaemia was the most common cause of thrombocytopenia followed by malaria in the ages above than 1 year. Petechiae was the most common clinical manifestation in 60%. 55% of patients received blood, 26% of which was packed red cells, as 89% were anaemic. The overall mortality rate for this phase 2 population was 29%.

### **Conclusion**

Thrombocytopenia is a common finding in PMGH. Patients with moderate thrombocytopenia do bleed and must be watched. In general the mortality rate is high and this was mainly due to the lack of proper treatment of acute leukaemia.

*No conflict of interest to disclose*

P131

## Translocation $t(1;19)(q23;p13.3)$ in pre-B Acute Lymphoblastic Leukaemia

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The rearrangement  $(1;19)(q23;p13.3)$  is the second most common non-random translocation associated with acute lymphoblastic leukaemia(ALL), occurring in 5 – 6% of paediatric ALL , 3% of adult ALL and 20-25% of pre-B-ALL overall. This rearrangement occurs in two forms: a balanced reciprocal translocation,  $t(1;19)$ , in ~25% of cases and an unbalanced form,  $der(19)t(1;19)$ , in ~75% of cases. Approximately 90-95% of cases with the balanced and unbalanced form of  $t(1;19)$  are attributed to structural interruption of the E2A (TCF3) gene at 19p13.3 and its subsequent fusion to the PBX1 gene at 1q23. With the exception of occasional cases, breakpoints are clustered in the E2A gene. In contrast, the breakpoints in the PBX1 gene are more widely dispersed, although still clustered in two regions.

We report six patients aged from two to thirteen years who presented with pre-B ALL at the Royal Children's Hospital between December 2004 and March 2009. Three had  $t(1;19)(q23;p13.3)$  and three had  $der(19)t(1;19)$ . Additional cytogenetic abnormalities were present in two patients who had a hyperdiploid karyotype and demonstrated clonal evolution.

The TCF3-PBX1 fusion transcript was detected in the four patients tested.

FISH studies using the Cytocell E2A breakapart probe showed the expected signal pattern consistent with the rearrangement in two patients. The remaining four patients had aberrant signal patterns, suggesting variability of breakpoints within the E2A gene.

All patients achieved complete remission and remain in continuing remission.

*No conflict of interest to declare*



**P132**

## **Automated, High-Throughput Extraction of RNA from TRIzol®-Stabilized Leukocytes**

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### **Aim**

To develop and validate a fully automated RNA extraction method from monophasic TRIzol®-stabilised leukocytes.

### **Method**

RNA was extracted in parallel from 18 paired, TRIzol®-stabilised leukocyte specimens (14 from bone marrow, 4 from peripheral blood), using both the manufacturer's protocol and a novel protocol suitable for automated high-throughput applications. The novel protocol utilises QIAGEN's RNeasy® chemistry, and does not require separation of the aqueous phase from monophasic TRIzol® prior to binding to a silica-based membrane. RNA purity and yield was determined spectroscopically, with RNA purity evaluated by  $A_{260}/A_{280}$  and  $A_{260}/A_{230}$  ratios. The suitability of purified RNA for RT-PCR and subsequent qPCR was evaluated by preparation of cDNA from 1 µg RNA using M-MLV reverse transcriptase, and subsequent quantification of *ABL* gene expression. Automated extraction from monophasic TRIzol® was performed using an epMotion 5075 fluid handling robot, and the RNeasy® 96 BioRobot® 8000 kit.

### **Results**

RNA yield between the two procedures was not significantly different using Wilcoxon signed rank test ( $P=0.0877$ ) from  $10^7$  leukocytes, but spectroscopic purity was significantly higher ( $p<0.0001$ ) from the RNeasy®-based procedure. A mean  $A_{260}/A_{230}$  ratio of 1.652 (SD 0.5472) indicates low levels of contamination by phenol or guanidine hydrochloride following the RNeasy®-based method. Quantification of *ABL* expression in paired samples indicated no significant differences on a paired t test, with a mean of 11400 (SD 5917) from the manual extraction, and 10496 (SD 3823) from the RNeasy®-based procedure.

The procedure was successfully automated starting from  $5 \times 10^6$  leukocytes, with no detectable cross-contamination or reduction in RNA quality.

### **Conclusion**

A novel method for column-based extraction of high-quality RNA from monophasic TRIzol®-stabilised leukocytes was developed, with no significant variation in yield from the conventional TRIzol®-based protocol. This novel extraction protocol is compatible with automated 96 well plate formats, and is suitable for high-throughput applications.

*No conflict of interest to disclose*

**A364**

P133

## Cardiac Biomarkers in AL Amyloidosis

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### Aim

Cardiac involvement is a critical determinant of prognosis in AL amyloidosis, but conventional methods of assessment with ECG and echocardiography are relatively insensitive. We aimed to assess the prognostic utility of serum cardiac biomarkers in AL amyloidosis.

### Methods

Baseline levels of troponin-I (TnI), NT-ProBNP and BNP were measured in patients enrolled on a Phase II study of risk-adapted melphalan in AL amyloidosis (ALLG MM8 study) and their impact on survival was analysed. Cardiac involvement was defined according to International Society of Amyloidosis criteria (biopsy positive or interventricular septal thickness on echocardiography >12mm).

### Results

20 patients with a median age of 61yrs were enrolled. Organ involvement by standard criteria was cardiac 50%, renal 80%, liver 10%, neurologic 35% and gastrointestinal 10% with a median of 2 organ systems involved by amyloidosis. Median baseline cardiac biomarker levels were: TnI 0.041microg/L (range, 0.006-0.455); NT-ProBNP 1440ng/L (range, 49-35000); and BNP 278ng/L (range, 40-3250). Baseline levels were significantly higher in patients with cardiac involvement as defined by standard criteria: TnI 0.019 vs. 0.084 (p=0.001); NT-ProBNP 333 vs. 5997 (p=0.02); and BNP 63 vs. 439 (p=0.01). A high NT-ProBNP (>332ng/L) was present in 100% and 45% of patients with and without cardiac involvement, respectively.

With a median follow-up of 18 months, median overall survival is 19 months (95% CI, 17-65months). Inferior overall survival was significantly predicted by increasing TnI (p=0.002), NT-ProBNP (0.034) and BNP (0.025). Using a published risk score (JCO 2004;22:3751) 6, 10 and 4 patients had a low (TnI<0.1 and NT-ProBNP<332), intermediate (either TnI≥0.1 or NT-ProBNP≥332) and high (TnI≥0.1 and NT-ProBNP≥332) cardiac biomarker risk. This risk score predicted outcome with 18 month survival of 100%, 47% and 0% for low, intermediate and high risk, respectively (p=0.008).

### Conclusions

TnI, NT-ProBNP and BNP, as well as the published cardiac biomarker risk score strongly predict survival and should be incorporated into routine assessment of patients with AL amyloidosis.

*No conflict of interest to disclose*

P134

## Safety and Feasibility of Outpatient Delivery of Hyper CVAD for Lymphoid Malignancy

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### Aim

HyperCVAD is a short term, dose intensive regime with alternating hyperfractionated cyclophosphamide ("A" cycle) and high dose cytarabine and methotrexate ("B" cycle). We aim to compare the toxicities and feasibility of outpatient versus inpatient delivery of HyperCVAD to patients with acute lymphoblastic leukemia and lymphoblastic lymphoma.

### Methods

Consecutive patients with newly diagnosed ALL or lymphoblastic lymphoma, undergoing treatment with hyper CVAD between 1999 and 2007 were retrospectively identified. Data was collected regarding location of treatment (inpatient versus outpatient), number of inpatient days per cycle, cycle length, and grade  $\geq 3$  toxicities.

### Results

A total of 142 treatment cycles were analysed in 26 patients, including 63 A cycles and 79 B cycles. A higher proportion of B cycles were administered in the inpatient setting (50.6% vs 30.2%;  $p=0.0167$ ). As expected, the average number of admission days per cycle for patients who received chemotherapy in the outpatient setting was significantly shorter (4.37 vs 16.47 days;  $p < 0.0001$ ). Toxicities were more likely to occur in B cycles, however there was no difference when B cycles were delivered in the inpatient or outpatient setting ( $p = 0.80$ ). Similarly, no significant difference in the rates of toxicity were found comparing administration of A cycles as an inpatient or outpatient ( $p = 0.35$ ). Cycle time was no different comparing the inpatient and outpatient setting (median 22 and 23 days respectively;  $p = 0.9977$ ).

### Conclusion

In appropriately selected patients, both hyper CVAD A and B cycles can be delivered as an outpatient, requiring significantly fewer overall hospital admission days and no significantly increased toxicity or treatment delay.

*No conflict of interest to disclose*

P135

## Successful Use of High Dose Methotrexate in a Haemodialysis Dependent Patient with Primary CNS Lymphoma

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<sup>2</sup>*Renal Medicine Department, Princess Alexandra Hospital, Brisbane*

### Aim

High dose methotrexate (HDMTX) is a crucial component of many chemotherapy regimens, particularly lymphoid malignancies and osteosarcomas. However, because it is primarily renally excreted and serious toxicities may develop in end stage renal failure (ESRF), it is relatively contraindicated in this setting. We aimed to demonstrate that HDMTX can be successfully delivered in ESRF using high flux dialysis for methotrexate clearance.

### Methods

Case Report

### Results

A 52 year old female had previously received a heart-lung transplant complicated by ESRF secondary to calcineurin inhibitor nephrotoxicity. She was commenced on haemodialysis. She subsequently presented with a Primary Central Nervous System Lymphoma. As part of her treatment, 2 cycles of HDMTX (1g/m<sup>2</sup>) two weeks apart were given. Long sessions of high flux dialysis were used, starting 1 hour after the HDMTX infusion and then daily for a total of 6 days. A high flux dialysis membrane (Fresenius Fx 80) was used with an initial 8 hour session on Day1 in cycle 1 (10 hours in Cycle 2) and then 6 hour sessions on Days 2 to 6. The methotrexate was successfully cleared achieving serum levels of 4.2micromol/L and 0.55microMol/L at 24hrs and 48 hrs during cycle 1. During Cycle 2 the levels were 3.4 and 0.55microMol/L at 24 and 48 hrs respectively. In both cycles the level was reduced to 0.1microMol/L by 72 hours post infusion. There was no rebound in the serum methotrexate levels following each dialysis session. There were no methotrexate-related toxicities during the 1<sup>st</sup> cycle. In the second cycle she developed grade 2 mucositis, Grade 4 neutropenia and thrombocytopenia (both lasting one day) and Enterococcus bacteraemia despite achieving target MTX clearance levels. These toxicities resolved with standard therapy and were not considered unusual for patients with post-transplant lymphoproliferative disease. The patient subsequently died in complete remission from a perforated CMV gastric ulcer 4 months later.

### Conclusion

HDMTX can be safely delivered in dialysis dependent patients using high flux dialysis to clear the serum methotrexate.

*No conflict of interest to declare*

**P136**

## **Type 1 Cryoglobulinaemia: Peripheral Blood Picture Ends the Diagnostic Dilemma**

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### **Aim**

This case study is to raise the awareness of the importance of peripheral blood picture in the diagnostic work up of any disorder.

### **Case study**

Ninety year old lady presented with complaints of severe ischaemic pain of the legs and toes for the past one and a half years, with aggravation on exposure to cold and associated with presence of skin lesions and cold urticaria. She was treated with steroids on the basis of a clinical diagnosis of vasculitis of unknown cause, but without much improvement. Physical examination revealed skin lesions of the leg (biopsy reported earlier as non specific vasculitis) with no evidence of organomegaly/lymphadenopathy.

A Complete Blood Picture (CBP) performed this time showed spurious high white cell count ( $18 \times 10^9/L$ ) with presence of amorphous deposits of cryoglobulin throughout the film, which disappeared on warming at  $37^\circ C$ . Further immunological investigations confirmed IgGk M-protein (5.5g/L) with reduction of other globulins, type 1 cryoglobulin (IgG) with cryocrit 15%. Her RA factor, ANA, anti ds DNA and hepatitis C serology were all negative. There was no evidence of related organ or tissue impairment.

Peripheral blood immunophenotyping showed presence of a very small population of bi-clonal B cells demonstrating CD 5- B cell population with no light chain restriction and about two third with a slight lambda chain restriction of uncertain significance(? Age related). Bone marrow was not performed due to age and physical debility.

A diagnosis of Type 1 Cryoglobulinaemia, possibly due to underlying Monoclonal Gammopathy of Undetermined Significance/ Monoclonal B-cell Lymphocytosis was ascertained. Low dose Melphalan has been initiated. Currently patient is tolerating her treatment and feels more comfortable.

### **Conclusion**

This interesting blood picture once again highlights the importance of peripheral blood film in the diagnostic work up, and how this subtle finding could contribute to the solving of this elusive disorder.

*No conflict of interest to disclose*

**A368**

P137

## A Pilot Prospective Study of Full Blood Examination Parameters in Pregnant Women in the National Capital District of Papua New Guinea

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### Introduction

Anaemia in pregnancy, a haemoglobin concentration below 110 g/L, has a global prevalence of 41.8%, affecting some 56 million women (1). This can result in an adverse mortality and morbidity for both mother and child, especially in low resource countries, where the millennium development goals strive to reduce this impact. Papua New Guinea has high maternal and infant mortality with little recent data on anaemia in pregnancy and virtually no data on the white cell and platelet counts.

### Aim

To quantify the full blood examination (FBE) parameters of pregnant mothers attending antenatal clinics in the National Capital District

### Methodology

All samples were processed centrally at the pathology department of the Port Moresby General Hospital, using a Sysmex KT-1000 automated analyser. The age of the patient, if known, as well as the sample origin was recorded. All data was statistically analysed using Epi-info.

### Results

A total of 400 samples were analysed. The mean haemoglobin was 9.76 g/L, median of 9.9 g/L with a standard deviation of 1.9 g/L. 79% of the mothers were anaemic. Mean total white cell and platelet count was  $8.2 \times 10^9/L$  and  $234 \times 10^9/L$  respectively.

### Conclusion

Anaemia is a significant problem in pregnant women in National Capital District and has worsened since the last survey over a decade ago (2). A further national survey investigating the prevalence and aetiology, in collaboration with the antenatal service, is warranted. An anaemia prevention and treatment program requires to be designed and implemented.

### References

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No conflict of interest to declare

**P139**

## **A Prospective Analysis of a Papua New Guinean BCR-ABL1 positive Chronic Myeloid Leukaemia (CML) Cohort Within the Glivec International Patient Assistance Program (GIPAP)**

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### **Introduction**

GIPAP provides free Imatinib mesylate (Glivec) to Papua New Guinean citizens with BCR-ABL1 positive Chronic Myeloid Leukaemia (CML) in chronic or accelerated phase or Gastrointestinal Stromal Tumour (GIST).

This is the first such cohort created in Papua New Guinea for this patient group and no previous data on the demographic, clinical and molecular features in this Melanesian population has ever been presented internationally.

### **Results**

Currently 8 patients are enrolled in the GIPAP cohort, with a mean age of 32 years, age range from 13-45 years, median age of 35 and a modal age of 40 years. The male to female ratio was 1:0.6. 60% of the cohort presented in chronic phase CML. BCR-ABL transcript analysis was carried out in all 8 patients, 87% of which showed the presence of b3a2 transcript, with only one patient in accelerated phase with b2a2. The mean RNA yield was 52.8 m g/trizol. The cut off was chose at 40 ct values and the assay had a sensitivity of 0.001%. The mortality rate is 25% with two deaths in accelerated phase. Three patients are receiving Glivec therapy two of whom have gained a complete haematological response.

### **Discussion**

Prior to April 2009, the only treatment options available in PNG were Hydroxycarbamide and Busulphan. GIPAP has transformed the management of CML in over 80 developing countries and as of April 2009 GIPAP commenced in PNG. This is the first ever data analysis of such a cohort. Early molecular data demonstrates a predominance of b3a2 transcript type and a 100% haematological response for those patients registered and taking Glivec. A BCR-ABL1 transcript molecular monitoring study for this cohort is currently in design using the GeneXpert PCR system.

*No conflict of interest to disclose*

**A370**

P140

**Early Dose-Escalation in Chronic Myeloid Leukaemia Patients with Low Plasma Imatinib Levels Results in 3 month Drug Levels and BCR-ABL PCR Results Equivalent to Patients with Optimal Levels**

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**Aim**

Low trough plasma imatinib levels have been associated with a poorer response to treatment in chronic myeloid leukaemia (CML) in chronic phase. We hypothesised that dose-escalation in patients with low day 22 trough levels could improve outcomes.

**Method**

Imatinib trough and peak levels were measured in 71 chronic phase CML patients at day 8, day 22, and 3 and 6 months after commencing imatinib 600mg. Patients with a day 22 imatinib trough level of <1000ng/ml were dose escalated to 800mg daily where tolerable.

**Results**

The median follow-up was 35.5 weeks, with 60 of 71 patients having 3-month data and 44 having 6-month data. At day 22, 10 (15%) patients had an imatinib trough level <1000ng/ml, with 7 of these being dose-escalated to 800mg. At 3 and 6 months, the mean ( $\pm$ SD) imatinib trough level in those who had been dose escalated to 800mg due to a day 22 trough level of <1000ng/ml was no longer statistically different from those with a day 22 trough level of  $\geq$ 1000ng/ml (1728 $\pm$ 436 vs 1760 $\pm$ 949 ng/ml at 3 months,  $p=0.89$ ; 1715 $\pm$ 626 vs 1535 $\pm$ 755 ng/ml at 6 months,  $p=0.62$ ). At 3 and 6 months there was no significant difference in BCR-ABL PCR results between dose-escalating patients and those with optimal imatinib trough levels at day 22 (4.76  $\pm$ 7.59 vs 1.68  $\pm$ 2.77 at 3 months,  $p=0.37$ ; 2.09  $\pm$ 3.99 vs 2.12  $\pm$ 9.56 at 6 months,  $p=0.99$ ).

**Conclusion**

These data suggest that selective dose-escalation based on Day 22 imatinib trough levels of <1000ng/ml results in subsequent plasma concentrations equivalent to patients with day 22 levels of  $\geq$ 1000ng/ml. Given that there was no difference in BCR-ABL PCR at 3 and 6 months between these two groups, we speculate that this strategy may ameliorate the adverse prognostic impact of low imatinib plasma concentrations on standard dose.

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P141

## Routine Use of Ancillary Investigations in Staging Diffuse Large B-cell Lymphoma Improves the International Prognostic Index (IPI)

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### Aim

This study aims to determine the effect of the routine use of staging investigations such as flow cytometry, immunohistochemistry and molecular studies upon the International Prognostic Index (IPI) in patients with Diffuse Large B-cell Lymphoma (DLBCL).

### Methods

Bone marrow trephines of 156 histologically proven DLBCL cases at initial diagnosis were assessed on routine histology, and immunohistochemistry (IHC) using T-cell markers, B-cell markers and kappa and lambda light chains. Raw flow cytometry data on all samples were reanalysed and reinterpreted blindly. DNA extracted from archived paraffin-embedded trephine biopsy samples was used for immunoglobulin heavy chain (IgH) and light chain (IgL) gene rearrangement analysis.

### Results

Immunophenotyping (flow cytometry and immunohistochemistry) upstaged 30 (19.2%) cases to stage IV and a further 8 (5.1%) cases were upstaged using molecular studies. IPI was upgraded in 18 cases (11.5%) on immunophenotyping alone, and 22 (14.1%) cases on immunophenotyping and molecular testing. Comparison of revised IPI models using immunophenotyping alone (rIPI1), and immunophenotyping with molecular studies (rIPI2), with baseline IPI performed using a Cox regression model showed that rIPI1 provides the best differentiation between the IPI categories.

### Conclusions

Improved bone marrow staging improves the predictive value of the IPI.

*No conflict of interest to disclose*

P142

### **Acquired Von Willebrand Disorder in a Multiple Myeloma Patient**

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#### **Background and Aims**

Acquired Von Willebrand's disease is a rare complication of multiple myeloma. We present a case report of a patient with a severe form of this condition requiring surgery.

#### **Patient Details**

A 63 year old male with a previous diagnosis of Multiple Myeloma required semi-urgent cholecystectomy prior to autologous transplantation. A baseline coagulation screen was abnormal with a prolonged aPTT of 45 seconds with a normal INR. Further investigation demonstrated a FVIII Coagulant level of 0.10 IU/ml, a VWF Factor Antigen of 0.11 IU/ml, RiCOF activity of <0.05 IU/ml and Collagen Binding Activity of <0.02 U/ml. At the time he had a IgG kappa serum paraprotein of 6g/l, and a serum free light chains level of 3600 mg/l.

#### **Management**

An initial Biostate half-life study was performed. A discordant rise in VWF:Ag and RcoF levels was observed at 30 minutes to 1.80 IU/mL and 0.66 IU/mL respectively. The half life of VWF:Ag was 6.5 hrs, with the RcoF half-life less than one hour. The patient then underwent plasmapheresis on 3 consecutive days with VWF:Ag and RiCoF activity levels post the third cycle of pheresis rising to 0.38 and 0.33 IU/mL. A combination of pheresis and intravenous IgG (dose 1gm/kg) was then utilised pre-operatively with normal levels of all measures of VWF parameters being achieved pre-operatively and surgery being uncomplicated. Prior to transplantation his VWF Ag and RiCoF activity again fell. A further dose of IV IgG alone was this time given, with prompt normalisation of VWF level and function.

#### **Conclusions**

We describe the successful management of acquired von Willebrand's disease using IV IgG infusion, both alone and in combination with plasmapheresis. Treatment of bleeding complications using clotting factor replacement alone is unlikely to be successful in such patients.

*No conflict of interest to disclose*

**P143**

## **Severe Small Bowel Enteropathy of Unknown Aetiology**

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A previously fit 19 year old female, presented with a three week history of bilateral knee/femur pain and lethargy. Blood count revealed Hb 120g/L, platelets  $241 \times 10^9/L$ , WCC  $15.6 \times 10^9/L$ , neutrophils  $2.8 \times 10^9/L$  and a blast count of 37%. A bone marrow biopsy confirmed acute lymphoblastic lymphoma and remission induction chemotherapy as per ALL VII protocol was commenced. This consisted of standard weekly doses of Vincristine and Daunorubicin for four weeks, a single dose of subcutaneous pegylated Asparaginase, and high dose Prednisone over 4 weeks.

The patient was readmitted on day 17 of remission induction with odynophagia, nausea and mild abdominal pain, and was treated with Fluconazole 200mg daily for oral thrush. Five days later there was a rapid clinical deterioration with development of severe abdominal pain and distension, associated with profound hypotension. Examination revealed a generalised peritonitic abdomen, and an abdominal CT (Fig 1 poster) showed a dramatic pan-wall thickening of the entire small bowel with hyperenhancement. This extended from the duodenum to the terminal ileum. There was less marked enhancement of the colonic mucosa, without wall thickening

Despite aggressive IV rehydration, ICU admission was required for inotropic support and management of a complete paralytic ileus and associated small bowel enteropathy. She was off inotropic support within 48 hours and made a slow recovery over two weeks. A repeat CT, four weeks post event, showed a marked resolution in the bowel wall changes (Fig 2 poster).

The pathophysiology of this patient's small bowel changes remain elusive. The extent of bowel involved crossed multiple vascular territories, and with the superior mesenteric artery being patent on CT, the likelihood of ischaemia was very low. The picture was also not consistent with a neutropenic enterocolitis. At no stage prior to this deterioration was the patient severely neutropenic, she was not febrile at presentation, nor did she have evidence of mucositis elsewhere.

A potential explanation for this presentation is an idiosyncratic drug reaction. Vincristine and Daunorubicin have been used extensively in the past without note of these adverse effects. It is possible that the newer Pegylated Asparaginase is responsible. An extensive literature search showed no reported cases, and discussion with the manufacturers revealed only cases of enterocolitis associated with neutropenia. The patient received further doses of Vincristine and Daunorubicin without incident. She is currently on consolidation therapy with no Asparaginase.

*No conflict of interest to disclose*

**A374**

**P144**

**Haematological Diagnosis in Community Paediatrics – Patterns of Referral; Diagnostic Trends, Traps and Tribulations**

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The spectrum of haematological abnormalities identified on laboratory testing in community paediatric practice differs significantly to that of tertiary referral centers. In the community setting, a higher proportion of samples are the first presentation of a patient, taken earlier in a disease process, with less access to clinical information and further sampling. Identifying those who require follow-up, and/or additional testing is therefore a high priority. We report the diversity of abnormalities encountered at a busy private pathology laboratory servicing urban, rural and remote Queensland.

Over a period of 18 months, samples received in children aged 15 years or less were referred for central review according to strict criteria. After a senior scientist review, selected samples were referred for a consultant opinion. Age, FBC parameters and outcome were recorded for 750 consecutive samples. The majority (86%) represented the first and only sample received during a 6 month period. Approximately equal numbers of samples reviewed were requested by general practitioners (45%) and community paediatricians (42%). A minority were outpatient hospital reviews (8.5%) including haematology/ oncology patients (7%) with a known diagnosis.

More than a third of samples requiring review were from infants <1 yr (33%), including neonates days 0-2 (6%), days 2-28 (7%), infants 1-3 months (10%), and 3-6 months (10%). A wide variety, that included rare diagnoses, was seen in the early neonatal samples (days 0-2), whereas older infants were more commonly assessed for neutropenia, haemolytic anaemia and polycythaemia. An increasing incidence of iron deficiency due to cow's milk enteropathy was noted in recent months. Samples from older patients were dominated by cytopenias (60%), the majority (>70%) receiving consultant review having more than one haematological abnormality. Problems encountered in accurate and prompt diagnosis included sampling, transport and referral issues. Haematological diagnosis in community paediatrics is facilitated by a well-structured approach for central referral and expert scientist review.

*No conflict of interest to disclose*

**P145**

## **Endogenous Thrombin Potential (ETP) for the Evaluation of Anticoagulant Properties of Pseudechis australis (Mulga Snake) Venom**

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### **Aim**

Snake venoms can be activators or inhibitors of the coagulation pathway. Pro-coagulant venoms which cause thrombin generation have been studied using Endogenous Thrombin Potential (ETP). Mulga snake envenomation is common in Australia, with marked prolongation of coagulation assays PT and APTT, normal fibrinogen and no clinical bleeding. The aim was to develop an assay to evaluate venoms which do not generate thrombin and to elucidate the effect of mulga snake venom on haemostasis.

### **Methods**

Thrombin generation was measured using chromogenic substrate on biochemistry analyser Cobas Mira. PT reagent (Innovin), APTT reagent (Actin FSL-low phospholipid and Actin FS-high phospholipid) was used to trigger thrombin generation with subsequent addition of venom to study inhibitory properties. Coagulation studies performed were PT, APTT and Factor assays. Measurement of Factor Xa was performed using a chromogenic substrate.

### **Results**

Mulga snake venom did not generate thrombin. The venom inhibited thrombin generated by Innovin and Actin FSL but not with Actin FS. More prolongation was noted on PT as compared to APTT. Furthermore, factor assays showed marked phospholipid dependent inhibition of Factor VIII and IX. The venom effect was reversible with antivenom and Factor IX on ETP. Lack of Factor Xa generation was also noted.

### **Conclusion**

Modification of ETP using Innovin and Actin to generate thrombin and subsequent addition of venom is likely to be a useful assay in analysing properties of anticoagulant venoms. Mulga snake venom inhibits thrombin generation potentially by inhibiting the interaction of Factor VIII, IX and phospholipid and subsequent generation of thrombin generation.

*No conflict of interest to disclose*

**A376**

P146

## Clinical Consequences and Phenotypic Manifestation of Compound Heterozygous Genetic Haemochromatosis

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Haemochromatosis is an autosomal recessive condition and the *HFE* gene is localized to chromosome 6. The typical haemochromatosis patient carries 2 copies of the C282Y mutation of the *HFE* gene (C282Y homozygote). A minor mutation, H63D, has also been described. Compound heterozygotes (C282Y/H63D) and H63D homozygotes most commonly have normal iron tests, but mild-to-moderate iron overload has been described with these genotypes. Compound heterozygotes for the C282Y and the H63D mutations may have a higher risk of iron overload or genetic haemochromatosis than single heterozygotes for the C282Y mutation.

We describe a study of 82 patients with CHGH with evidence of phenotypic manifestations particularly in men. Table 1 summarises the demographic details and Table 2 shows ferritin, transferrin saturation, liver function, glucose levels.

There were 32 female and 51 male patients; 25 patients had at least one liver function abnormality and 14 had elevated glucose level.

### Conclusion

CHGH is an uncommon genetic abnormality but can contribute to marked hyperferritinaemia and liver function abnormality in some patients, particularly amongst men. Some of these patients require careful evaluation and phlebotomy to normalise phenotypic manifestations of CHGH.

*No conflict of interest to declare*

**P147**

## **Peripheral Blood CD34 Enumeration on Patients with Very Low White Cell Counts Identifies Patients with Early Stem Cell Mobilisation But May Be Unnecessary**

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### **Aim**

To review whether CD34 enumeration on peripheral blood to guide the institution of apheresis for stem cell mobilisation was necessary and/or beneficial prior to recovery of the white cell count to  $1.0 \times 10^9/L$ .

### **Method**

All peripheral blood stem cell collections and transplants between 2005 and 2009 performed at the Princess Alexandra Hospital were reviewed. Our policy is to monitor peripheral blood CD34 counts daily immediately the white cell count begins to rise from the nadir following chemotherapy. We commence apheresis for stem cell collection when the peripheral blood absolute CD34 count is greater than  $10 \times 10^6/L$  (or  $>5 \times 10^6/L$  for patients with delayed count recovery or predicted poor mobilisation). Patients who achieved this threshold when the white blood count (WBC) was  $< 1.0 \times 10^9/L$  were identified. These patients ("early mobilisers") were reviewed in detail to determine if the collection at this early timepoint was necessary to achieve an adequate overall stem cell collection. The subsequent transplant engraftment data for the early mobilisers was compared to the rest of the transplants to identify any differences in short term and long term engraftment.

### **Results**

Between January 2005 and March 2009, 119 patients who achieved successful stem cell collection were analysed. Eleven patients achieved successful stem cell collection when the WBC was less than  $1.0 \times 10^9/L$ , following chemotherapy, on the first day of collection. One patient had a single day of stem cell collection. On all other patients the stem cell yield improved on the second and subsequent days. Nine of the ten patients assessed would have achieved an adequate yield in a single day of stem cell collection ( $>4 \times 10^6/kg$  for myeloma;  $>2 \times 10^6/kg$  for other diseases) had the collection been deferred to the second day. The WBC on first day of collection had no effect on platelet engraftment or neutrophil engraftment, whether analysed as a categorical variable ( $>$  or  $< 1 \times 10^9/L$ ) or as a continuous variable or whether adjusted (using Cox regression) for the number of CD34 positive stem cells infused. The only positive correlation in our data was between the number of CD34 positive cells infused and the time to neutrophil recovery.

### **Conclusion**

These data demonstrate that early stem cell collection is feasible with useful stem cell yields achieved. It suggests, however, that early mobilisers will invariably mobilise well and that delaying CD34 enumeration until the WBC is  $>1.0 \times 10^9/L$  is unlikely to be detrimental to the total stem cell yield. The quality of stem cells collected early, in terms of both neutrophil and platelet engraftment, did not differ from stem cells collected later.

*No conflict of interest to disclose*

**A378**

**P148**

**The Sysmex XE 2100 Optical Platelet Count – Our Experience at the Royal Brisbane and Women's Hospital**

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**Aim**

To review the accuracy and usefulness of the optical platelet count for patients with certain haematological conditions.

**Method**

The optical platelet count is measured on the Sysmex XE 2100 in the reticulocyte channel. A polymethine fluorescent dye stains the RNA/DNA in the platelet membrane and granules. The optical count, impedance count and in some cases an estimate from the blood film, were compared in five patients with the following conditions: immune thrombocytopenia (ITP), thrombotic thrombocytopenia purpura (TTP), cryoglobulinemia, thrombocytopenia secondary to pregnancy and SLE, and a myeloproliferative disorder.

**Result**

The optical count was higher and more accurate compared to the impedance count for the first two cases (ITP and TTP) due to the presence of very large platelets and fragmented red cells respectively. The cryoglobulins present in the third patient, interfered with the impedance count, resulting in a falsely high count. The optical count overcame this interference and gave a much lower and accurate count. There was little difference in the counts for the fourth patient as her platelets, though large, were not outside the size thresholds for the impedance count. The MPD patient had very large, pleomorphic and variably granulated platelets so that the optical count, although higher than the impedance count, still underestimated the total number of platelets.

**Conclusion**

The optical count obtained from the Sysmex analysers is very useful in certain haematological conditions such as ITP or TTP. However it will not accurately count platelets that are partially granulated or agranular.

*No conflict of interest to disclose*



P149

## Microbial Contamination Rate of Haematopoietic Progenitor Cell (HPC) Cord Blood $\leq 0.5\%$ Over a Decade of Cord Blood Banking

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### Aim

To examine by historical data review the microbial contamination rate and organisms identified over a decade of Cord Blood Banking at the Queensland Cord Blood Bank At The Mater (QCBB).

### Method

Manufacturing data for HPC Cord Blood (n= 7,278) for the period 1 July 1999 to 30 June 2009 were examined. Validated methods are used for anti-antisepsis/disinfection of the umbilical cord prior to cord blood collection in a Class II Biohazard Safety Cabinet and for the sampling methods for inoculation of blood culture bottles. Antisepsis/disinfection comprises a 30 second wash with 70% sterile Isopropyl Alcohol and a 30-second scrub with a 20% Povidone-Iodine swabstick with 30-second drying intervals between; timers are used to ensure consistency. Processing involves centrifugation, volume reduction and component fractionation on the Fenwall Optipress II (Baxter HealthCare CA USA) to provide a RBC, plasma, and buffy coat fraction in separate heat-sealed bags. Routine sampling includes an *in-process* sample (3mL RBC inoculum) into both an adult aerobic and anaerobic blood culture bottle and a *post-processing* sample of the final product (0.5mL inoculum) into a paediatric aerobic blood culture and an adult anaerobic bottle.

### Result

The BacTec microbial detection system was in use from 1999 to March 2009 and the BacTAlert<sup>TM</sup>

3D system from April to June 2009. The overall contamination rate was 0.43% (31/7,298) with an annual contamination  $\leq 0.5\%$ . Contaminants identified included vaginal and peri-anal organisms likely to represent contamination at collection or true intra-uterine infection of the baby and skin and environmental contaminants likely to represent contamination by QCBB personnel at collection or during processing or inoculation of the blood culture bottles.

### Conclusion

The use of validated methods for antisepsis/ disinfection for cord blood collection, sampling techniques for culture inoculation and efficient microbial detection systems can virtually eliminate the potential for microbial contamination during manufacture of HPC Cord Blood and avoid the loss of products otherwise suitable for use in allogeneic transplantation.

*No conflict of interest to disclose*

A380

P150

## The Clinical Course and Response to Treatment of P53 Gene Deletion Patients with Multiple Myeloma

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### Aim

We reviewed the cytogenetics and FISH analysis in 120 consecutive myeloma patients who were under the age of 70 years and being considered for auto-transplantation. A full range of FISH probes was utilised and the number of patients with P53 deletions was analysed. We then looked at therapy response to compare with the rest of the group and also to see if novel agents might improve the prognosis.

### Method

Cytogenetic analysis was performed at diagnosis using 24 hour unstimulated cultures. FISH studies were performed on fixed cells from cultures using probes for 11q22 (ATM); P1221 13q deletion, 14q32 (IgH); TP53 and t(14:16). The 6q23 (MYB) and MYC (8p21) were used more recently, as was column purification.

### Result

Data was available on 120 patients (men = 68, women = 52). FISH abnormalities detected in 72% of the cases. The incidence of t(4:14) was 8%, t(11:14) in 7% and P53 deletion detected in 16 or 13%. Five of the P53 patients had a plasma cell leukaemia at presentation (PCL). The median survival of the P53 patients was only 9 months. Three of the patients were treated with Bortezomib after early alkylator failure. One had a limited response and died at 8 months. The two other patients are still alive – both >12 months. One is receiving maintenance Bortezomib post auto.

### Conclusion

The incidence of P53 patients in our cohort is similar or slightly higher than other studies (usually 10%) There was a marked association with PCL and poor median survival. Bortezomib is known to overcome high risk cytogenetics and seems to be useful in this group of patients.

*No conflict of interest to declare*

**P151**

## **Granulocytic Sarcoma: Early Diagnosis and Systemic Therapy Improve Outcome**

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### **Aims**

To raise awareness of the diagnostic difficulties and current recommended treatments in granulocytic sarcoma (GS).

### **Methods**

We present three cases of GS seen at our institution and a brief literature review.

### **Results**

We describe three cases of GS, presenting in the skin and gallbladder, breast and stomach, respectively. The first two cases presented with isolated GS that was treated locally, but evolved nonetheless to AML over months to years. In one case breast GS recurred on three separate occasions after radiotherapy, before evolving to AML. The third patient was initially diagnosed with Chronic Myelomonocytic Leukaemia then found, ten months later, to have gastric GS with concurrent AMML. All patients were treated with standard remission induction chemotherapy for AML; one died of sepsis after achieving second complete remission, the other is still alive and remains in complete remission. The third patient received induction chemotherapy for secondary AML but did not achieve remission, even after further salvage chemotherapy. He is currently receiving palliative care.

### **Discussion**

GS may be diagnosed in isolation or develop from a myeloproliferative neoplasm or myelodysplasia. Isolated GS inevitably develops into AML although the interval varies from weeks to months.

The most common locations of GS are skin, soft tissue, bone, periosteum and lymph nodes. Primary breast involvement is uncommon and involvement of the gallbladder is extremely rare. In cases of primary isolated GS, diagnosis is often difficult and requires a high degree of suspicion. Most cases of GS can be reliably detected and correctly identified by a standard panel of commercially available monoclonal antibodies.

Prognosis of GS is generally poor, with younger age and administration of systemic therapy the only factors found to influence survival. Local therapy only is associated with an extremely high relapse rate and, although often done in the past, is no longer recommended. Current literature supports systemic therapy for all cases, where tolerated, leading to markedly improved outcomes

*No conflict of interest to disclose*

**A382**

**P152****Spontaneous Splenic Rupture in Waldenstrom's Macroglobulinaemia: A Case Report****David Joseph Russell**, Michail Charakidis*Department of Haematology-Oncology, Royal Hobart Hospital, Tasmania***Introduction**

Splenic rupture in Waldenstrom's Macroglobulinaemia (WM) is a previously unreported phenomenon. We report a case of WM complicated by spontaneous splenic rupture

**Case Presentation**

A 49 year old Spanish woman presented after being referred by her general practitioner, with a three week history of malaise, night sweats, 6kg of weight loss, intermittent nausea and vomiting, progressive upper abdominal pain, and easy bruising. The blood film revealed a leukocytosis of predominantly small atypical lymphocytes and plasmacytoid cells. Flow cytometry confirmed this to be a clonal B-cell population. Serum electrophoresis demonstrated markedly elevated IgM protein and immunofixation highlighted a monoclonal IgM kappa band, consistent with a diagnosis of WM. On the fourth day post admission the patient had a rapid clinical deterioration; she was found profoundly hypotensive and complaining of generalised abdominal pain. An ensuing CT scan exposed an extensive haemoperitoneum with active bleeding from a ruptured spleen. An emergency splenectomy was performed with subsequent histology demonstrating widespread splenic parenchymal infiltration consistent with that of WM.

**Conclusion**

Spontaneous splenic rupture is a complication of rapid disease progression, and therefore is not an expected complication of low-grade lymphoplasmacytic lymphomas (LPL's) such as WM. This case highlights that despite the typical disease course of low-grade haematological malignancies, signs and symptoms of imminent splenic rupture should be considered when formulating a clinical assessment.

*No conflict of interest to disclose*

**P153**

## **Imatinib Mesylate Causes Growth plate closure *in vivo***

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### **Aims**

While imatinib therapy is well-tolerated in children, emerging data suggest that long-term therapy may result in side effects that are specific to the paediatric setting. Three recent case studies report decelerated growth in juvenile CML patients undergoing imatinib therapy (1,2,3). However, to date there are no reports suggesting a mechanism for altered growth in imatinib-treated paediatric patients. Here we describe growth plate changes observed in female Sprague-Dawley rats treated with imatinib by daily gavage for up to 12 weeks.

### **Methods**

Rats were administered imatinib (100mg/kg/day) or vehicle by gavage. Animals were then sacrificed for micro-computed tomographic ( $\mu$ -CT) and histological analyses of the proximal tibial growth plate. To determine if imatinib directly inhibits chondrocyte activity, the effects of imatinib on basal and platelet-derived growth factor (PDGF)-induced glycosaminoglycan (GAG) production was examined in the murine pre-chondrocyte cell line ATDC5.

### **Results**

Whilst  $\mu$ -CT revealed no change in trabecular bone volume at the growth plate, histological analysis showed that the width of the growth plate was significantly narrower in imatinib-treated group than in the control group after 4 weeks (115 $\mu$ m v 141 $\mu$ m;  $p < 0.05$ ), 8 weeks (45.1 $\mu$ m v 159 $\mu$ m;  $p < 0.001$ ) and 12 weeks (11.8 $\mu$ m v 147 $\mu$ m;  $p < 0.0001$ ). By 12 weeks, the growth plate had almost completely fused.

In ATDC5 cultures, a decrease in GAG production was observed in imatinib-treated cultures ( $p < 0.05$ ). Treatment with PDGF-BB induced a 3.5-fold increase in GAG levels ( $p < 0.0001$ ), which was partially reversed by addition of 4 $\mu$ M imatinib ( $p < 0.05$ ).

### **Conclusions**

Taken together, these results suggest that inhibition of PDGFR- $\beta$  signalling by imatinib may contribute to the observed decrease in growth plate thickness through the inhibition of chondrocyte activity. The dramatic effect of imatinib on growth plate morphology in rats suggests that growth plate closure should be investigated as a potential mechanism for inhibited growth in pre-pubescent patients receiving imatinib.

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**A384**

P154

## The Major Metabolite of Imatinib: CGP74588 has Kinase Activity, a Long Half Life, and is Actively Transported via OCT-1

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### Aim

In CML patients receiving imatinib (IM), IM trough levels associate with response rates. Pharmacokinetic variability of IM is poorly defined, in particular little is known about the primary metabolite, CGP74588 (CGP). Here we investigate the potency, influx mechanisms, and interaction with IM of CGP.

### Methods

IC50s were determined in cell lines by assessing the in vitro reduction in p-CrkI in response to either CGP or IM. The intracellular uptake and retention of IM (IUR) and OCT-1 activity (OA) was performed using <sup>14</sup>C-labeled drug. Day 22 peak and trough IM and CGP plasma levels were measured in 61 patients enrolled to TIDEL II (600mg IM) using HPLC.

### Results

Day 22 trough levels indicate that the median %CGP relative to IM was 19.7% (Range 7.7 to 39.7%). The % CGP was greater in trough than peak measurement, and the fold change between peak and trough was less for CGP (IM 0.52, CGP 0.7), suggesting a prolonged half-life when compared to IM. IC50 analysis in K562 cells revealed CGP had similar potency to IM (IC50<sup>imatinib</sup> 5.64±0.85 µM, IC50<sup>CGP74588</sup> 6.35±0.70 µM (n=7)). Furthermore IUR analysis with either 2µM of IM or CGP revealed CGP had a significantly higher IUR than IM in K562 cells, (33.6±5.2 (n=19) and 25±4.7 ng/200,000 and n=26 cells respectively (p<0.0001)). Inhibitor studies demonstrated that like IM, CGP is a substrate for OCT-1 (OA IM=10.3±2.5 and CGP74588=17.1±7.8ng/200,000 cells (n=10)), and ABCB1. In combination CGP74588 increased intracellular imatinib by 26.92%, however the reverse was not true (n=5). In preliminary regression analysis the levels of IM, CGP and the %CGP appear to factor significantly in 3 month molecular response p=0.029, p=0.033 and p=0.007.

### Conclusion

These studies indicate that CGP has similar kinase activity to IM, a prolonged half-life and good target cell penetration mediated by OCT-1. While the relative % of CGP is only approximately 20%, the potency of this metabolite suggests that it may contribute to outcome. The maturing TIDEL II dataset should help elucidate this further.

*This research was supported by Novartis Pharmaceuticals. The company had no role in analysing the data or preparing the abstract.*

**P155**

## **Intravenous Busulfan and Fludarabine Appears To Be A Safe and Efficacious Conditioning Regimen for Allogeneic Transplantation In Older Patients With High Risk AML**

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### **Aim**

Allogeneic stem cell transplant (Allo-SCT) is a potential curative treatment in acute myeloid leukaemia (AML). The toxicity of myeloablative conditioning restricts its use to younger patients. Non-myeloablative regimens offer a transplantation option to older patients. However, the optimal regimen has yet to be defined. We report here our experience with IV busulphan and fludarabine (Bu/Flu) conditioning in older patients with high risk AML.

### **Method**

Nine AML patients have undergone peripheral blood Allo-SCT with iv Bu/Flu. Median age was 56 years (range 40-62 years). Six patients were in CR1 and one patient had refractory AML. Adverse risk cytogenetics were present in 8 patients. Six and three patients received matched sibling and unrelated donor transplants, respectively. Flu 40mg/m<sup>2</sup> and Bu 130mg/m<sup>2</sup> were administered from D-5 to D-2. GVHD prophylaxis was Cyclosporin and Methotrexate, with addition of ATG in unrelated donor transplants. Standard antifungal, anti HSV, PCP, CMV and VOD prophylaxis were given.

### **Results**

Conditioning was well tolerated with no infusional or CNS toxicity observed. Four and three (one had GI perforation) patients had grade III and grade IV mucositis, respectively. In this limited study there was no evidence of VOD after IV Bu, however, one had self limiting cholecystitis. All patients developed febrile neutropenia. Median time to neutrophil and platelet engraftment were 19 days (range 12–26days) and 13 days (range 8–21days), respectively. Acute skin GVHD occurred in 4 patients progressing to chronic GVHD in two. One patient developed chronic hepatic and GI GVHD. With a median follow-up of 315 days, 5 patients remain in CR. Four patients have relapsed with 2 relapses occurring before day 100.

### **Conclusion**

In this small cohort of patients IV Bu/Flu was well tolerated with no VOD and manageable toxicity. Preliminary results are encouraging in this older population of patients with high risk AML.

*No conflict of interest to disclose*

**A386**

P156

## Angiogenesis in Breast Cancer: Is there Potential Prognostic Value in Assessment of Tissue Factor?

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### Background

There is accumulating evidence that TF plays an important role in cancer biology including cancer progression, angiogenesis and metastasis, and may also have clinically useful prognostic significance. Targeting TF, or TF initiated pathways may offer new therapeutic approaches in cancer treatment.

### Aim

To perform a preliminary study of the presence of TF and VEGF in invasive ductal carcinoma of the breast as assessed by immunohistochemistry. Normal breast tissue and fibroadenoma were used as control tissues.

### Method

The study material included paraffin embedded tissue sections from 150 representative breast cancer, 20 fibroadenoma and 10 specimens of normal breast tissue from reduction mammoplasties. Immunohistochemical sections stained for TF and VEGF were examined and graded with a 3 point scale.

### Results

TF staining was scored in the cytoplasm and the nucleus of both stromal and tumour cells. No clear difference was evident in the total TF score between the different disease grades. However a Kruskal-Wallis test including only the cytoplasmic level of TF in the tumour cells of different grades and normal tissue showed that there was a trend towards a difference between these groups, that did not quite reach significance ( $p=0.078$ ).

### Conclusion

While this is consistent with previous reports, further work particularly including a larger number of normal tissue samples would be needed to establish a clearer result concerning the significance of cytoplasmic TF staining in breast tumour cells and its potential prognostic value.

*No conflict of interest to disclose*



P157

## The Role of FISH to Identify *MYC* and *BCL2* Rearrangements in High Grade Non-Hodgkin Lymphoma

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### Aim

Double hit lymphoma is a rare type of aggressive non-Hodgkin Lymphoma demonstrating genetic rearrangements of both the *MYC* and *BCL2* oncogenes. Fluorescence in situ hybridisation (FISH) has been used to identify these genetic aberrations in samples from a single centre and then the clinical and laboratory features of the disease characterised.

### Method

Rearrangements incorporating the *MYC* and *BCL2* genes were identified in lymphoma samples by FISH using Vysis break apart probes on paraffin embedded tissue sections or fixed cultured cell suspensions.

### Result

Double hit rearrangements were detected in 12 patients (7 male, 5 female, age 34-89) by FISH analysis. All cases demonstrated a diffuse infiltration of intermediate to large malignant B cells that were CD10, CD20 and Bcl2 positive on tissue staining. Ki67 staining was variable between cases with a mean value of 75% (range 40-100%). Eleven cases presented *de novo* with genetic double hits present in the first tissue biopsy whereas in 2 cases the genetic abnormality was observed at the time of disease transformation in patients with known follicular lymphoma. Karyotype was available in 8 of the 12 cases and confirmed the presence of the *MYC* and *BCL2* rearrangements. Karyotypes were highly complex with a median of 7 aberrations per karyotype (range 3-22). Patients were treated with a variety of intensive lymphoma chemotherapy protocols with 6 patients achieving a complete response. Overall prognosis was very poor with the only long term survivors managed with allogeneic bone marrow transplantation.

### Conclusion

FISH facilitated identification of the rare double hit lymphoma in 12 cases during the laboratory investigation of aggressive B cell lymphoma. This has important prognostic implications and should initiate consideration of early aggressive therapy as the prognosis of this type of lymphoma is very poor.

*No conflict of interest to declare*

P159

## Two CML Secondary Resistance Mechanisms Have Been Recapitulated *in vitro* in the KU812 Cell Line, after Long-Term, Gradually-Increasing Exposure to Imatinib Mesylate

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<sup>1</sup> Haematology, SA Pathology, IMVS; <sup>2</sup> University of Adelaide; <sup>3</sup> University of South Australia; Adelaide, South Australia, Australia

### Introduction

The treatment of chronic myeloid leukemia (CML) has been revolutionised in recent years with the introduction of imatinib mesylate (IM). Despite excellent overall responses many patients develop resistance to IM therapy. Postulated mechanisms of secondary resistance include increased BCR-ABL expression, mutations in the kinase domain of BCR-ABL, increased expression of drug-efflux proteins (such as ABCB1 and ABCG2) or other mutations that render the cell independent of BCR-ABL activity.

### Method

Cultures of the KU812 cell line were initiated in 0.1µM imatinib, and this concentration was increased every ~10 days by 0.1µM. After 160 days these cells were insensitive to 2µM imatinib. A variety of experiments were used to determine the mechanisms of resistance in this cell line, including IC<sub>50</sub><sup>imatinib</sup>, the intracellular uptake and retention (IUR) of imatinib, Annexin V staining, and kinase domain sequencing.

### Results

Three mutations were present in the kinase domain of the imatinib-resistant cell-line (KU812-R), namely E450Q (70%), E459 (80%) and E470K (60%). Furthermore, BCR-ABL transcript number (with respect to the BCR control gene) was increased from 384% in KU812 cells to 2662% in the KU812-R line. Quantitative DNA-PCR results show an increase in BCR-ABL copy number from 493% in KU812 cells to 4018% in the KU812-R line, thus confirming that amplification of BCR-ABL has occurred.

The IC<sub>50</sub><sup>imatinib</sup> in KU812 cells is 6.1µM, but has increased to 52µM in KU812-R cells, confirming resistance in these cells is mediated by a BCR-ABL-dependant mechanism. Interestingly, the KU812-R cells also exhibit resistance to nilotinib, with the IC<sub>50</sub><sup>nilotinib</sup> increasing from 84.5nM to 3500nM.

### Conclusion

Our results suggest that imatinib resistance in the KU812-R cell line is mediated by kinase domain mutations, and/or increased BCR-ABL copy number (Philadelphia chromosome duplication), but the dynamics of these events are unclear. Notably, this study demonstrates resistance by these means can be recapitulated in-vitro by steadily increasing IM concentrations.

*No conflict of interest to disclose*

**P161**

## **LIMMIT® Tablets as a Cause of Severe Lead Poisoning Manifesting as Severe Anaemia**

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We report a case of a 46 year-old Indian lady who presented with intermittent epigastric pain, fatigue and exertional dyspnoea. She has a history of type 2 diabetes mellitus and a previous history of menorrhagia. She had substituted her prescribed metformin for Ayurvedic medications (LIMMIT) obtained from India over the preceding year.

Peripheral blood morphology showed a severe microcytic hypochromic anaemia, with coarse basophilic stippling. Her whole blood lead level was markedly elevated (5.05µmol/L, normal < 1.2µmol/L).

With cessation of her Ayurvedic medications, her anaemia improved. A follow-up blood film 2 months later showed resolution of the basophilic stippling, along with declining lead levels (3.00µmol/L). An analysis of her Ayurvedic medications using Inductively Coupled Plasma Atomic Emission Spectrometry showed extremely high lead content.

Ayurvedic medicine, the traditional Hindu system of medicine, emphasises balance in bodily systems, and utilises dietary, herbal and yogic breathing to achieve that balance. Cases of lead poisoning resulting from ingestion of Ayurvedic medications have been reported in the literature. The haematological, nervous and renal systems are commonly affected. Assessment of suspected lead poisoning should include examination of the blood film, whole blood lead levels, and erythrocyte protoporphyrin levels. Basophilic stippling is an indication of severe lead poisoning, and was previously used to assess the degree of lead poisoning. However, imaging studies e.g. X-ray fluorescence may give a better indication of total body lead content. Treatment of lead poisoning includes identifying and isolating the source of exposure from the patient, and considering chelation therapy in appropriate situations.

Traditional medicines authorised for supply in Australia are required by the TGA to meet set standards of manufacturing and quality that aim to ensure that medicines do not contain unsafe levels of heavy metals. This case highlights the importance of adequate policing of alternative medications available for consumption.

*No conflict of interest to disclose*

**A390**

P162

**The Australian Cancer after Stem Cell Transplantation (CAST) Study: Examining the Incidence and Risk of Second Cancer in Recipients of Haematopoietic Stem Cell Transplantation**

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**Background**

Haematopoietic stem cell transplantation (HSCT) provides a favourable treatment outcome for many adult and childhood cancers as well as inherited and constitutional marrow failure syndromes, immunodeficiencies and metabolic diseases. However, the development of subsequent malignancies is now recognised as one of the most serious late complications of HSCT. Previous investigations of cancer incidence after HSCT have been limited by varying selection and evaluation criteria, small sample size or short follow-up. This population-based cohort study will provide unbiased estimates of the incidence, risk factors and survival of malignant neoplasms following HSCT in Australia.

**Aims**

1) To determine the overall and site-specific incidence rates of malignant neoplasms occurring in recipients of HSCT. 2) To evaluate the risk factors and prognosis for individuals diagnosed with a malignant neoplasm following HSCT.

**Method**

More than 14,000 patients treated between 1992 and 2008 will be included in our analyses. Incident cases of malignant neoplasms will be identified through data linkage between the Australasian Bone Marrow Transplant Recipient Registry, the National Death Index and the National Cancer Statistics Clearing House allowing calculation of standardised incidence ratios, risk factor analyses and survival analyses for specific cancer types.

**Summary**

The study presents a unique opportunity to provide population-based estimates of the incidence, risk factors and survival for malignant neoplasms following HSCT. Knowledge of the range of cancers, and the magnitude of the increased risks, is important in developing appropriate health-care and follow-up for HSCT recipients.

*No conflict of interest to disclose*

P163

## Bortezomib plus Melphalan-conditioned Autologous Transplantation in Patients With Multiply-Relapsed Multiple Myeloma

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High-dose Melphalan (HDM) and autologous haematopoietic stem cell transplant (ASCT) is an option for multiply-relapsed multiple myeloma patients. Bortezomib (Bz) and Melphalan act synergistically. The addition of bortezomib to HDM is safe in up-front HSCT (Lonial *et al* 2008).

We conducted a retrospective review of nine HDM+Bz-conditioned ASCT in eight heavily pre-treated patients, median age 56.5yrs (49-61), median lines of therapy 3.5 (1-5), including prior ASCT (n=6), from 16/1/2008-3/6/2009, at Peter MacCallum Cancer Centre (PMCC). We examined whether bortezomib could be safely added to a HDM regimen (140-200 mg/m<sup>2</sup>), particularly evaluating platelet and/or neutrophil recovery. Six patients received bortezomib 1.3–1.6mg/m<sup>2</sup> pre-stem cell infusion (day -12 to -1), 1-3 doses. Median number of CD34s infused was 5.84x10<sup>6</sup>/Kg (2.37-16.24). Two patients also received bortezomib 1.3mg/m<sup>2</sup>, 1-2 doses post-stem cell infusion (Day +1 to +4).

Median time to neutrophil and platelet recovery was 10 (9-11) and 12 days (8-12), respectively. Median number of platelet and red cell transfusions was 1 and 0 respectively. There were no apparent differences in platelet recovery times or transfusion requirements in the two patients who received post-transplant bortezomib. In second ASCTs, bortezomib/HDM did not affect platelet recovery times or the number of red cell/platelet units transfused, however neutrophil recovery was delayed one day and median length of hospitalisation increased by two days compared to the first ASCT. No unexpected non-haematological toxicities were seen. Compared to HDM-ASCT at PMCC 2001-2008 (n= 257), there were no apparent differences in neutrophil recovery time or other toxicities. Platelet recovery was delayed 2 days. Seven patients were evaluable at Day 100 with no deaths, an ORR 100%, 4 PRs and 1 VGPR.

Our results suggest that the addition of bortezomib to HDM pre- and post-ASCT for myeloma is safe and merits further study to determine the optimal schedule of administration and efficacy.

*No conflict of interest to disclose*

A392

P164

## Cytogenetic Work-up in a Centenarian with Limited Clinical History

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### Aim

We present a case report of a patient with Mantle Cell Lymphoma, highlighting the use of peripheral blood cultured with a B-cell stimulating mitogen (TPA) as a method of detecting chromosome abnormalities from B-cell lymphoproliferative disorders when bone marrow is not successful or is unavailable for cytogenetic analysis.

### Method

The bone marrow biopsy of a 102 year old male was submitted for examination for Haematology, Cytogenetics and Cell Surface Markers. Limited clinical history was available at that time.

### Results

Examination of the aspirate and trephine found a low grade B- cell lymphoproliferative disease, best fitting either an atypical CLL or Mantle Cell Lymphoma. Due to the low cell numbers in the aspirate, no cell divisions were available for conventional cytogenetics. Molecular (FISH) analysis of interphase nuclei detected the CCND1/IGH gene fusions seen in t(11;14). This translocation is a common abnormality seen in Mantle Cell Lymphoma. After consultation with the patient's physician a heparinised peripheral blood sample was submitted for further cytogenetic studies. The white cell "buffy" coat was cultured for 5 days with the B-cell stimulating mitogen TPA. Conventional cytogenetic studies found major structural and numerical abnormalities in two related clones. These results would suggest the t(11;14) is the primary cytogenetic lesion with clonal progression into two new cell lines containing additional cytogenetic changes. This finding would fit a Mantle Cell Lymphoma with evidence of clonal evolution.

### Conclusion

Culture of peripheral blood for cytogenetic analysis is performed for constitutional analysis and for patients as a screen mostly for myeloid neoplasms such as CML. With the use of peripheral blood stimulated by the B-cell mitogen TPA we were able to provide, in this case, a clearer picture of the disease than was available from the bone marrow aspirate. TPA could have been used with the bone marrow aspirate but at the time of culture no indication of B cell involvement was available, and marrow from the centenarian was limited in volume and quality. In suboptimal conditions, where a B-cell LPD is suspected, TPA stimulated peripheral blood may be an alternative option to bone marrow biopsy.

*No conflict of interest to disclose*

**P165**

## **T cell Malignancies.....The Fremantle Hospital Experience**

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The goal is to assess clinical presentation and concomitant diagnosis, demographic characteristics, flow cytometry, TCR gene rearrangement, histology findings, treatment and survival.

T cell malignancies are relatively uncommon and are particularly difficult to identify as the T cell antigen receptor lacks a surrogate marker for clonality. Immunophenotypic and molecular studies have become important tools in the identification of these haematopoietic neoplasms. Consequently abnormal T cell populations are identified with flow cytometry by the expression of aberrant T and NK cell antigens. These findings may now be verified with the demonstration of T cell clonality using TCR V $\beta$  flow cytometry or molecular genetic assessment of TCR gene rearrangements. However, reactive T cells may also express aberrant markers and chronic stimulation may result in false-positive clonal TCR rearrangement.

PathWest Laboratory Medicine, at Fremantle Hospital, services Fremantle Hospital, Kaleeya Hospital and several small laboratories in the Fremantle area. Since 2002 we have identified 50 patients with abnormal T cell flow cytometry and T cell gene rearrangement studies. There were also 5 patients with abnormal flow cytometry and TCR rearrangement with a minor population at the limits of detection. These cases were retested 3 months after the initial presentation and were found to be TCR positive.

This study will result in a better understanding of the clinical features, laboratory findings and outcomes in patients with aberrant T cell populations.

*No conflict of interest to disclose*

P166

## Failure to Achieve a Threshold Dose of CD34<sup>+</sup>CD110<sup>+</sup> Progenitor Cells in the Graft Predicts Delayed Platelet Engraftment after Autologous Stem Cell Transplantation for Multiple Myeloma

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### Aim

To predict delayed platelet engraftment more accurately post autologous stem cell transplantation using a relatively homogeneous population of myeloma patients.

### Methods

We retrospectively analysed the CD34<sup>+</sup>CD110<sup>+</sup> (CD110 or c-mpl, thrombopoietin receptor) content in the grafts of 70 patients undergoing transplantation for multiple myeloma with an in-house flow cytometric assay.

### Results

Infusing at least  $3.0 \times 10^4$  CD34<sup>+</sup>CD110<sup>+</sup> cells/kg separated the cohort into those who achieved platelet engraftment before or after 21 days. This early megakaryocyte cell marker correlated more closely with early versus delayed platelet engraftment than CD34<sup>+</sup> measurements. Of the 70 patients, 4 required  $\geq 21$  days to achieve platelet transfusion independence. Three of the four received a CD34<sup>+</sup>CD110<sup>+</sup> cell dose of  $< 3.0 \times 10^4$  cells/kg while 66 of 70 patients who received  $> 3.0 \times 10^4$  CD34<sup>+</sup>CD110<sup>+</sup> cells/kg achieved platelet transfusion independence in  $< 21$  days ( $P < 0.001$ ). Infusing  $> 3.0 \times 10^4$  CD34<sup>+</sup>CD110<sup>+</sup> cells/kg was sensitive (100%) and specific (75%) for achieving platelet engraftment within 21 days. Patients with delayed platelet engraftment received a median of  $2.28 \times 10^4$  CD34<sup>+</sup>CD110<sup>+</sup> cells/kg versus  $12.1 \times 10^4$  cells/kg in those without this complication ( $P = 0.033$ ). No effect was seen with neutrophil engraftment. Patients with early engraftment required a median of 1 platelet transfusion post transplant versus 2.5 in those with late engraftment ( $P = 0.009$ ).

### Conclusion

A subthreshold absolute CD34<sup>+</sup>CD110<sup>+</sup> cell dose in the graft is a reliable predictor of delayed platelet engraftment and could be used to guide the timing and number of peripheral blood stem cell collections for patients with multiple myeloma undergoing a stem cell transplant.

*No conflict of interest to disclose*



**P167**

## **An Unusual Cause of Anaemia in an Infant**

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### **Case Report**

A 7 month-old infant presented with a severe macrocytic anaemia, mild neutropenia and thrombocytopenia, and lactic acidosis. The haemoglobin F was raised and the bone marrow was moderately hypocellular with prominent vacuolation of both myeloid and erythroid precursors, dyserythropoiesis and increased ring sideroblasts. Cytogenetic analysis of bone marrow metaphases was normal. Quantitative PCR analysis of mitochondrial DNA demonstrated a characteristic deletion. The patient was diagnosed with Pearson's Syndrome, a rare congenital multi-system disorder characterised by refractory sideroblastic anaemia, pancreas, liver and kidney dysfunction.

### **Pathophysiology**

Pearson's syndrome is a generalized mitochondrial disorder that occurs in infancy due to a deletion or duplication of mitochondrial DNA and is characterized by hypoplastic macrocytic anaemia alone or associated with thrombocytopenia, granulocytopenia, lactic acidosis, exocrine or endocrine pancreatic insufficiency, proximal tubular renal insufficiency, hyperlipidaemia with liver steatosis or skin lesions. The child usually demonstrates failure to thrive.

### **Treatment and Prognosis**

Therapy is largely supportive, however, allogeneic bone marrow transplantation has been attempted with variable success. The course is usually fatal in early childhood.

### **Conclusion**

Pearson's syndrome should be suspected in any infant with bone marrow dysfunction, metabolic acidosis and characteristic bone marrow morphology. Mitochondrial DNA testing should be performed to assess for causative deletions. A multidisciplinary team approach should be instituted with sufficient psychological and social support for family members.

*No conflict of interest to disclose*

P168

## A Comparison of RQ-PCR and DNA-PCR for the Detection of BCR-ABL in Sorted Populations of Primitive Haematopoietic Cells in Patients with Chronic Myeloid Leukaemia (CML)

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### Aims

BCR-ABL levels in CML patients are conventionally monitored using Reverse Transcriptase quantitative PCR (RQ-PCR). However, RNA quality can be problematic with small sample sizes, resulting in unreliable RQ-PCR's. DNA-PCR (DQ-PCR) for BCR-ABL utilises patient specific primers, tailored to an individual's exact BCR-ABL breakpoint. DQ-PCR requires the identification of the breakpoint in every patient, thus being more complex initially. However, high quality DNA can be isolated from small cell numbers, making sensitive detection possible. Here we assess and compare BCR-ABL levels in corresponding DNA and RNA from MNC and CD34+.

### Methods

Cryopreserved MNC's were thawed, with selected subpopulations isolated by FACS. RNA and DNA extractions were then performed. RQ-PCR was performed using BCR as the control gene, with DQ-PCR using GUSB.

### Results

Preliminary experiments established that in 100 cells, the amount of DNA extracted is 2-fold higher than RNA and of superior quality. The DNA breakpoint has been successfully identified in 7 patients to date using Long PCR and sequencing, demonstrating the applicability of this approach. In total, DQ and RQ-PCR have been compared in MNC and CD34+ cells of 7 patients. In 5 of 7 MNC samples the % of BCR-ABL (relative to control gene) detected by DQ-PCR was higher than RQ-PCR (average increase of 3.2-fold). Comparing the level of BCR-ABL transcript in MNC vs. CD34+ cells demonstrated that 4/6 patients had a higher level in CD34+ cells than MNC (average 1.0-fold increase by RQ-PCR). The same increase was not observed in DNA suggesting, as expected, that the rise in BCR-ABL observed and previously reported in CD34+ cells is transcriptionally related.

### Conclusions

DQ-PCR is a viable approach for looking at BCR-ABL gene copy number and is particularly applicable to small sample sizes. In this continuing study, DQ-PCR appears more sensitive than RQ-PCR and thus may be readily applicable to the detection of MRD. These results are ongoing.

*No conflict of interest to disclose*

**P169**

## **Addition of Aprepitant to Standard Anti-Emetic Therapy in Patients Undergoing High Dose Chemotherapy and Stem Cell Transplantation Does Not Affect Engraftment and Survival**

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### **Aim**

The neurokinin-1 antagonist Aprepitant has demonstrated efficacy in reducing nausea and emesis with highly emetogenic chemotherapy. Aprepitant is an inhibitor of CYP3A4 and therefore may interact with drugs used for high dose conditioning chemotherapy. This study was performed to determine whether use of Aprepitant affects engraftment and survival in patients undergoing high dose conditioning chemotherapy and stem cell transplantation.

### **Methods**

The study population consisted of patients undergoing high dose chemotherapy and stem cell transplantation at Liverpool Hospital from January 1995 to December 2008. Pharmacy records were used to identify patients who received Aprepitant in addition to standard anti-emetic therapy commencing day 1 of the conditioning therapy and continuing until 48 hours post chemotherapy. The Aprepitant group was compared to the non-Aprepitant group with respect to age, sex, diagnosis, conditioning regimen, time to neutrophil engraftment, red cell transfusions, platelet transfusions, length of stay and overall survival using Stata 10 software.

### **Results**

Two hundred and seventy patients were treated with high dose chemotherapy and predominantly autologous stem cell transplantation (2 syngeneic) for mainly haematological malignancy (94%) of which 32 patients received Aprepitant. The 2 groups were similar with respect to median age and sex distribution however the Aprepitant group had proportionally more patients with multiple myeloma (47%) and Melphalan conditioning chemotherapy (50%) versus 35% multiple myeloma and 37% Melphalan conditioning in the non-Aprepitant group ( $p=0.1$ ) and less patients with non-Hodgkin's lymphoma (38%) and BEAM conditioning chemotherapy (41%) in the Aprepitant group versus 43% non-Hodgkin's lymphoma and 47% BEAM conditioning chemotherapy in the non-Aprepitant group ( $p=0.3$ ). There was no significant difference in time to neutrophil engraftment ( $p=0.83$ ), red cell transfusions ( $p=0.34$ ), platelet transfusions ( $p=0.25$ ), length of stay ( $p=0.78$ ) and overall survival ( $p=0.60$ ).

### **Conclusion**

Use of Aprepitant anti-emetic therapy does not affect outcome in patients undergoing high dose conditioning chemotherapy and stem cell transplantation.

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**A398**

P170

**AML Molecular Mutation Screening in a Routine Haematology Laboratory**

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**Aim**

With the identification of mutations in cytogenetically normal AML patients that infer either poor or favourable prognosis, there is a need for screening tests able to be performed in the routine laboratory. We investigated alternative methods to the labour intensive sequencing method for detection of FLT3 and NPM1 mutations in order to establish a robust and less labour intensive method.

**Methods**

Seventy-five cytogenetically normal AML and MDS patient samples of bone marrow, peripheral blood or paraffin embedded tissue (PET) were analysed for FLT3 ITD, FLT3 D835 and NPM1 mutations. DNA from blood and bone marrow was purified using the Promega Maxwell Platform. DNA from PET tissue was extracted using commercial kits from Qiagen. After PCR amplification, DNA was analysed for FLT3 mutations by 2 methods: polyacrylamide gel electrophoresis (PAGE) and/or capillary electrophoresis on the Beckman Coulter CEQ8800 Genetic Analyser using the fragment analysis software. DNA for NPM1 mutations was analysed by capillary electrophoresis.

**Results**

Twenty-two samples were tested by both methods for FLT3 ITD and 18 samples were analysed for FLT3 D835 mutations by both methods and gave 100% concordance. NPM1 mutations were clearly detected by CEQ analysis.

Mutation Type	PAGE number tested (positive)	CEQ number tested (positive)	Both methods number tested (positive)
FLT3 ITD	66 (11)	40 (10)	22 (10)
FLT3 D835	66 (7)	31 (5)	18 (4)
NPM1	-	18 (4)	-

Both of these methods were less operator dependent and time consuming compared with sequencing. However, capillary electrophoresis is preferred for FLT3 ITD detection as PAGE may fail to detect ITD of <20 base pairs.

**Conclusion**

PAGE and capillary electrophoresis have been demonstrated as robust, less time consuming methods to screen for FLT3 ITD and D835 and NPM1 mutations and are more widely applicable to routine laboratories. It is planned to compare our NPM1 mutation results with those obtained by real time PCR followed by melt curve analysis.

*No conflict of interest to disclose*

**P171**

## **Unusual Cases of Haemolytic Anaemia in Children**

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Haemolytic anaemia in children is a relatively rare occurrence, but there can be a wide variety of causes. The disorder can be classified by determining whether the shortened erythrocyte survival is due to an intrinsic abnormality of the red cell or an extrinsic factor acting on the normal red cell.

Six cases of haemolytic anaemia are presented here – five are acquired and one is an inherent component of an uncommon syndrome.

Four cases of immune mediated haemolysis, a rare but recognised complication of transplantation, are described. Two cases (a brother and sister) occurred following a haematopoietic stem cell transplant for the metabolic disorder, Krabbe disease. They both developed severe cold agglutinin disease but behaved very differently in response to therapy. One case of warm AIHA following a bone marrow transplant for aplastic anaemia is also presented. The final case of transplant associated immune haemolytic anaemia occurred in the context of a liver transplant with severe anti-A mediated haemolysis following transplantation of a liver from a group O donor.

The last case of acquired haemolytic anaemia was due to undiagnosed Wilson's disease.

The sixth case is a boy with Evans syndrome. AIHA and immune thrombocytopenia (ITP) occur concurrently, with an immune neutropenia also occurring in some children. Recent evidence suggests that autoimmune lymphoproliferative syndrome (ALPS) may be the underlying cause in a substantial proportion of these cases.

The causes and mechanisms of the haemolysis will be discussed along with the treatment. The haemolysis has resolved in all six cases, some within a matter of days, but in one of the cases it took many months.

*No conflict of interest to disclose*

P172

**Clinical Features, Prognosis, And Outcome Of 37 Patients With Cardiac Amyloidosis**

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**Background**

Cardiac amyloidosis is a relatively rare, but significant cause of heart failure, in that it often heralds a rapid clinical decline with a paucity of successful therapeutic options currently available.

**Aim**

To complete a retrospective audit of patients who presented to St Vincent's Hospital for endomyocardial biopsy, and were discovered to have cardiac amyloid deposition. Then to discuss the baseline demographics, medical history, presenting symptoms, amyloid subtype, investigation results, treatment, and outcomes of these patients.

**Method**

Thirty-seven patients were identified over the period 1992-2009 from anatomical pathology records. The case records were reviewed for reason for referral, demographic information, clinical features, co-morbidities, pathology, imaging, treatment, and outcomes. Following consultation with the Birth, Deaths, and Marriages Registry, a letter was sent to those patients who were still alive, and to the immediate family of those patients who had passed away, informing them of our intent to follow with a telephone survey regarding symptoms, clinical features, and family history of the patient.

**Results**

The patients ranged in age from 41-84 years, with a median age of 62 years, and the majority of patients were male (62.1%). The majority of patients had presumed AL type amyloid with evidence of a clonal plasma cell population, while only two of thirteen patients tested, showed immunohistochemical evidence of AA amyloid. Six patients who presented after 2005, had serum free light chain ratios performed, with four having abnormal kappa/lambda ratios. A history of carpal tunnel syndrome, and gastro-esophageal complaints, predating diagnosis was common. In more recent years, treatment regimes for AL amyloidosis most commonly included melphalan, and prednisolone. Further information is to follow when survey data is correlated with clinical records.

**Conclusion**

Amyloidosis is an important disease requiring further investigation to provide better treatment outcomes. Increased understanding of the clinical features, and co-morbidities associated with amyloidosis may offer an opportunity for earlier diagnosis. The advent of the serum free light chain ratio may also improve the sensitivity of diagnosis in AL amyloidosis, and earlier diagnosis may provide more opportunities for trials of novel therapies.

*No conflict of interest to disclose*

**P173**

## **A Single Centre Review Investigating Whether the Transport Times of MUD Stem Cells Influences Engraftment Times Post HSCT**

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### **Aim**

To analyse the impact that travel times and the wait time before infusion has on neutrophil and platelet engraftment and overall mortality in recipients of unrelated donor stem cells.

### **Background**

Approximately a third of all Allogeneic stem cell transplants performed now use unrelated donors. Usually donors have stem cells collected at centres distant from the transplant centre. Registry data indicates that the length of travel time and the wait time before infusion does not affect neutrophil engraftment time. A higher overall mortality was associated with bone marrow grafts which had a combined travel and wait time of  $\geq 26$  hours [1].

### **Method**

A retrospective chart review was undertaken at Wellington Hospital looking at consecutive MUD transplants between March 2004 and June 2009. Data were collected on the disease, donor location, stem cell source, travel times, wait time at the transplant centre before infusion, neutrophil and platelet engraftment; and early (prior to Day +100) and overall mortality.

### **Result**

There were 26 consecutive MUD transplants performed during the study period. A single cord blood transplant was excluded from analysis. 52% donor stem cells had travel times to the transplant centre of  $\geq 20$  hours. The time between the arrival of stem cells at the transplant centre to infusion into the patient ranged from 1 – 18 hours. Extended travel and wait times did not appear to impact on neutrophil or platelet recovery (median time to neutrophil engraftment = 16 days, platelet engraftment = 20 days) or mortality.

### **Conclusion**

The majority of collections for our centre were from Germany. In all cases, combined travel and wait times before infusions were greater than 30 hours. With the caveat of small numbers, there appeared to be no difference in neutrophil and platelet engraftment times or to early or overall mortality. In some cases there were considerable delays between stem cells arriving at the transplant centre and infusion into the patient. Our remote location and the logistics related to flight arrival times and TBI timing make this unavoidable.

### **Reference**

Lazarus, H. M. et al (2009): Rapid transport and infusion of hematopoietic cells is associated with improved outcome after myeloablative therapy and unrelated donor transplant. *Biology of Blood and Marrow Transplantation*, 15 (5), 589 – 596.

*No conflict of interest to disclose*

**A402**

P174

## Slow Uninterrupted Chemotherapy for Myelodysplasia/AML in Older Patients with Co-morbidities (SUMO)

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### Aim

Improvements in outcomes for Myelodysplasia and Acute Myeloid Leukaemia in elderly patients with decreased performance status lags behind that for other age groups with higher rates of toxic deaths and mortality from relapses. This study aims to define the role of reduced intensity chemotherapy in the treatment of AML and RAEB in this patient group, in a protocol derived from the one originally published by Kanemura et al.

### Method

The design is a single centre, open label prospective study. Patients over the age of 65 who had significant co-morbidities or with ECOG  $\geq 2$  having been diagnosed with AML or RAEB were enrolled into the study. Induction treatment begins with a 7 day continuous infusion of cytarabine (20mg/person/day) and etoposide (50mg/person/day) with concurrent G-CSF. Response was assessed haematologically after 7 days and treatment was continued for a further 7 days if clearing of marrow blasts was not achieved. Consolidation was given to those who attained CR, either with the same regiment or using 5:2.

### Results

6 patients were treated between November 2007 and July 2009. Mean age was 73.5yrs (70-77). 4 patients had 1 or more major co morbidities, including cardiac failure, renal impairment and prior history of cancer. 5/6 patients had ECOG 2-3.

3/6 patients attained morphological remission and transfusion independence, and 2 attained cytogenetic remission after 5:2 consolidation. 2/6 patients had progressive disease and 1 patient died during induction due to sepsis. Duration of 5/12 cycles given were 14 days, the rest being 7 days.

In 4/6 patients who were not neutropenic when starting induction therapy, the mean duration of subsequent neutropenic period was 5 days per cycle (0-14).

Overall, 3/6 patients were alive at 6 months.

### Conclusion

Elderly AML/MDS remains a management challenge despite improvement in outcomes for other age groups. This study has demonstrated dose reduced combination chemotherapy is well tolerated with a low rate of toxic death and a reasonable response rate for these patients. However, complete remission rates were sub-optimal and responses only durable if more intensive consolidation was given.

*No conflict of interest to disclose.*

### Reference

Kanemura N, Tsurumi H, Kasahara S, et al. Continuous drip infusion of low dose cytarabine and etoposide with granulocyte colony-stimulating factor for elderly patients with acute myeloid leukaemia ineligible for intensive chemotherapy. *Hematol Oncol.* 2008 Mar;26(1):33-8.



**P175**

## **Positron Emission Tomography Findings in Patients with Lymphoma-Associated Hemophagocytic Syndrome**

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### **Introduction**

Haemophagocytic syndrome (HPS) is a clinicopathological entity characterized by systemic proliferation of histiocytes, fever, cytopenia, liver dysfunction, hepatosplenomegaly and often coagulopathy. It is often associated with infection, autoimmune diseases and haematological malignancies, particularly non-Hodgkin's lymphoma. Lymphomas manifesting initially with HPS often pose a diagnostic challenge as the majority of cases have no significant lymphadenopathy for early histological diagnosis. There is paucity of data on specific features of Positron Emission Tomography (PET) in patients with lymphoma-associated HPS (LHPS).

### **Case Report**

Herein, we describe 3 cases of LHPS and their characteristic PET scan features. These 3 patients had pyrexia of unknown origin and pancytopenia. Extensive workup did not reveal any infection or autoimmune aetiology. Computed Tomography (CT) findings of all 3 patients were largely unremarkable for significant disease. Their PET scans however showed extensive and diffuse fluorodeoxyglucose (FDG) uptakes in bone marrow of the axial skeleton with little involvement in lymph nodes. Bone marrow biopsies showed evidence of haemophagocytosis in all 3 cases, but only 2 of the marrow trephines confirmed the diagnosis of large B cell lymphoma and peripheral T-cell lymphoma (unspecified). The third case of anaplastic large cell lymphoma was diagnosed by biopsy of a FDG-avid supraclavicular node.

### **Discussion**

Patients with LHPS often present with fever of unknown origin, associated with cytopenia and liver dysfunction. Lymphadenopathy may be absent or minimal. Histological diagnosis of the lymphoma is often delayed due to its atypical presentation. Bone marrow biopsy is required to confirm the presence of haemophagocytosis and to detect any lymphomatous infiltration. CT scan may not be sufficient for appreciation of the full extent of the disease. Our 3 cases illustrate the usefulness of PET scan which shows distinctive features of extensive lymphomatous involvement in LHPS, which may otherwise be missed on conventional CT scan.

*No conflict of interest to disclose*

**A404**

**P176**

## **Nilotinib Increases Dasatinib Intracellular Concentrations in CML cells: Implications for Combination TKI Therapy**

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### **Aims**

Both nilotinib (NIL) and dasatinib (DAS) have the potential to induce remissions in up to 50% of the CP-CML patients who fail imatinib. However, the sequential use of tyrosine kinase inhibitors (TKI) can result in compound mutations, potentially resistant to all currently available TKI's. Weisberg *et al* previously demonstrated the increased potency of imatinib (IM) and NIL combination compared to single drug. We subsequently demonstrated that IM increased the intracellular concentration (IUR) of NIL and postulated this as the cause of synergy between these drugs. Similarly, Bradeen *et al* reported that the combination of low-dose DAS and NIL minimized the outgrowths of resistant mutations. In this study we assess the effect of IM and NIL on <sup>14</sup>C-DAS IUR in BCR-ABL<sup>+</sup> (K562) and negative (CEM) cells along with their ABCB1 overexpressing (K562-Dox and VBL-100) cells-lines.

### **Method**

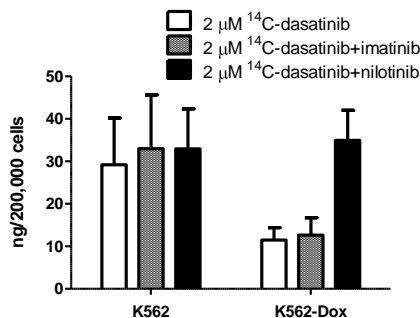
The intracellular uptake and retention (IUR) was determined using <sup>14</sup>C-DAS, +/- unlabelled NIL or IM as previously described.

### **Result**

NIL significantly ( $p \leq 0.01$ ) increased <sup>14</sup>C-DAS IUR in the ABCB1 overexpressing K562-Dox (Fig.1) and VBL-100 cell lines but not in the parental K562 and CEM ( $p > 0.05$ ). In the MNC of CP-CML patients studied to date, nilotinib did not change <sup>14</sup>C-DAS IUR ( $p = 0.8$ ,  $n = 7$ ). DAS did not significantly effect either the <sup>14</sup>C-NIL or <sup>14</sup>C-IM IUR in any of these cell lines ( $p > 0.05$ ). Similarly IM did not alter <sup>14</sup>C-DAS IUR.

### **Conclusion**

These data suggest that NIL increases DAS IUR most likely by inhibiting ABCB1 mediated DAS efflux, as this observation is only significant in ABCB1 expressing lines. These in-vitro studies suggest that the combination of low dose DAS and NIL may provide additive/synergistic antileukemic activity with minimal toxicity. Importantly leukemic stem cells are refractory to TKI therapy and known to overexpress ABCB1. The combination strategies as suggested here may overcome TKI refractoriness and effect better stem cell targeting.



**Fig 1:** Nilotinib significantly increases <sup>14</sup>C-dasatinib IUR in ABCB1 overexpressing K562-Dox cells ( $11.47 \pm 2.91$  vs.  $34.93 \pm 7.1$ ;  $p = 0.01$ ) but not in their in parental K562 cells ( $29.20 \pm 10.97$  vs.  $32.94 \pm 9.43$ ).

### **Conflict of Interest Statement:**

This research was supported by Novartis and Bristol-Myers Squibb. The company had no role in analysing the data or preparing the abstract.

**P177**

## **Patient Acuity Tool Project. The BMT Network, NSW**

**Chloe Ahern**

*BMT Network NSW*

### **Introduction**

The Patient Acuity Tool project began as a pilot study in 2005. "Patient Dependency Systems" or "Patient Acuity Tools" have been developed for many different specialties. However, very few have been detailed or focused on Haematology/Bone Marrow Transplant (BMT). Describing patients in the Haematology/BMT setting in terms of a combination of tasks to complete, as well as physiological factors such as blood pressure and respiratory rates, can give an indication of a patient's "acuity". Grading this acuity can give an accurate measurement of the workload of each individual patient.

### **Objectives**

This qualitative study was undertaken to demonstrate patients that would ordinarily be on a unit with a higher level of care i.e. a High Dependency Unit (HDU) or Intensive Care Unit (ICU), are kept for periods of time on the Haematology/BMT Units, due to the complex and specialised care that they require.

### **Method**

A 4 page assessment tool was developed from an earlier 2005 pilot study, and was completed daily between 1300 and 1600 hrs on all haematology/BMT patients on each of the 9 units that were participating in NSW. The Tool was completed if a patient was admitted as an emergency or was transferred to a higher level of care outside of the usual recording times. The assessment tool graded patients from level 1-4 (lowest to highest), with levels 3 and 4 equating to a HDU type patient. The study was continued for 24 weeks across the participating sites. Interrater reliability was used to assess reliability of the tool and a modified Delphi Method was used to ensure the validity.

### **Results**

Over 11000 tools (patient days) were completed in the 24 week period. 6012 tools were level 1, (54%) of the total. 4626 were level 2, (42%) of the total, which was not unexpected, as most patients would fluctuate between the levels on a daily basis according to their individual stage of treatment. 335 (3%) of the tools were level 3, and 79 (0.7%) of the tools were level 4. There were 1947 patients admitted and over the period. 79 (4%) patients were transferred to either ICU or HDU,

### **Conclusion**

This validated and reliable tool is useful for demonstrating the acuity of Haematology/BMT patients, and can be used as a predictor for the degree of care required for this group of patients.

*No conflict of interest to disclose*

P178

## Snapshot of Haemophilia A Patients with hepatitis C in South Australia

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### Background

Hepatitis C is the most common transfusion transmitted viral infection seen in patients with bleeding disorders who were treated with plasma-derived blood products prior to 1990. Many patients and their families are now dealing with the consequences of chronic liver infection; of the six patients with haemophilia and hepatitis C who have died in the last 36 months, five of the deaths were directly related to their liver disease.

### Aim

Our adult treatment centre has 131 haemophilia A patients, of which 85 have tested hepatitis C antibody positive. We recently conducted a hepatitis C update to determine more closely the needs of our patients.

### Results

Of the 85 adults that are hepatitis C antibody positive, 37 have cleared the virus, with more than half of these (20) doing so without treatment. Some 34 patients have been treated, with 17 achieving a sustained viral response. The other 17 patients failed therapy, due to poor or no response, relapse after therapy, or complications of side effects, and are now being monitored. Three patients are currently on therapy, with another five planning to commence. Three patients current status are unknown. Of the remaining 20 patients who have not received treatment, eight are yet to have an initial hepatology consult (median age=38 years), seven are considered by their hepatologists to be inappropriate for treatment, and two have declined treatment. The genotype of 13 patients is unknown, with only three of these ineligible for treatment.

### Conclusion

It is crucial that this patient group is educated and aware of treatment potential and the importance of looking after their liver health. Obstacles to achieving this were found to be distance and poor compliance. These barriers need to be overcome to give all patients the opportunity for surveillance and effective early treatment before liver damage occurs.

*No conflict of interest to disclose*

A407

P179

## Creating an Accurate Haematology Patient Database: A Continuing Project

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### Background

Haematology units need to have accurate and timely diagnostic information of patients under their care. An accurate database has the ability to reduce workload, be a valuable tool for measuring outcomes for patients, and to allow units to make comparisons with national and international standards.

### Aim

This project was commenced to obtain comprehensive diagnostic information of all patients attending the unit from January 2009 onwards. The updated database will allow identification of patients for clinical trials and/or new treatments as they occur within the unit.

### Method

A Project Officer with Haematology nursing experience was appointed for a period of one year. Using a specific database designed for oncology and haematology units, patients were identified that did not have accurate diagnosis classification. Primary diagnostic reports were sought to ascertain diagnosis, date of diagnosis and method used. Using the internationally recognised coding system, ICD-10-AM, patients were coded according to that diagnostic information.

### Results

From April 2008- April 2009, 3356 patients attended the unit. Of those patients 57% had a diagnosis on the database at the commencement of the project. Four months into the project 77% of patients now have a diagnosis. The five most frequent diagnoses were Non-Hodgkins lymphoma, Chronic lymphocytic leukaemia, Disorders of iron metabolism, Polycythaemia and Multiple myeloma. The most frequent obstacle in determining diagnosis was patients being diagnosed at an outside institution. The database itself proved difficult as the most recent ICD-10-AM codes were not available. The coding system was also found to be not specific enough especially in identifying patients for clinical trials.

### Conclusion

The Haematology patient database project is still in progress but has already shown to be a useful tool within our Hospital. Planning is ongoing on how it will be maintained as an accurate and suitable resource tool.

*No conflict of interest to disclose*

**P181****Case Study: Goodpasture's Syndrome Treated with Regular Consecutive Therapeutic Plasma Exchange****Zoe Grinter**, Annabel Horne*Haematology Oncology Ambulatory Care, St Vincent's Hospital Sydney, NSW, Australia***Background**

Goodpasture's Syndrome (GS) is a rare disease (0.5/million/year). Typical presentation involves haematuria and rapidly progressing renal failure. Additionally, haemoptysis, cough and respiratory failure are not uncommon. Reports in the literature describe a variable clinical course, with mortality rates as high as 50%.

Mrs JS, a 57 year old woman, presented to the emergency department of St Vincent's Hospital with acute onset cough, haemoptysis, myalgia, fevers, pleuritic chest pain, and headaches. Full blood count, renal, liver and coagulation blood tests were undertaken, along with a renal biopsy and lung function tests. A diagnosis GS was made. JS was admitted by a renal physician and commenced aggressive therapy. A referral was made to a haematologist with a view to commencing therapeutic plasma exchange (TPE).

**Aim**

To reduce the number of circulating anti-glomerular basement membrane (GBM) antibodies in order to stop any further deterioration in kidney and lung function.

**Method**

JS underwent three courses of five consecutive TPE using the Cobe Spectra cell separator. Two litres of 4% Albumex was used as replacement fluid for each TPE. The apheresis was used in combination with high dose pulse steroids, oral cyclophosphamide, and three times weekly haemodialysis. A double lumen Vascath was used for both apheresis and haemodialysis. TPE procedures were well tolerated and not associated with any complications.

**Results**

The patient has been discharged from hospital and her respiratory and renal function is much improved. This has been associated with a reduction in the number and frequency of haemodialysis required. Her anti-GBM level is now undetectable.

**Conclusion**

This case illustrates that TPE is a safe and successful procedure. The combination of oral chemotherapy, steroids, haemodialysis and TPE prescribed for JS reflects current best practice for the treatment of fulminant GS.

*No conflict of interest to disclose*

**A409**

P182

## Humidified Air in the Transplant Setting

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### Aim

A pilot study was developed to determine whether the use of humidified air for patients undergoing haemopoietic stem cell transplantation (HSCT) would reduce mucositis, associated pain and febrile episodes.

### Method

A randomised controlled trial was used, with patients randomised to either standard hospital protocol as control, or standard hospital protocol plus humidified air as the treatment arm. Twenty patients were entered into the study at the Royal Adelaide Hospital Bone Marrow Transplant Unit. Baseline data analysis included determining if there were significant differences between the two arms by disease type and conditioning chemotherapy. Outcome data between the two arms were analysed to determine if there were significant differences with regard to body temperature, mucositis grading and oral pain.

### Result

The major findings of this study concluded that there was no statistically significant reduction in pain, mucositis grade and mean temperature in the treatment group. There was some clinically significant reduction in reported pain at the World Health Organisation (WHO) grade 2 mucositis toxicity grade in the treatment group. There was also some clinical significance at WHO grade 4 mucositis in a reduction of mean temperature in the treatment group patients. Some patients on the treatment arm reported improved oral comfort when wearing the humidified air.

### Conclusion

Compliance was a limiting factor in this study, as was the number of patients enrolled. Further research would be beneficial in exploring the clinical significance of the differences in grade 2 and 4 mucositis, with regard to pain and temperature. While this research has not led to changes in the standard management of mucositis in patients during HSCT, there have been instances where Ear Nose and Throat specialist consultation has resulted in the use of humidified air for selected patients.

*This research was supported by Fisher and Paykel. The company had no role in analysing the data or preparing the abstract*

**P184**

## **Designing and Implementing a Haematology Specific Nursing Assessment Form**

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### **Background**

Essentials of Care (EOC) is a practice development framework encompassing nine domains of person-centred care focusing on evaluation and improvement. EOC follows a two year cycle of five components, the first of which commenced in May 2008, which included a one-week period of observation and audits of nursing documentation. Results showed varied compliance in completion of nursing care plans for haematology patients. Following feedback of this information a number of issues were identified with the current generic hospital wide care plan and its use in the haematology setting.

### **Aim**

To design and implement a haematology specific nursing assessment form. In addition, improve documentation of patient care and communication between nursing staff, particularly during patient handover.

### **Method**

- Researched use of haematology specific care plans within South Eastern Illawarra Area Health Service
- Developed a haematology specific nursing assessment form reflecting the nursing care provided and fulfilling documentation requirements
- Collaboration and feedback sought from haematology nursing staff
- 3 month trial commenced February 2009
- Audit completed May 2009

### **Results**

The initial audit of the haematology specific nursing assessment form illustrated increased completion of nursing assessments and documentation by staff. Utilising the form during handover has enhanced communication between staff regarding patient care. Nursing staff also provided positive feedback regarding the streamlined and user friendly format of the new form. Additionally, new and agency staff found the assessment form to be beneficial when completing nursing assessments of haematology patients.

### **Conclusion**

The implementation of a haematology specific nursing assessment form has facilitated compliance in documentation and communication between nursing staff. It has also shown to be a valuable tool in assisting clinical handover.

*No conflict of interest to disclose*

**A411**



**P185**

## **Developing a Pathway for Monitoring Hyperglycaemia in Patients Receiving High Dose Steroids**

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<sup>2</sup>SA Pathology, Adelaide, South Australia.

### **Aim**

High doses of steroid medications are frequently used alone or in combination to treat malignancies or manage associated symptoms. One potential side effect of steroid administration is hyperglycaemia. Consequently patients already diagnosed with diabetes may need to adjust hypoglycaemic medications while those patients without a diabetes diagnosis may require insulin support while receiving high dose steroids. Previously, no formal pathway for monitoring of hyperglycaemia in this patient population existed. Our aim was to collaborate with staff from the Endocrine Department to develop a pathway that appropriately identified patients requiring further management and/or treatment of steroid induced hyperglycaemia.

### **Method**

A pilot project conducted in 2007/2008 for all patients commencing VAD chemotherapy demonstrated that not every patient requires self monitoring of their blood glucose levels. A new pathway was then developed to identify patients requiring referral for self blood glucose monitoring based on an elevated random serum blood glucose level. The pathway was introduced in May 2008 and was specifically designed for any patient receiving high dose steroid medication and only those with hyperglycaemia referred for self blood glucose monitoring education.

### **Result**

To date only one patient has required referral for self blood glucose monitoring instruction using this new pathway. Patients referred via this pathway will be audited and the results presented.

### **Conclusion**

A formal pathway for monitoring and appropriate referral of patients experiencing steroid induced hyperglycaemia has assisted in ensuring that these patients receive appropriate management of this potential side effect of treatment.

*No conflict of interest to disclose*

**P186**

## **A Case Study of a 48 Year Old Male Developing Zygomycosis after 2nd Cycle of Chemotherapy for AML**

**Gillian Parkin**

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### **Presentation**

The 48yr old male patient presented to the unit after being referred by his GP. The patient had been feeling tired and lethargic for a few months, with minor skin and throat infection with delayed healing. The patient had recently been given a course of antibiotics by his dentist for gingivitis. Blood tests on arrival showed an Hb 90, WBC 0.6, Neut 0.11 and Platelet count of 101. A Bone Marrow aspiration was performed which showed a blast count of 54%. The diagnosis was discussed with the patient and his partner, a Hickman line was inserted and DA 3+10 chemotherapy commenced.

### **Treatment**

The chemotherapy was given without major incident; the patient did receive IV antibiotics. Having had a prolonged episode of neutropenia and no count recovery a repeat BMA was performed on 22/04/09. The marrow showed a failed response with 42% blasts and a second cycle of DA3 +8 chemotherapy was commenced, Etoposide for 3 days was added on the 30/4/09. The patient developed a fever and a CT scan on the 11/05/09 was performed, a respiratory review was called for, and a bronchoscopy was performed on 12/05/09. A diagnosis of Zygomycosis was confirmed and the patient commenced on IV Ambisome. The patient had a severe reaction on the first dose, receiving pre medication for the remainder of the course. A further CT performed on 26/05/09 showed an increase in size of the lesion and the patient underwent surgery: a right upper lobe lobectomy was performed. The patient came back to the ward 2 days later and was commenced on Posaconazole, and discharged on the 02/06/09.

### **Zygomycosis**

Zygomycosis is a fast growing fungi that can occur in patients who are immunosuppressed, suffer severe burns, diabetes, lymphoma and leukaemia. The fungi can invade the vessels of the arterial system causing embolisation and necrosis of the surrounding tissue. Rapid treatment is needed to treat successfully. At the time of this abstract the patient is back on the unit, neutropenic after a course of MACE chemotherapy.

*No conflict of interest to disclose*

**A413**

P187

## Challenges Faced in Developing a Haematology Cancer Nurse Coordinator Role in Western Australia

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In 2006 there were 848 reported cases of malignant haematological disease in Western Australia. Haematological malignancies are a diverse group of neoplastic disease that often develop with little warning and require immediate, intensive and lengthy treatment.

Many cancer patients experience confusion and lack of information in their dealings with cancer care services and they are often unable to access appropriate care in a timely manner. In Western Australia, the Cancer Nurse Coordinator (CNC) position was implemented in 2006 as both a strategic and clinical role to facilitate continuity of care and access to appropriate resources for cancer sufferers.

This paper will discuss the challenges faced in the development and alignment of this role within current service provision and resources, being mindful to avoid duplication of services or confusion. An initial scoping exercise identified a key aim of the Haematology CNC is to promote the role as an accessible point of contact for both patients and health professionals in the provision of informational, practical and emotional support; health system navigation; and onward referral. Further, the role is viewed as pivotal in facilitating timely communication with General Practitioners, primary health care providers and the treating centre. Many gaps in service provision were also identified and a follow-up to the scoping exercise has been the development of a study to: a) identify specific unmet needs of haematology patients and b) to promote future evolution of the CNC role. A brief presentation of the key elements of this proposed study will also be presented.

*No conflict of interest to disclose*