P001

Addressing Emerging and Re- Emerging Threats to the Blood Supply

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In Australia, the safety, quality and efficacy of blood-derived therapeutics is overseen by the Therapeutic Goods Administration (TGA) through the *Therapeutic Goods Act 1989*. These measures have ensured that the classical blood borne pathogens, particularly HIV, HCV and HBV pose only little risk to patients. Through donor selection, testing and, when possible pathogen reduction, fresh blood components and fractionated plasma derivatives the transmission of these pathogens have become a hypothetical reality rather than a real risk, where estimation relies on mathematical modelling rather than actual observation of prevalence/incidence. As these risks have abated, often other infectious have attracted attention. These risks are linked to the effects of globalisation, travel, environmental changes and commerce delivering potential infections occurring rapidly ahead of an understanding of their epidemiology which might allow a better application of donor selection measures. In addition some pathogenic entities such as prions are not captured by mass screening as test are present not available.

The example of plasma derivates shows that pathogen elimination renders blood therapeutics safe in the absence of effective selection and testing methodology. While new selection measures such as donor deferral and new tests like bacterial release testing continue to be introduced, an applied technology to eliminate pathogens from fresh components will do much to approach the nirvana of a ‘zero-risk’ blood supply.

No conflict of interest to disclose

P002

Transfusion Risks: a UK Perspective

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Background
The purpose of this presentation is to showcase initiatives which have been adopted within the UK to address Transfusion Risks. This perspective is based on personal experience working within the field of transfusion for over 21 years. By identifying the risks and controls governing the UK and comparing them to findings within Australia may establish common learning opportunities.

Situation
Serious Hazards of Transfusion (SHOT) within the UK has consistently identified Incorrect Blood Transfusion as the major error within the transfusion process. This is consistent with experience at a local level within the UK. Initial studies within Australia have also identified a similar scenario.

Action
Several local and regional initiatives have been adopted in the UK to help not only identify gaps and high risk areas but also adoptable measures to reduce the practical risks associated with transfusions. Initiatives include baseline knowledge assessment of key nursing and support staff. This has lead to the development of education strategies to: (i) address different learning styles, (ii) to accommodate day and night shift workers and (iii) a training target of more than 75per cent of nursing and support staff be trained in transfusion practice. Other initiatives featured in this presentation include exploring the pros and cons of IT technology and highlighting experiences learnt within a regional hospital setting. This presentation endeavours to explore the role audits and patient involvement play in transfusion practice and how they can be effectively harnessed in the management of transfusion practice.

Learning
The focus of the presentation is to impart real information based on personal experience and knowledge into the practical applications adopted to reduce the risk of transfusion in the UK. It endeavours to present a balanced view of the issues, experiences and learning of transfusion practice in the UK as learning opportunity for transfusion practice in Australia.

No conflict of interest to declare

P003

HIS and LIS Application for Improving the Safety of Blood Transfusion in Blood Bank at Shin-Kong Wu Ho-Su Memorial Hospital in Taiwan

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Aim
The purpose of this research is analysis reasons of transfusion error then application new technology lie: HIS, LIS, barcode system and automated blood bank analyser for eliminating such risks improving transfusion safety.
Method
We collected and analysed the causes of transfusion errors and the incidence of error in the transfusion process from 2001 to 2007. Our information department developed computerized system monitoring and reporting during the process of blood transfusion, which could check history blood typing of patient and audit blood bag information automatically. In addition, we adding an automated blood bank analyzer system for ABO/Rh testing to ensure that there is no manual step or technologist input into the process from the entry of the sample for testing into the laboratory system, until the final report is obtained and downloaded into the laboratory computer record.
Result
The incidence of error in the transfusion service was 0.11% from 2001 to 2007 of data collection. Of these events 46.09% occurred within the blood bank, the majority of classified are key in wrong data or error transcription were 13.91% ; the error rate happened outside the blood bank was 53.91%, of these classified as major events like doctor order error was 21.74%; mislabeling events was 19.13% most of mismatched specimen/ request form etc. Since 2004, we set up the LIS and barcode system computerized the procedures of transfusion, and increased automated analyzer at blood bank. The incidence of error in the transfusion service was decreased from 0.14% to 0.08%. The key in wrong data and error transcription were by human error was decreased to 0.01%, the error rate including doctor nurse order error and mislabeling events was largely decreased to 0.01%. Exceptional human error intercepted rate up to 91.8% by the system.
Conclusion
The small number of studies integrating new technology for the transfusion process in laboratory and hospital had obvious preventing human errors. Further we expect to increasing computerize procedures to help identification during transfusion for ensuring transfusion safety, reducing the medical errors.

No conflict of interest to disclose

P004

Informing Emergency Blood Supply Contingency Planning: Bloodhound on the Trail

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Background

Ensuring adequate availability of blood components during times of greatly reduced supply or increased demand, such as pandemic influenza or natural disasters, requires an understanding of blood usage and careful emergency supply contingency planning.

Aims

To collect Australian data to identify clinical indication of red cell use, assess the urgency of clinical need for transfusion and determine the proportion of usage that would potentially be deferrable in an emergency.

Study design and materials

A random sampling approach was developed and piloted. Red cells (RBCs) were tagged with a case report form (CRF) during production, and distributed to Victorian transfusion laboratories. At the time of unit issue for transfusion, the CRF was completed by laboratory scientists with recipient demographics, clinical indication, and urgency of need. Machine readable forms were returned to ARCBS for collation and analysis.

Results

Of 5132 RBCs tagged, 5052 CRFs were returned (response rate 98.4%), of which 4829 units (95.6%) were transfused.

Clinical indications for transfusion were haematology/oncology (1623 units, 33.6% of transfused units); surgery and trauma (1442, 29.9%); other medical/miscellaneous indications (834, 17.3%); unspecified anaemia (616, 12.7%) and unknown (314, 6.5%).

Clinical urgency of transfusion was acute (timeframe of requirement: within 1 hour) in 605 cases (12.6%); urgent (1-24 hours) in 2516 (52.1%); semi-urgent (24 hours-1 week) in 1431 (29.6%) and non-urgent (>1 week) in 169 (3.5%). In 108 cases (2.2%) the urgency was unknown.

Transfusions for elective surgery and non-urgent conditions together accounted for 472 units (9.8% of transfused units).

Conclusions

Bloodhound provides comprehensive and current Australian data regarding indications and urgency of red cell usage to inform emergency supply contingency planning. These data suggest that cancellation of elective surgery and deferral of non-urgent transfusions would have only a short-term and moderate impact on usage. Consideration of alternative strategies to ensure blood availability is required.

No conflict of interest to disclose

P005

Queensland Rural and Remote Emergency Blood Supply

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Aim
The project's aim was to review the blood service (including emergency donor panels) in rural and remote Queensland to ensure the provision of a high quality, cost effective blood service in a clinical emergency.
Method
Data were collected through surveys, site visits, workshops and literature searches. Surveys examined the usage and preferences of blood supply options. ARCBS and QH provided information re emergency donor panel (EDP) donor testing frequency and EDP activations. Literature searches examined international equivalents.
Result
Survey results showed a division in opinion with respect to the preference of O Rh(D) Negative red cells (57%) to EDP (17%) with 20% having a preference for both and 6% for retrieval services. A strong view amongst rural doctors was that fresh whole blood (via EDP) was able to replace all blood components, which were not available as individual fresh blood components. In 2007, there were 22 active EDPs in Queensland and their average donor continuity (percentage of a year with a screened donor panel) was 82%. The average number of O Rh(D) Negative donors was 8/site with 8 sites having <6 O Rh(D) Negative donors. From 1999 to 2007, the average number of EDP activations was 12/year. The main indications for activation were gastro-intestinal, obstetric and trauma related haemorrhage. Legal opinion indicated jurisdictions (eg QH) would hold the majority of the operational liabilities with EDPs.
Conclusion
A significant focus of the review was on EDPs and their place in the rural and remote blood supply. The risk versus benefit is considered to be in favour of EDPs with an emphasis on improving the quality and safety of EDPs.

No conflict of interest to disclose

P006

Haemovigilance in Queensland: Completion of the Pilot Program of Queensland Incidents in Transfusion (QiiT)

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Aim
To report on the progress made towards implementing a haemovigilance system in Queensland.
Method
A six month pilot program of the proposed haemovigilance system for Queensland (QiiT) was completed by December 2007. This pilot program was a collaborative project involving stakeholders from Queensland Health, public and private pathology services, ARCBS and private and public hospitals. The four pilot hospital sites have continued reporting to QiiT since completion of the pilot program. The public hospitals via an electronic link from the Queensland Health incident reporting system (PRIME).
Result
During the six month, pilot program 14 reports were received, with reports from all four pilot sites. The system allowed identification of duplicate reporting, despite the use of de-identified data reporting. Only one follow up analysis could not be completed. Of the remaining 12 individual reports, six were validated against the haemovigilance data set. The remaining six reports did not meet the data set definition for the reported condition. Since completion of the pilot program the data set has been adapted to the proposed national haemovigilance data set, and to date a total of 33 reports have been received from the four sites. Data received from PRIME has allowed quantification of the number of validated and non-validated reports received against the complete haemovigilance data set for a 12 month period.
Conclusion
The pilot program demonstrated that QiiT can effectively collect, analyse and validate incidents and adverse events relating to transfusion practice. The system will now be implemented in Queensland. QiiT will form an integral part of the strategies to ensure quality transfusion practices in Queensland, and will allow Queensland to contribute data to the national haemovigilance system.

No conflict of interest to disclose

P007

Serious Transfusion Incident Reporting: A Growing Process

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Introduction
Blood Matters, an initiative of the Department of Human Services, Victoria and the Australian Red Cross Blood Service, aims to improve patient outcomes by enhancing safety and appropriateness of blood use. A haemovigilance framework was developed in 2005 and piloted in Victoria in 2006, providing a central system for reporting events related to administration and handling of fresh blood components and pre-transfusion samples. This event data should direct future initiatives for practice change.

Method
The STIR (Serious Transfusion Incident Reporting system) database captures de-identified notifications from health services, with an initial generic electronic questionnaire and subsequent submission of detailed information on defined transfusion-related events. All reports are reviewed by an expert group to validate clinical features, and determine causality and severity of the incident. An annual aggregated report is provided to the participating hospitals, including any recommendations for practice change.

Results
Following promotion and refinement of the system, participation has grown from 14 pilot hospitals to 48 public and private hospitals, and now extends across three jurisdictions (VIC, TAS and ACT). In 2006-2007 STIR was notified of 155 transfusion adverse events. Acute reactions have been the most frequent (49% of all reports, most of which were allergic or febrile non-haemolytic), followed closely by combined procedural adverse events (45% of all reports, incorporating incorrect blood component, wrong blood in tube and near miss events). No deaths related to transfusion were reported.

Conclusion
The first two years of the STIR system have captured some inherent risks with blood products, and real and potential risks in the processes used in clinical transfusion practice STIR has proved successful in its capacity to educate and recruit participation from health services. Several challenges remain, including improvements to data validation and minimising the burden of data collection.

No conflict of interest to disclose

P008

Transfusion Reactions Manifesting Predominantly with Pain

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Two patients with similar transfusion reactions of an under-recognised type were detected in 2007 through the New Zealand Blood Service haemovigilance reporting system. **Case 1:** An 86 year old man with MDS, β-thalassaemia trait, Parkinson's disease and TIA twice had similar reactions to RBC transfusions. These consisted of lower back pain, dyspnoea and agitation. On both occasions his observations remained stable and the pain settled without specific treatment within an hour of stopping the transfusion. He was known to have anti-knops alloantibody and a pan-reactive autoantibody. No obvious cause for his symptoms was found. Despite symptomatic anaemia he declined further transfusions and was treated with rhuEPO. **Case 2:** A 56 year old man post-total knee replacement received 3 units of RBC. Shortly after starting the 3rd unit he developed severe joint, abdomen, chest and loin pain. There were no other symptoms but his blood pressure rose from 185/102 to 217/125 mm Hg. Observations and investigations post-reaction were unremarkable. There was no obvious explanation for his symptoms and the pain settled within a couple of hours of stopping the transfusion without specific treatment.

Discussion and Conclusions

Post-RBC transfusion two patients had similar reactions with severe pain as the major component. They resemble cases described as acute pain transfusion reactions (APTR) [1] in a lone report in the literature . APTR was described in patients with diverse conditions receiving RBC and platelet transfusions. In addition to the acute, severe axial and proximal limb pain, hypertension, dyspnoea or tachypnea, tachycardia and restlessness may also occur. Symptoms subside quickly after stopping the transfusion and symptomatic treatment. The pathogenesis is unknown. In our cases, on clinical grounds, infective or neurological causes seem unlikely and metabolic, immunological or cytokine-mediated mechanisms seem possible. In conclusion, APTR are poorly-recognised, significant but short-lived reactions of unknown aetiology, we suspect they occur more frequently than are reported.

Reference

1 Orton MD, Andres T, Bielski M, et al. Acute pain transfusion reactions: An under recognized adverse transfusion event associated with leukoreduced components. Blood 2001; 98 (suppl): 57a

No conflict of interest to disclose

P009

Apheresis FFP: Double Trouble?

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Aim
Investigation of anaphylactic reactions in two patients within 15 hours, receiving clinical FFP from one apheresis donation divided into two units.
Method
Patient histories, patient transfusion reaction investigations and product culture for bacterial contamination were performed at RPH. Donor history and laboratory investigations were organised by ARCBS-Enterprise
Results
Case 1: 74 year old female had a right hemihepatectomy and bile duct reconstruction and received 1 unit RBC, 2 units FFP during operation. Post-operative INR 1.9, 2 units FFP requested. 100ml transfused when patient had symptoms of hypotension 75/53mmHg (BP pre-op: 181/87), O_2 saturation 89%, tachycardia 140bpm, urticaria, wheeze. The transfusion was ceased, adrenaline administered. Initial laboratory investigation included a blood group check.
Case 2: 12 year old female with scoliosis, elective admission for posterior spinal fusion and fixation correction (T3-L5). During the procedure she received 250ml of cell salvaged blood, 2 units RBCs. 100ml FFP was transfused when patient had symptoms of severe hypotension, tachycardia 150bpm. Adrenaline and gelofusine were administered. Peripheries shut down and the patient was resuscitated for 40 minutes, no trace on arterial line for 20mins. Patient transferred to ICU, chest Xray clear. Laboratory investigations: blood group check= compatible; mast cell tryptase= mildly elevated, serum IgA level= normal; Anti-IgA1, IgA2 antibody titres= no antibodies detected, no granulocyte antibodies detected. FFP: 2 units from the same apheresis donation. Bacterial culture of FFP bags: negative. Donor: male, multiple platelet and plasma donations. Laboratory investigations: negative for antibodies to HLA class I and II, granulocytes, IgA1and IgA2; IgE level: normal.
Conclusion
Investigations for ABO incompatibility, Anti-IgA antibodies, TRALI, circulatory overload, and bacterial contamination, failed to identify cause. This may be the first reported case in Australia of one FFP donation associated with anaphylactic reactions in 2 patients. It highlights one of several important aspects of reporting transfusion reactions. Quarantine of associated blood products, whilst not current practice, could be considered in light of our experience.
No conflict of interest to disclose

P010

Pneumatic Tube Delivery System: Validation and Use to Transport Blood Components

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Aim

To validate the Pneumatic Tube System for the transportation of blood components, to ensure that proper temperature, tracking and integrity of the product is maintained.

Method

Sixteen red cell concentrates and eleven platelet concentrates were transported through the pneumatic tube system from Central Pathology to the destination and back. The elapsed time and temperature was measured before dispatch, on arrival, and on return. Samples were collected from each pack prior to sending and after arriving at the destination. Red cell concentrates were tested for potassium levels, LDH, red blood cell count, haemoglobin and blood film analysis. Platelet concentrates were tested for platelet count and platelet activation using flow cytometry with Annexin V and CD62P as markers of activation.

Results

The average time taken to send a unit of red cell concentrate outward via the longest route was 2 minutes. The average temperature increase of all outward trips was 1.5 ºC and of all round trips was 3ºC. There was an average of 0.2% change in the red cell count and a 0.1 % change in the haemoglobin. The potassium level increased on average by 4%, while the LDH results were inconclusive. Morphological review did not reveal significant differences in the specimens post transport.

The average outward trip for platelet concentrates was 2 minutes and the temperature increase for a round trip was 0.2 ºC. There was an average change in platelet count of 1.2%. The mean percentage of platelets positive for Annexin V pre dispatch was 45 and 48 post trip and for CD62P was 25 and 25 respectively.

Conclusion

Results indicate that we can safely transport cellular blood products from the transfusion service to distant patient care areas in the hospital as our testing indicates that components are not adversely affected by pneumatic tube transport. Of interest may be the relatively high level of activation in the platelet concentrates prior to transportation of 45 and 25% using Annexin V and CD62P, respectively.

No conflict of interest to disclose

P011

Saving the Platelet: St Vincent's Hospital Initiative to Reduce Platelet Expiry Rates

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Background
Platelets have had historically high expiry rates due to short lifespan and shorter regulated approved transfusion times St Vincent's hospital historically has had usual expiry rates of 15-20% for platelets. This is a problem of a wasted finite resource that has been donated for altruistic reasons. Additionally there is a cost consideration since the NSW government has devolved its share (37%) of the cost for blood products; wastage has become an increased concern.

Aim
To reduce platelet expiry levels to fewer than 10%

Method
Interventions to reduce wastage included restrictive ordering to one bag (four units) of platelets per time unless exceptional circumstances use of Group O when possible, education for prescribing doctors. The use of taxis to order additional units as needed has enabled reduced stock ordering of platelets therefore reduced wastage. Blood bank staff initiating discussion with doctors re need of products. This allows the redirection of products not needed by the initial order that may be utilized by another patient.

Results
The results of this project have been very pleasing with a reduction in expired platelets from 17% baseline to approximately 7.4 % per month as average for last financial year with some months demonstrating less than 5% expired platelets.

Conclusions
St Vincent's Hospital has developed and maintained benchmark figures for platelet expiry. This has been a concerted effort from Transfusion Medicine staff in conjunction with ordering doctors. It will be seen if this intervention is sustainable over time, particularly with the commencement of bacterial testing of platelets, which could change the distribution, and supply of platelets.

No conflict of interest to disclose

P012

DiaMed Assays for Anti-IgA Antibodies and IgA Deficiency: Comparison with a Reference Method

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Aim
Anti-IgA antibodies are rare but can cause transfusion-associated anaphylaxis. The detection of anti-IgA antibodies has traditionally been performed in a limited number of reference laboratories. In this study our aim was to compare the performance of a simple DiaMed gel assay with a reference method.
Methods
Two simple gel card assays are now available which can be used to screen for anti-IgA antibodies and IgA deficiency. A total of 24 sera which had previously been assayed for anti-IgA antibodies over a 3 year period were used to assess the DiaMed anti-IgA and IgA deficiency assays.
Results
The DiaMed assays correctly identified patients (n=6) who had significant IgA deficiency and anti-IgA antibodies. All patients with an abnormal anti-IgA titre by hemagglutination assay and who were also IgA deficient had anti-IgA antibodies detected using the DiaMed screening test. One patient, previously shown to have an IgA level of <0.067g/L failed to be detected as IgA deficient in the DiaMed IgA deficiency test, however anti-IgA antibodies were not present. Samples with slightly increased anti-IgA titres tended to have normal IgA levels.
Conclusions
The DiaMed gel card assays simplify screening for anti-IgA antibodies and are an appropriate tool for the investigation of transfusion related anaphylactic reactions in any routine Blood Bank laboratory.

No conflict of interest to disclose

P014

Referral Human Immunodeficiency Virus (HIV) Screening for Blood Donors in Indonesia Years 2005-2007

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Background
In 2001, global approximately 14,000 new cases of HIV were found every day, 95% in developing countries. In Indonesia the first HIV/AIDS case was found in April 1987 and until March 2008 there were 17,998 people with HIV/AIDS, of whom 2,486 have died.* HIV infection could be transmitted through blood transfusion, therefore all donor's blood should be screened for HIV before transfusion. Central Blood Transfusion (CBTS) Indonesian Red Cross is a referral centre of HIV blood screening and helps the Ministry of Health (MOH) to run surveillance of HIV infection among blood donors.
Aim
To see the repeated reactive of anti HIV blood screening result on donors blood that will reported as a surveillance data of HIV positive among blood donors.
Method
The Initial Reactive samples from the Branch Blood Transfusion Units in Indonesia was sent to the CBTS for re-testing using similar reagent that was used by the Branch BTUs and another Elisa reagent. If one or both the test showed reactive results, samples were indentified as Repeated Reactive samples.
Result
In the period of 2005-2007, there were 1,797, 1,683 and 1,418 Initial reactive samples consecutively sent to the CBTS. 24% of these samples was tested using Rapid test, 70% using ELISA, 1,5% using both Rapid test and ELISA and 4,5% using Chemiluminesence. The repeated non reactive result was found in 32% of the samples, while 41% gave repeated reactive result. The remaining 23% of the samples were indeterminate, and 4% were not able to be tested due to poor quality samples.
Conclusion
32% non reactive results showed that false reactive rate in the Branch BTUs is very high that will impact to cost inefficiency of blood service. The false reactive results was suspected caused by high percentage of rapid test being used. Centralizing the Transfusion Transmissible Infection (TTI) blood screening by using the standardized ELISA is believed could increase the safety of blood. Meanwhile, the 41% repeated reactive results showed that donor selection criteria and method need to be improved in order to defer high risk blood donors.
*Data from Ministry of Health until March 2008
No conflict of interest to disclose

P015

Phlebotomy Patterns in Haemochromatosis Patients and Their Contribution to the Blood Supply

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Background and Aim
Regular phlebotomy is the treatment for haemochromatosis (HC) and some patients may be eligible to be blood donors. We examined (a) how many whole blood donations (WBD) HC patients contribute and (b) phlebotomy patterns with the different genotypes associated with HC.

Methods
Chart review of HC patients at NZBS Christchurch between 01/02/2007 to 31/07/ 2007. Generally, patients had phlebotomies to maintain serum ferritin < 50 mcg/L and transferrin saturation < 50%. They were eligible to donate if otherwise suitable and serum ferritin was < 500 mcg/L and LFT normal. Information on WBD was derived from NZBS computer records.

Results
Of the 410 HC patients 247 (60.2 %) were male and 163 (39.8%) female. 244 (59.5%) were C282Y homozygotes, 15 (3.6%), C282Y heterozygotes, 6 (1.4%), H63D homozygotes and 48 (11.7%), C282Y/H63D compound heterozygotes. In 97 (23.6%), genetic test results were unavailable. 228 / 410 (55.6%) were considered eligible to donate for at least a period during the 6 months. 387/730 (53%) phlebotomies were therapeutic (0.15 units/patient/month) and 343/730 (46.9%), WBD (0.25 WBD/patient eligible to donate/month). A total of 10927 WBD were collected from 9095 donors during the study period of which 343 (3.1%) units came from 228 eligible HC patients and 10584 from 8867 non-HC donors (0.19 WBD/non-HC donor/month). Phlebotomies/patient/month ranged from 0 - 2.6. Mean phlebotomies/patient/month in C282Y homozygotes, C282Y heterozygotes, H63D homozygotes, C282Y/H63D compound heterozygotes, those without genetic test results and overall were 0.30, 0.16, 0.11, 0.33, 0.28 and 0.29 respectively.

Discussion and Conclusion
HC patients contribute small but significant numbers of WBD but the proportion eligible to donate and the number of WBD/patient eligible to donate/month are less than reported in other studies.[1] C282Y heterozygotes and H63D homozygotes had fewer phlebotomies than C282Y homozygotes while C282Y/H63D compound heterzygotes had as many.

Reference
Leitman SF, Browning JN, Yau YY, et al. Hemochromatosis subjects as allogeneic blood donors: a prospective study. Transfusion 2003; 43: 1538-1544

No conflict of interest

P016

Improving the Yield of Factor FVIII Recovery in Whole Blood Donations

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Aim

Factor VIII (FVIII) is a coagulation protein required for the control of blood clotting and the prevention of severe bleeding in haemophiliac patients. FVIII levels within Anti-Haemophilic Factor (AHF) plasma must, on average, be greater than 0.7 IU/ml. Process control data for FVIII levels in Fresh Frozen Plasma (FFP) for some sites indicated a gradual decrease in the reported level of FVIII. The aim of this study was to identify possible causes for the apparent decrease in FVIII levels and determine potential process improvements.

Method

Investigation of FVIII loss focused on component manufacture. This required sampling and workflow analysis including donor sample collection, whole blood (WB) unit sampling post-donation, pre-filtration, post-centrifugation, plasma freezing analysis and time and motion studies.

Results

A comparison of total protein and FVIII from both plasma units and test segments showed an 11% decrease ($p=0.0008$) in segments, attributed to dilution resulting from residual red cell preservative remaining in segment tubing. When comparing units tested prior to freezing, those separated within 8 hours displayed a 7% loss in FVIII ($p=0.007$) with no additional loss after 16 hours ($p>0.05$). Mixing of units post phlebotomy and stripping of attached tubing were identified as possible areas of improvement. The 24h holding study indicated that the time taken to WB separation had significantly impacted on FVIII levels with decreases of 2% ($p=0.0008$) per hour within the first 6 hours followed by a 1% loss per hour ($p=0.004$).

Conclusions

Significant factors impacting on FVIII levels in the processing of plasma from WB collection bags were identified. They include FVIII loss during the hold prior to separation and freezing, freezing process and test segment preparation. Minimising the time WB is held prior to checking, consignment and processing is expected to have a positive impact on FVIII levels.

No conflict of interest to disclose

P017

Evaluation of a Spectrophotometric Method for Determining the Extent of Red Blood Cell Contamination in Clinical Fresh Frozen Plasma

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Aim

The Council of Europe Guidelines 13th (CoE v13) edition states that the residual red blood cells (RBC) in clinical Fresh Frozen Plasma (FFP) must be at a concentration of < 6 x 10^9 RBC/L. This study aimed to compare the current Flow Cytometric method with the Harboe spectrophotometric method (Harboe Method) for the detection of residual RBC in plasma.

Method

Serial dilutions of whole blood were performed in triplicate using Phosphate Buffered Saline (0.05 - 25 x 10^9 RBC/L). The accuracy, linearity and reproducibility of samples were then tested. The Harboe Method measures the optical density of oxyhaemoglobin at 415nm, with background correction for impurities. Through using known standards, the concentration of residual RBC in plasma can be determined. The Flow Cytometer method uses fresh plasma mixed with a known number of fluorescent beads and the antibody glycophorin A to detect RBC. Non-lipaemic and lipaemic plasma was tested for absorbance interference by spiking with RBC of known concentrations.

Results

The Harboe method displayed linearity in the range of 1 – 25 x 10^9 cells/L with a correlation coefficient of 0.99 and %CV < 0.3. In the range of 1 – 10 x 10^9 cells/L, the Harboe method displayed mean differences of 0.08 x 10^9 RBC/L and a standard deviation of ± 0.38 x 10^9 RBC/L, comparable to the flow cytometric method. The underlying haemoglobin content within lipaemic plasma was found to obscure the resolution of red cell contamination.

Conclusion

The Harboe method appears cost effective and requires less labour than the Flow Cytometer method. Tested across the range of 1 - 25 x 10^9 cells/L, the Harboe method was found to be linear, reproducible and accurate for the analysis of RBC contamination in FFP. These results have shown that the method should be suitable to assess plasma against the CoE v13 criterion of <6 x 10^9 RBC/L.

No conflict of interest to disclose

P018

Evaluation of Leuco-depleted Fresh Un-refrigerated Whole Blood Using the Terumo WB-SP Blood Bag with In-line Filter

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Aim
ARCBS provides fresh un-refrigerated whole blood in special circumstances e.g. neonatal exchange transfusion. Due to implementation of 100% leuco-depletion of red blood cell units, the evaluation of platelet saving in-line filter bags was required to produce whole blood containing viable platelets.
Method
Whole blood units (n=19) were collected into Terumo WB-SP bags. Units were filtered at 4 (n=9) or 20 hours (n=10) post collection and held to a maximum of 24hrs at 20-24ºC. Samples were collected and tested for red cell, platelet and plasma markers, contact activation markers and cytokines to assess component quality. Testing was performed pre and post filtration at 8, 20 and 24 hours (if applicable).
Results
Acceptable results were obtained at both filtration times after the maximum 24 hour hold. Whole blood red cell parameters such as 2,3-DPG, ATP, Haemolysis, pH and Potassium levels were within defined acceptance limits. Platelet recovery was 76±10 %, Hypotonic Shock Response was 57±8% and CD62P (P Selectin) expression was low indicating acceptable platelet quality. Plasma met acceptance criteria with FVIII levels of 86±20IU/L, Factor V at 87±15IU/L and Fibrinogen at 2.81±0.5g/L. Contact activation marker levels of Factor XIIa and C3a were 11.66±1.5U/L and 564±177ng/mL respectively with no increase post filtration. sC5b-9 levels increased post filtration but remained stable. Prothrombin F 1 & 2 decreased on filtration and reduced further at 24 hours. Cytokine levels TNF̃alpha, IL-8, RANTES and sCD40L were within normal plasma levels. TGF beta1 was above normal plasma levels at 7.27±8.24ng/ml but below levels in platelet concentrates and those reported to cause adverse events.
Conclusion
The Terumo WB-SP bag is suitable for preparation of leuco-depleted fresh un-refrigerated whole blood. All units showed minimal platelet activation. Other quality indicators were within acceptable ranges at expiry (24 hours post collection), including: cytokines, contact activation markers, red cell content and plasma factors.

No conflict of interest to disclose

P019

Prenatal Fetal *RhD* Typing for RhD Negative Pregnancies: Comparing Two Methods of Maternal Genomic DNA Extraction to Control for the Absence of the Maternal D Gene

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Background

Although the majority of phenotypically RhD negative mothers are genotypically RHD negative, a small proportion have a non-functional rather than absent RHD gene. This could lead to a false positive result in prenatal free fetal *RHD* gene testing from maternal blood. A non-functional maternal RHD gene could be excluded by testing maternal genomic DNA (mgDNA.

Aim

To determine levels of ffDNA contamination resulting from two different methods of mgDNA extraction from the blood of pregnant women.

Methods

The comparison of techniques involved maternal blood samples from RhD negative women known to have an RHD positive fetus. DNA was extracted from whole blood (n=10) or white cells (n=8) using the automated QIAGEN EZI DNA extraction method. *RHD* testing of mgDNA involved amplification of three regions of the D gene and testing in replicates of four. Testing for the fetal associated hyper methylated RASSF1A gene was performed in triplicate.

Results

The whole blood method showed evidence of fetal contamination, with a positive RHD result, in 4/10 patients (5 of 120 replicates) and positive RASSF1A result in 1 of 30 replicates. One sample, collected at 37 weeks gestation, had a positive signal for Exons 5, 10 and RASSF1A. None of the mgDNA samples extracted from white cells showed any evidence of contamination. (Odds Ratio 9.190, p value 0.0673 {Fisher's Exact Test})

Conclusions

mgDNA extraction from whole blood is prone to contamination with free fetal DNA and cannot therefore be used to determine whether the mother has a non-functional RHD gene. The risk of contamination may be greater at later gestations, where ffDNA levels are known to be higher. In this small series, there was no evidence of fetal contamination with mgDNA extraction from white cells.

No conflict of interest to disclose

P020

DAR-E on the Red Cells of a Sudanese Child

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Aim

To determine the RhD status of a Sudanese boy whose red cells showed discrepant reactions with different monoclonal anti-D reagents.

Method

Weak RhD typing of red cells from a 9 year old Sudanese boy was initially identified by testing his red cells with CSL Epiclone 2 IgM anti-D by a tube immediate spin method. Subsequent testing was performed using several commercial monoclonal anti-D by tube immediate spin and Diamed gels (Table 1). The RhD specificity was confirmed using a panel of monoclonal anti-D reagents (Alba Bioscience, Scotland).

Result

Initial testing of the child's red cells by tube immediate spin against CSL Epiclone 2 IgM anti-D containing RUM-1 showed very weak agglutination (grading +/-). The HM10 clone in Diagast IgM II anti-D reacted, by tube immediate spin, more strongly than RUM-1. The ESD-1M clone used by Diamed to detect DVI was strongly positive; non DVI detecting anti-D clones were very weak or negative. Table 1 summarises the reactivity pattern of monoclonal anti-D reagents used in the initial determination of RhD status. DAR-E was identified using a selection of 12 monoclonal anti-D reagents from Alba Bioscience (Scotland).

Anti-D Brand	Clone	Method	Grading
CSL Epiclone 2 IgM	RUM-1	Tube Immediate spin	+/-
Diagast IgM I	P3X61		0
Diagast IgM II	HM10		2
Diamed ABO/D (VI-) + reverse grouping	LHM 59/20 (LDM3) / 175-2	Diamed gel (I/S)	0
Diamed ABD(VI-)	TH-28, RUM-1, LDM1		+/-
Diamed ABO/Rh (VI+)	ESD-1M + 175-2		4
CSL Epiclone 2 IgM/IgG	RUM-1, MCAD6	Diamed gel (15' 37^0C)	4

Table 1. Reactivity patterns of monoclonal anti-D reagents used for initial determination of the RhD status (Grading 0-4).

Conclusion

This case highlights the different reaction strengths of commercial monoclonal anti-D reagents and the value of using different clones to elucidate RhD status. DAR-E was described in 2005 as a partial D found in Ethiopians. As DAR-E is part of the Weak D type 4 cluster this child may form immune anti-D if transfused with RhD positive red cells and therefore should be considered RhD negative.

No conflict of interest to disclose

P021

Flow Cytometric Determination of Feto-Maternal Haemorrhage in a Rhesus D Positive Patient with an Increased Level of Adult F Cells

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Aim

To determine the size of feto-maternal haemorrhage (FMH) by flow cytometry in a 33 week gestational Rhesus D Positive patient presenting with abdominal trauma and suspected abruption.

Method

FMH was initially quantitated by flow cytometry (BD FACS Canto, USA) using single colour FITC conjugated Hb F (Silenus, Australia). As a consequence of increased adult F cells dual colour flow cytometric analysis was performed (IQP, Netherlands). Briefly, red cells were fixed with formaldehyde, permeabilised with sodium dodecyl sulphate and stained with FITC conjugated carbonic anhydrase (CA) and PE conjugated Hb F. Samples were then analysed on a Becton-Dickinson FACS Canto. Controls were analysed in parallel, including a negative, 0.2%, and 1% fetal cells.

Results

	Method	% fetal cells	% adult F cells	Volume FMH
Patient	**Single colour** **Dual colour**	**0.40** **0**	**30** **22**	**8.8 mL** **< 1.0 mL**
Negative	**Single colour** **Dual colour**	**0** **0**		
0.2% control	**Single colour** **Dual colour**	**0.19** **0.20**		
1% control	**Single colour** **Dual**	**1.14** **1.10**		

Conclusion

The advantages of flow cytometry for the quantitation of FMH in antenatal patients have been well documented. Flow cytometric analysis using anti-D FITC or PE can be used routinely for FMH quantitation of Rhesus D negative women who have delivered a Rhesus D positive infant. However, in this case the woman was Rhesus D positive necessitating the quantitation of FMH by a flow cytometric Hb F based method. Using single colour Hb F flow cytometry the patient was found to have increased levels (30%) of adult F cells, making it difficult to quantitate the number of fetal cells present. The dual colour flow cytometric method utilizes Hb F and carbonic anhydrase, which is an enzyme present only in adult red cells, allowing the differentiation between true fetal cells and adult F cells. Although this dual colour methodology is expensive, it is a useful tool for FMH quantitation in Rhesus D positive women with increased levels of adult F cells as highlighted by the case described.

No conflict of interest to disclose

P022

Improving Transfusion Practice through Education

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Aim

Improvement in the safety and quality of clinical transfusion practice in Australia and to assist hospitals with accreditation requirements for transfusion training and credentialing.

Method

Development of an on-line (e-learning) tool based on established educational principles and utilising a range of media and activities designed to engage the learner emotionally, psychologically and physically.

Result

This resource was made available at www.bloodsafelearning.org.au in late 2007. It has been widely accepted with significant uptake across Australia. To date approximately 6,000 users from medical, nursing, laboratory and other professions have registered and used the tool on-line. Feedback on the instructional design and media delivery has been very positive. This has resulted from input by a wide range of transfusion experts across medical, nursing and laboratory disciplines, involvement of qualified and experienced educators and the use of professionals for graphic design, photography, video production, computer programming, acting and production.

Conclusion and Future Directions

Funding has been received from the National Blood Authority (NBA) via the Jurisdictional Blood Committee (JBC) to further develop this as a national resource. This will be used to determine long term sustainability including financial and governance models. Development of additional learning modules is also underway.

Initial development of this tool was funded by SA Health as a component of the BloodSafe program. All authors are affiliated with the BloodSafe program which is a collaborative transfusion safety and quality improvement program between SA Health entities and the Australian Red Cross Blood Service.

P023

The Role of Cell Salvage and Iron Therapy in Patients Undergoing Elective Orthopaedic Surgery

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Background
In view of the ongoing shortage of blood supply and to minimize risk factors associated with anaemia in elective surgery with subsequent blood transfusion requirements and its complications/hazards, we have prospectively offered cell salvage and iron therapy to patients with elective orthopaedic surgery.
Patients and Methods
At a single institution, the Launceston General Hospital, we report on 16 patients who underwent elective hip- (7), knee-(6) replacement and spinal surgery (3). The median age was 69 years (range 46-82) with a median body weight of 80 kg (range 61-90). The OrthoPAT (Medtel, Australia) is an Orthopaedic Perioperative Autotransfusion System which is a fully automated, compact, portable, cell salvage machine that can be used during both the intra-operative and post-operative periods.
Results
The median pre-admission Hb level was 114 g/L with a median post-op Hb of 96 g/L (range 66-104). The average amount of blood loss during the operation is 700 ml (range 590-2010 ml). The average amount of reinfused blood was 330 ml (range 200-440). Five patients received allogeneic blood transfusion, 3 of whom received 2 units, 1 patient required 3 units and other patient 4 units, while the rest did not receive any blood transfusion. The median stay in hospital was 9 days with a range between 2-28 days. Factors that may affect the outcome of procedure such as pre-operative Hb level, iron status, amount of blood loss, blood transfusion, infection and length of stay in hospital as well as quality of life were studied.
Conclusion
Preliminary data suggest that cell salvage that employs modern technology is easy to apply and monitor in the context of elective surgery. Furthermore, cell salvage in combination with haematinics therapy (iron) may improve the outcome of elective surgery and decrease the necessity for blood transfusion. Further studies to confirm these preliminary findings are warranted.
The authors confirm that there is no conflict of interest in relation to this research

P024

Tranfusion and Elective Joint Replacement in South Australia

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Aim
To establish transfusion practice in elective joint replacement patients in SA.
Method
Retrospective case note audit of consecutive elective joint replacement patients was conducted in 4 SA teaching hospitals in 2005. Data was collected by hospital transfusion nurse consultants and reviewed by a haematologist.
Results
118 patients (62% female) were reviewed. Average age was 69 years (range 33-89 years). 38% underwent primary THR, 54% primary TKR. 12% donated autologous blood.
44% of primary THR and 36% of primary TKR received a transfusion. 18% of patients had pre-operative anaemia (transfusion rate 76%).
98% of transfusion episodes in stable patients were within NHMRC guidelines (compared to 96% in 2003 and 82% in 2002 audits). 11% of patients had a post transfusion Hb>115g/L (compared to 14% in 2003 and 22% in 2002 audits).
Conclusion
Transfusions within the NHMRC guidelines and rates of over-transfusion have improved significantly since 2002. Pre-operative anaemia if addressed in advance of elective surgery could lead to further reductions in transfusion rates.

No conflict of interest to disclose

P025

Factors Affecting Platelet Increment After Transfusion of Whole-Blood Derived vs Apheresis Platelets in an Oncology Set-up

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Background
The therapeutic efficacy of platelet preparations depends on various factors related to the product and patient.
Aim
To analyze factors affecting platelet increment after transfusion of whole-blood derived (RDP) vs Apheresis Platelets (SDP) in cancer patients.
Methods:
All platelet units were evaluated for Platelet count, WBC content and volume prior to issue. The CCI was evaluated at 1 hour and 18 or 24 hours. Type of platelet product issued for transfusions was decided as per their availability. Multiple linear regression analysis of CCI with variables related to the patient and platelet product was performed.
Results
279 transfusion episodes in 69 patients were studied. SDP's had higher (p=0.003) platelet content. 62% of platelet transfusions were ABO compatible. 5.7% transfusions were therapeutic and bleeding was controlled in all patients irrespective of the platelet preparation used. CCI at 1 hour and 24 hours was equal with RDP and SDP transfusion. Leucocyte content of RDP's was greater than SDP's. 29% of the platelet products were leucodepleted. Transfusion reactions occurred in 17% patients and were associated more (p = 0.001) with RDP transfusions. 5.8% patients had refractoriness to platelet transfusion. The mean interval between two platelet transfusions showed no correlation with the platelet type and dose transfused. Multiple linear regression analysis revealed platelet dose, leucodepletion, shelf life of product, fever, infection, splenomegaly, bleeding, and number of transfusions received in the past as key factors affecting the CCI.
Conclusion
The factors affecting CCI are independent of the type of platelet preparation used. Patient related factors are probably more important than the type of platelet product; and these factors should therefore be considered for planning an appropriate platelet transfusion support strategy in cancer patients.

No conflict of interest declared

P026

The Victorian Experience of Special Platelet Support - It's a Team Effort!

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Background
ARCBS issues both pooled and apheresis platelets, with blood group A and O apheresis platelets usually in stock. Special platelet support refers to individual patient support, with targeted collection of compatible, single donor apheresis platelets to support platelet refractoriness or in special clinical circumstances e.g. fetomaternal (neonatal) alloimmune thrombocytopenia (FMAIT). In order to provide this special platelet support ARCBS relies on a group of committed volunteer donors who donate as required for specific patients.

Description
Human leucocyte antigen (HLA) typed platelets may be required by patients who are refractory to pooled and ABO-matched apheresis platelets due to the presence of HLA alloantibodies. Human platelet antigen (HPA) typed platelets are required for intrauterine or neonatal transfusion in FMAIT or for the management of post-transfusion purpura. Some patients require B group platelets (not routinely collected) to support ABO-mismatched stem cell transplants. The provision of timely and appropriate components requires the involvement of the treating clinician, hospital blood bank and ARCBS. Within ARCBS, compatible platelet collection involves a number of teams to perform clinical assessment, patient and donor immunogenetic testing (HLA/HPA typing, antibody screening etc), donor search and selection, apheresis collection, testing, labelling, packaging and transport and clinical follow up. The number of requests for special platelet support has steadily increased since 1999. From July 2007 to June 2008 the Victorian ARCBS collected 936 platelets specifically for 51 Victorian patients. Support ranged from 1 to 101 units of platelets per patient (mean 18). The majority were to support platelet refractory haematology/oncology patients. An additional 86 platelets were sent to 23 interstate patients.

Conclusion
The demand for special platelet support is increasing in response to clinical needs. The provision of special platelet support requires the coordination of many personnel and activities within ARCBS and at the transfusing hospital.

No conflict of interest to disclose

P027

Platelet Refractoriness Post-Allogeneic Bone Marrow Transplantation Due to Recipient-Derived Anti-HPA-5a

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Human platelet antigen (HPA) antibodies are a well-established cause of both neonatal alloimmune thrombocytopenia and post-transfusion purpura, but have infrequently been implicated in platelet refractoriness in patients with haematologic malignancies. We report a case of platelet refractoriness potentially attributable to recipient-derived HPA antibodies in a multiparous female who received an allogeneic bone marrow transplant for acute myeloid leukaemia from an HLA identical brother. The conditioning regimen was fludarabine (25mg/m^2) / melphalan (140mg/m^2) with cyclosporine and methotrexate as graft-versus-host disease (GVHD) prophylaxis.

Following transplantation her platelets remained <5-10x10^9/L despite megakaryocyte engraftment on marrow biopsy and recovery of neutrophil count. Platelet transfusions from the donor and from unrelated HLA matched (by HLA matchmaker) donors failed to produce significant incremental rises in platelet count. DNA-based platelet genotyping performed on a pre-transplant sample of the recipient and donor revealed HPA-5bb and HPA-5aa respectively. Anti-HPA-5a of IgG subclass was demonstrated in the recipient's pre-transplant serum using a platelet glycoprotein ELISA. As the patient was highly HLA-immunised **(**lymphocytotoxicity testing – 88%-100% reactivity) platelets that were both HLA- and HPA-matched could not be sourced.

We hypothesise that the failure to obtain both platelet increments following HLA-matched transfusions and platelet count recovery despite adequate donor marrow engraftment was due to immunocompetent lymphocytes of recipient origin producing anti-HPA-5a, despite aggressive conditioning and GVHD prophylaxis and with molecular studies (day 30, 60 and 100 post transplant) demonstrating 100% CD3 donor chimerism. Anti-HPA antibodies are a rare cause of platelet refractoriness and should be considered in allograft recipients who fail to obtain either satisfactory increments after transfusion of HLA-matched platelets or platelet count recovery after engraftment.

No conflict of interest to disclose

P028

Supporting a Patient Refractory to Platelet Transfusion Through High-Dose Consolidation Chemotherapy for Acute Myeloid Leukaemia with Autologous Cryopreserved Platelets

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This 50-year-old woman, diagnosed with Acute Myeloid Leukaemia, became platelet-refractory during induction chemotherapy. She had no prior transfusions and 2 pregnancies. The patient presented with constitutional symptoms, haemoglobin 72g/L, white cell count $73.9x10^9$/L (blasts 93%) and platelets $132x10^9$/L. Bone marrow biopsy confirmed Acute Monocytic Leukaemia.

During induction chemotherapy (cytarabine/idarubicin), the first platelet transfusion was given on Day 8 with increment but was complicated by fever/chills. Until marrow recovery, multiple platelet transfusions were administered without significant increment. Single-donor, apheresis platelets were used for all but 3 of total 21 transfusions.

Owing to poor incrementation (possible HLA-sensitisation), autologous platelets were harvested to support consolidation chemotherapy.

Platelets were collected by apheresis and cryopreserved in a rate-controlled freezer with additive Dimethyl Sulphoxide (DMSO) to a final concentration of $1772x10^9$/L, divided into 80mL bags. In an effort to preserve platelets, minimal centrifugation was used and DMSO was used in low concentrations, so washing was not required prior to re-infusion. Sample analysis confirmed a relatively platelet-pure product.

Two courses of high-dose cytarabine-based consolidation therapy were completed with autologous platelet support. During first consolidation, platelets (2 bags) were thawed (37^oC) at the bedside and administered without manipulation on days 11, 12 and 14 with demonstrable increment. During second consolidation, platelets (2-3 bags) were infused on days 10, 12, 14, 15 and 16, again with increment. Platelet increments were smaller in second consolidation, possibly due to sepsis, differing antibiotic regimens, or inter-bag variability. The patient received HLA-matched platelets on days 21 and 25 of final consolidation. There was no significant bleeding.

This case demonstrates that patients refractory to platelet transfusion can be supported through high-dose chemotherapy with autologous products. The cryopreservation process is simple, does not require a large amount of freezer space and resulted in clinically useful increments, indicating a high yield.

No conflict of interest to disclose

P029

Combination Use of Apheresis Granulocyte and Buffy Coat Components for the Treatment of Neutropenic Sepsis

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1. Peter MacCallum Cancer Centre, 2. Australian Red Cross Blood Service

Whilst the issues surrounding the use of granulocyte transfusions remains varied, complex and controversial, in many organisations it remains current practice for the treatment of severe neutropenic sepsis. Management of granulocyte donors is an integral factor in providing a quality granulocyte component. With rigorous screening and donor availability it is difficult, and at times impossible, to provide apheresis granulocyte component for all patients in a timely fashion.

Two patients aged 65 and 76 were diagnosed with transformed AML from MDS in 2008, and were both treated with FLAG induction. Both patients developed neutropenic sepsis, for which they were treated with intravenous antibiotics. Subsequently, invasive fungal infections of the frontal sinus were identified, requiring surgical debridement.

As a result of the post-induction neutropenia and ongoing sepsis, it was decided to maximise supportive treatment with granulocyte and BC components.

Due to the urgency of the supportive treatment, BC components were used initially to bypass the wait for granulocyte donor screening and product collection. A target of 10 BC components formed a ‘dose’, providing a good result for the patients particularly in the peri and post operative phase of surgical debridement. An added advantage of this method was that BC contain platelets and this lead to a significant improvement in platelet count on each occasion as the patients had previously been relatively refractory to platelet transfusion. BC therapy however, is not without potential adverse effects, including alloimmunisation, due to the level of donor exposure.

The outcome of this combination of supportive therapies proved to be beneficial with prompt availability of BC components, whilst apheresis granulocytes were being arranged. This is a potential option for acute management of these seriously ill patients.

No conflict of interest to disclose

P030

Implementation of the National Intravenous Immunoglobulin (IVIg) Criteria: The IVIg and Transfusion Nurse Role

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Background

Intravenous immunoglobulin (IVIg) is a precious resource and Australia's use continues to increase. A dedicated IVIg Nurse role was created in 2005 by SA Health. Since 2006 Transfusion Nurses (TNs) at ARCBS have contributed to the management of IVIg. ARCBS plays an important role in clinical review of IVIg requests and distribution. The *'Criteria for the clinical use of intravenous immunoglobulin in Australia'* were approved for implementation in March 2008, with a six month transition period.

Aim

To support optimal patient care, assist clinicians, and improve reporting around IVIg, through design and implementation of a national process and specific tools for Criteria implementation.

Method and Results

Establishment of a multidisciplinary national working group to develop the process and supporting materials, including modifications of the national ARCBS STARS (Supply Tracking and Reporting System) database, for additional data collection. STARS contains national data on patients receiving IVIg (5170 active patients March-April 2008). Extensive communication is underway with clinicians regarding existing patient diagnoses, doses and treatment response in the context of requirements of the Criteria. ARCBS has developed and implemented:

- National education templates and clinical information packages
- National review letters (1) to clarify diagnosis & compliance with Criteria and (2) to document response
- 3 national request forms (haematological, neurological & immunological/general) and a weekly institutional infusion schedule.

IVIg/TNs and ARCBS medical specialists have provided extensive support, including educational presentations and clinical liaison (advice on indications, dosage & administration) that will continue beyond the transition period. ARCBS TN hours (initially 4 FTE nationally) increased in March 08 reflecting the additional workload. Hundreds of patient letters have been sent to treating clinicians, with interim response rates of 20-80%, reconfirming diagnoses.

Conclusion

The IVIg/TNs play a vital role in IVIg management for customers (patients & clinicians) and within the ARCBS TMS team.

No conflict of interest to disclose

P031

Is a Clinical Working Group Necessary for Implementation of the New Criteria for Clinical Use of IVIg in Australia?

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Aim

To review the referrals and outcomes of requests for IVIg made to the QH IVIg Working Group prior to the introduction of the New Criteria for IVIg use in Australia.

Method

All referrals to the IVIg committee since April 2006 were reviewed. Referrals were assessed against the AHMAC 2000 and new criteria released in 2008.

Result

Between April 2006 and March 2008, 135 requests for IVIg were referred to the committee. In 12 cases the clinician was advised to access IVIg via a Jurisdictional direct order, while in 8 cases a request for further information elicited no response from the clinician. Of the remainder, 36 requests were for further doses of IVIg in patients who had previously been approved access. These 36 requests related to 21 individual patients. 79 new referrals were reviewed by the committee; 17 cases were category 1 (IVIg indicated), 21 cases were category 2 (inconclusive evidence for IVIg use) and 1 was category 3 (IVIg not indicated) according to the AHMAC 2000 criteria. Of the remaining cases, 34 had conditions not listed in the AHMAC 2000 criteria and in 6 cases the diagnosis remained uncertain despite extensive investigations by the referring clinician. Of the 34 cases not listed in the AHMAC 2000 criteria, 25 (74%) would be eligible under the new criteria.

Conclusion

The clinical committee is only referred a small fraction of the requests for IVIg and so cannot make a significant impact on overall IVIg use in the state. However, the committee has allowed assessment of referrals against emerging indications for IVIg use in a real time manner and allowed a review of cases where the diagnosis is uncertain. This data highlights that criteria/guidelines soon become outdated, and that clinical involvement in approval processes can mitigate this problem.

No conflict of interest to disclose

P032

The Use of Intravenous Immunoglobulin (IVIg) in Dermatological Conditions in Australia

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Background
A range of serious dermatological conditions including dermatomyositis and Kawasaki's disease is effectively treated with IVIg. Some (bullous pemphigoid, BP, pemphigus foliaceus, PF, and pemphigus vulgaris, PV) have a known autoimmune basis, whilst for others (cicatricial pemphigoid, CP) a specific autoantibody is yet to be identified. IVIg has been shown to be effective in life-threatening toxic epidermal necrolysis (TEN) and Stevens-Johnson syndrome (SJS).
Aim
To analyse IVIg usage in dermatological conditions.
Methods
Review of IVIg usage in dermatological conditions reported to ARCBS from July 1 2006 to June 30 2008. Classification of conditions was according to recently implemented *Criteria for the Clinical Use of Intravenous Immunoglobulin* (NBA, 2008*).* Predicted demand for these conditions based on published epidemiological incidence rates was also calculated.
Results
Dermatomyositis was the most commonly supported dermatological indication (66 patients, 470 treatment episodes, ~25,000g IVIg). 46/66 received induction doses of 2g/kg; 50/66 commenced/or continued maintenance therapy reflecting a therapeutic response.
Kawasaki's disease was the second most common, with 297 patients (average age just over 4 years) and 362 treatment episodes using ~ 11,700g.
12 BP patients (~4500g), 2 CP patients (~330g) and 2 PV patients (~350g) were also supported with IVIg, estimated to account for 13.6% of patients with pemphigoid syndromes. 16 patients received IVIg for TEN or SJS (~2000g).
Conclusion
Demand for IVIg in Australia for dermatological conditions as immunomodulatory therapy is increasing as more conditions are identified as responsive, dermatologists become aware of the availability of IVIg and because of the better side-effect profile of IVIg compared with immunosuppressive treatments. Only a small proportion of patients with BP, CP and PV are currently treated with IVIg. It is important for clinicians to be aware of the inclusion criteria for use of IVIg, especially the role of a dermatologist in diagnosis.

No conflict of interest to disclose

NOTES

P074

Analysis of CD34 Viability: Fresh is Best?

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Enumeration of viable $CD34^+$ cell numbers by flow cytometry is routinely performed on peripheral blood stem cells, using 7AAD to identify dead cells. Comparison of estimates from assays performed on the product prior to cryopreservation and after thawing provides a measure of the number of stem cells that survive the cryopreservation/thaw process.

In this retrospective study of viability assays performed between January 2003 and May 2008, we reviewed the recovery data for patients infused with varying doses of CD34 cells, with the aim of determining if the post-thaw CD34/kg estimate was a more useful indicator of patient recovery than the pre-cryopreservation estimate.

The haemopoietic recovery timepoints used were days to neutrophil counts of 0.1 x 10^6, 0.5 x 10^6 and 2.0 x 10^6, and platelet counts of 50,000 and 150,000. The median recovery timepoints for patients receiving <2.0 x 10^6 CD34/kg, 2-3 x 10^6 CD34/kg, 3-4 x 10^6 CD34/kg, 4-5 x 10^6 CD34/kg and >5.0 x 10^6 CD34/kg , based on pre-cryopreservation estimates, were calculated. Within these groups, the patient data was sorted on the basis of post-thaw CD34/kg and the median recovery timepoints were compared.

We found that where patients had received an infusion of <2 x 10^6 CD34/kg based on the post-thaw estimate there was an increase in the time taken for neutrophils to reach each timepoint compared to patients receiving 2-3 x 10^6 CD34/kg. However this increase was not statistically significant. The time taken for platelet recovery was not increased for patients receiving <2 x 10^6 CD34/kg based on the post-thaw estimate.

We also compared the median recovery timepoints for each group, based on the pre-cryopreservation CD34/kg dose. We noted a significant increase in the time to neutrophil recovery to 2 x 10^6 (p = 0.0054) and for the platelet count to reach 50,000 (p = 0.0410) in the 2-3 x 10^6 CD34/kg group when compared to the 3-4 x 10^6 CD34/kg group. However we noted no increase in neutrophil or platelet recovery timepoints when 3-4 x 10^6 CD34/kg were infused compared to when 4-5 x10^6 CD34/kg were transplanted.

We conclude from these data that infusing greater than 3-4 x 10^6 CD34/kg has no significant effect on patient recovery timepoints.
No conflict of interest to disclose

P075

Collection of Cells, One Step Further

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The world has become a smaller place, with stem cells for transplantation coming from all over the world. Haemopoietic Progenitor Cells (HPC) collected either by apheresis (HPC-A) or by bone marrow donation (HPC-M) are best used as fresh as possible, however, in Australia, most cells from overseas are 24 hours or more away. It is even possible that cells collected in Australia can be 12 or more hours away. We know that there is deterioration the longer the cells are in storage. So how do we accommodate getting the cells safely to our patients? This paper looks at how donated HPC's make it from donor to patient. It will look at the role of the courier, how cells are stored for their journey, and what can go wrong. There will be discussion on who is best to care for the cells for the passage from the donation centre to the patient.

No conflict of interest

P076

Impact of Gating Strategy on Enumeration of Viable CD34 in Cryopreserved Haemopoietic Progenitor Cells

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On behalf of Katherine Marsden[3], the RCPA Haematology QAP staff[3] and Participants of the RCPA CD34 QAP
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Aim

Viable CD34 (V-CD34) enumeration based on membrane integrity is one of the proposed assays for quality control of processing, cryopreservation or storage of haemopoietic progenitor cells (HPC), and for assessment of engraftment potential. This pilot study is to assess the logistics for introducing a V-CD34 QAP, and the impact of gating strategy on V-CD34 enumeration, independently of reagents and method.

Methods

56 participants in the current RCPA CD34 QAP were asked for feedback for a V-CD34 QAP using cryopreserved samples. For list mode data (LMD) analysis, apheresed HPC were cryopreserved, thawed, and then stained with CD45-FITC, CD34-PE and 7-AAD in Trucount tubes. Parameters acquired by Cellquest/FacsCalibur were FL1, FL2, FL3, FL4, FSC and SSC. LMD files were uploaded on the RCPA QAP website for participants to analyse by any BD software, Coulter CXP or Expo32. Data were collected on the gating strategy, events acquired for various populations, total and V-CD34 per ul. The acceptable limits of performance were median ± 25%.

Results

21 of 25 respondents were interested in a V-CD34 QAP. 17 centres submitted results for LMD analysis, showing 2-19% CV for total number of events acquired, bead events, total CD34 events, total CD45 events and total CD34/ul. The V-CD34 events and V-CD34/ul showed 32-65% CV. Comparing the different gating strategies, the number of results within acceptable limits of performance were 6 of 6 for NSW BMT Network template, 3-5 of 7 for single platform and 1 of 4 for dual platform.

Conclusion

A majority of respondents showed interest in a V-CD34 QAP. The gating strategy is a contributing factor to variations in enumeration of total and V-CD34. This pilot study demonstrated that the adoption of a common gating strategy such as the NSW BMT Network template would contribute towards standardisation of V-CD34 enumeration in cryopreserved/thawed samples.

P077

Haemopoietic Progenitor Cell Infusion: What Could Possibly Go Wrong?

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Aim
To investigate (and hopefully validate) a worst-case scenario in the infusion of Haemopoietic Progenitor Cell's (HPC). Specifically, the patient being medically unable to receive already thawed HPC.
Method
Thawed HPC were handled according to 3 different proposals.
Proposal 1: Store thawed bag in the refrigerator (4^{o}C) until required.
Proposal 2: Store thawed bag at room temp (23^{o}C) until required.
Proposal 3: Refreeze the bag.
The viability of the HPC were assessed by determining viable CD34 and CFU numbers.
Result
The percentage of viable CD34 cells remained relatively stable.
Clumping of nucleated cells was observed in stored samples not treated with DNAse. The clumping had a significant impact on the viable CD34 numbers, with a dramatic drop over time mirroring the increase in observed clumping. Conversely, the DNAse treated samples showed no visible clumping and appeared to show no adverse effect on viable CD34 numbers or CFU's.
The stored samples showed a small decrease in viable CD34's after overnight storage.
The difference between storage at 4^{o}C and RT was small, but 4^{o}C consistently yielded better results.
The refrozen sample showed only a slight decrease in number of viable CD34 cells and CFU's from the compared to the control.
Conclusion
Faced with a scenario where a thawed bag of HPC was unable to be infused, these results suggest two possible paths to preserve the HPC clonogenic ability and viable CD34 population;
Where the infusion is expected to proceed within hours, store the diluted, DNAse-treated cells at 4^{o}C.
Where the infusion is likely to be delayed overnight, immediately refreeze the cells.

No conflict of interest to disclose

P078

Lymphoid Blast Crisis of Chronic Myeloid Leukaemia in a Jehovah Witness Patient

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Lymphoid blast crisis of Chronic Myeloid Leukaemia (CML) occurs in about 30% cases and is generally reported to have a better prognosis than myeloid blast crisis. The presence of the b2a2 fusion transcripts and 5' M-bcr breakpoints also confers an improved outcome. The case is of a 57 year-old woman with Hereditary Angioedema presenting with a blood picture in keeping with myeloproliferative disease. Bone marrow examination was diagnostic for chronic phase Chronic Myeloid Leukaemia. In the case presented, a treatment regimen, no longer routinely used for CML (busulfan therapy), rather than the current imatinib mesylate therapy, undoubtedly resulted in rapid progression to blast crisis. The use of busulfan in chronic phase CML patients should be limited to those patients who are intolerant of, or resistant to other therapy.

The lymphoid blast crisis appeared 11 months after diagnosis of the CML. The patient was referred to a Haematologist and treated with vincristine, Glivec and prednisone. She was not given standard therapy which would have required substantial blood product support. The therapy caused a prolonged period of severe pancytopenia.

Erythropoietin (EPO) was used for the anaemia .The patient had a brief haematological remission but 3 months later she relapsed and succumbed.

The ethical issues of treatment which normally necessitates the extensive use of blood product support are highlighted in this case of a Jehovah Witness patient. Refusal to receive blood components greatly affects treatment options. The course of this patient's disease would undoubtedly have been quite different had she received current therapy (Imatinib mesylate) for CML at diagnosis.

No conflict of interest to disclose

P079

Monosomy 7 in Paediatric Patients: A Case Series With 5 Different Haematological Presentations

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Aim
Monosomy 7 and deletions of 7q occur in a variety of myeloid disorders, particularly AML and MDS, disorders which are more commonly found in adults than children. We aim to report 5 paediatric patients with haematological disorders, associated with a complete or partial monosomy 7.
Methods
Consecutive patients with patients with complete or partial monosomy 7 presenting to our institution over a 12 month period were identified. Data was collected regarding clinical features, bone marrow morphology and conventional cytogenetics plus FISH analysis.
Results
Five patients were identified, with ages 1 to 6 ½ years, 3 males and 2 females. Diagnoses were AML in 2 patients (one de novo AML with multilineage dysplasia and one with trisomy 21, and one patient each with MDS, JMML and aplastic anaemia. The patient with de novo AML had persistent disease following induction phase II, and underwent an unrelated umbilical cord in CR1. This patient relapsed at day 250 and died shortly afterwards. The patient with trisomy 21 AML had an additional ring isochromosome 7 and trisomy 8, and is in CR and completing chemotherapy. The patients with JMML and MDS have been successfully allografted and remain alive in CR1 at day 70 and 160, respectively. The patient with JMML had a small ring chromosome derived from chromosome 7, while the patient with MDS had Wiskott Aldrich Syndrome, confirmed on WASP gene sequencing. The patient with aplastic anaemia was treated with ATG and cyclosporin A, and remains in a complete haematological and cytogenetic remission after 9 months of followup.
Conclusion
Our 5 cases demonstrate that in addition to the more common presentation with AML or MDS, monosomy 7 /7q- can be associated with more unusual presentations in childhood, including aplastic anaemia and Wiskott-Aldrich syndrome.

No conflict of interest to disclose

P080

Recurrent Transfusion-Related Spontaneous Remission of Acute Myeloid Leukaemia

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Aim

Spontaneous remission of acute myeloid leukaemia (AML) has been reported on a number of occasions – usually in the context of infection or blood transfusion. We report experience with a recent case and review previous published cases in an attempt to further delineate this relationship and postulate possible underlying mechanisms.

Method

We analysed circumstances surrounding induction of remission in our index case and performed a literature search using PubMed with keywords 'spontaneous remission', 'acute myeloid leukaemia' and 'transfusion'.

Results

An asymptomatic 81y.o. female presented with isolated neutropenia. Bone marrow biopsy (BM) revealed myelodysplastic features consistent with Refractory Anaemia with Excess Blasts (RAEB-I). Seven months later, she developed progressive pancytopenia. Repeat BM revealed progression to AML with normal cytogenetics. Transfusion of two units of non-leucodepleted and non-irradiated red blood cells (RBCs) was complicated by a febrile reaction. Within weeks and without other intervention, peripheral blood counts normalised. Two months later, the pancytopenia recurred. After further RBC transfusion and associated febrile reactions, peripheral counts again normalised within weeks and repeat BM demonstrated leukaemic remission with no excess blasts. She has received no other treatment to date. Five months after the most recent transfusions, she has evidence of myelodysplasia with mild thrombocytopenia and leucopenia but no significant peripheral blast count. In the search undertaken, approximately 33 cases of spontaneous remission from AML have been published since 1980 where patients have invariably received a transfusion or presented with infection. Mean duration of remission was approximately seven months. Only three remissions occurred in the setting of myelodysplasia.

Conclusion

We conclude a possible immune basis for these remissions – due to the transfusion of immunocompetent anti-leukaemic leucocytes with the red cells, cytokine induction by transfusion or infection, or the development of other anti-leukaemic cellular or humoral immune responses induced by infection or transfusion.

No conflict of interest to disclose

P081

Two Fatal Cases of Central Nervous System Scedosporiosis Occurring After Neutrophil Recovery Following Induction Chemotherapy for Acute Leukemia

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Scedosporium prolificans is an emerging opportunistic pathogen which disseminates quickly and is often refractory to therapy in the immunocompromised patient. It is being increasingly reported in patients with acute leukemia who are neutropenic, and involvement of the central nervous system (CNS) in these situations is almost universally fatal. We describe two recent cases of CNS Scedosporidiosis manifesting after neutrophil recovery which were ultimately fatal despite early identification and institution of appropriate therapy.

Case 1

A 28 year old female with acute myeloid leukemia developed rapidly progressive unilateral chemosis and visual loss due to endophthalmitis on Day 32 of her induction chemotherapy. The onset of symptoms occurred five days following achievement of a normal neutrophil count and whilst remaining on empiric liposomal amphotericin. Despite immediately changing to voriconazole at the onset of visual symptoms and subsequently introducing terbinafine when Scedosporium prolificans was identified from blood cultures, she suffered multiple and inevitably fatal intracerebral haemorrhages associated with ruptured mycotic aneurysms caused by a diffuse cerebral vasculitis.

Case 2

A 52 year old female received one cycle of HyperCVAD for precursor B-cell acute lymphoblastic leukemia. Approximately seven days after recovery of a neutrophil count to > $1.0x10^9$/L, she complained of a severe generalised headache. CSF and blood cultures demonstrated Scedosporium prolificans. Despite immediate commencement of voriconazole and terbinafine, she developed initial focal limb weakness followed by progressive loss of consciousness and death within a week of CNS symptoms.

Conclusions

CNS Scedosporiosis in immunocompromised patients is almost universally fatal.
Combination voriconazole and terbinafine is the currently recommended antifungal strategy. To our knowledge diffuse cerebral vasculitis with mycotic aneurysm formation and haemorrhage has not been described. The onset of clinical manifestations occurring after neutrophil recovery in both cases was unexpected. Increased awareness of this pathogen is encouraged.

No conflict of interest to disclose

P082

CD4+/CD56+ Haematodermic Tumour in Transformation?

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An 89 year old lady, previously well presented with a two week history of a rapidly growing cutaneous violaceous plaque on her mid-back region. This measured at 10x15cm when reviewed. She also described multiple small circumferential subcutaneous pink nodules up to 1cm in diameter appearing throughout her body over the same period. Skin biopsy revealed a dermal infiltrate with sheets of immature cells with immunohistochemistry profile of CD3-, CD4(weak), CD56 (weak), CD43+ and CD117-. FBC and E/LFTs were normal. BMAT was not involved morphologically or by flow cytometry. Haematodermic neoplasm was diagnosed.

Given her age she was treated with localised radiotherapy and two cycles of chlorambucil and prednisolone with good response. Ten weeks after her initial diagnosis a routine FBC showed leukaemic dissemination. Her smear revealed 80% "large blastic cells that appear to be of monocytoid origin". The immunophenotype was CD4+, CD56+, CD13+, CD14+, CD11b+, CD33+, CD34-, cMPO-, CD117- and CD3-. She died 4 days later.

CD4+/CD56+ haematodermic neoplasms (HDN) are uncommon; designated cutaneous lymphomas under the revised 2005 WHO-EORTC classification. Previously known as blastic NK cell lymphoma, they are more recently termed early plasmacytoid dendritic cell leukaemia/lymphoma. They are highly aggressive with a poor prognosis (median survival 12-14 months), usually presenting with skin lesions, rapid nodal and marrow involvement with leukaemic dissemination and progression to AML. This close relationship to acute myelomonocytic and monoblastic/monocytic leukaemia (FAB -M4/M5), is evident with up to 30% of this subgroup expressing CD56.

The patient had morphological features of transformation to AML with myelomonocytic differentiation (CD11b, CD13, CD14, CD33) but lacked the key myeloid antigens for a definitive diagnosis of AML (cMPO and CD117).

This is an unusual case of apparent/incomplete transformation of HDN to AML (described by Craig et al. In *Blood* April 15, 2008) It is uncertain whether repeat immunophenotyping, several days later (had the patient survived) would yield a similar finding or complete transformation to AML.

No conflict of interests disclosed

P083

Philadelphia Chromosome t(9;22) in AML with Additional Cytogenetic Abnormalities: A Report of 2 Cases

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Aim
We describe the characteristics of 2 patients with AML, having the Philadelphia chromosome t(9;22) either as a secondary abnormality or part of a complex karyotype.
Case 1
A 66 year old man presented with fever, sweats, bone pain and confusion from hypercalcaemia. Initial pathology results showed haemoglobin of 114gm/L; Platelet of 57x10^9/L; WCC of 6.8x10^6/L with 4% blasts. Marrow aspirate was consistent with a diagnosis of AML. Karyotype showed an apparent terminal deletion of 9q, a derivative of (22) t(9,22), and a t(12;13). A single BCR-ABL fusion gene was found on FISH. Induction therapy with 7-3-7 and Imatinab was given; followed by 2 consolidations with 5-2-5. Molecular remission (BCR-ABL) was achieved, but last only 6 months. The same clone was detected at relapse.
Case 2.
A 24 year old man presented with cervical lymphadenopathy. Pathology results showed haemoglobin of 90gm/L, WCC of 62x10^6/L with 75% blasts, monocytosis of 7.4x10^6/L, and platelet count of 26x10^9/L. Marrow aspirate showed AML, (Monoblastic variant with Eosinophilia). Cytogenetics revealed inv16 in all cells and an additional t(9;22;19) in 50% of cells, confirmed by PCR and FISH as p190 BCR-ABL. The patient was commenced on a Fludarabine/Cytarabine combination chemotherapy (ALLG AML13 protocol), later switching to 7-3 after developing pneumonitis to fludarabine; 2 consolidations with 5-2 were given. He relapsed 3 months after molecular remission with respect to both BCR-ABL and CBFB. At relapse, inv(16) was detected without evidence of t(9;22;19) on cytogenetics or FISH. The patient remains disease free after a double-cord cell stem cell transplant.
Discussion
These two cases describe the presence of BCR-ABL mutation in AML. Anecdotally, this mutation appears to confer a bad prognosis. In Case 1 the mutation appears to be part of a complex karyotype, with the BCR-ABL possibly playing a major role in pathogenesis. In the Case 2 the BCR-ABL mutation in the malignant clone was probably secondarily acquired in clonal evolution, being only present in 50% of leukemic cells at outset. The patient was initially classified as CBFB and treated as good risk. However, the presence of BCR-ABL may have played an enabling role to proliferation even though it was absent at relapse. This appears to be the only case reported of p210 BCR-ABL with inv(16).
No conflict of interest to disclose

P084

Secondary Donor-derived Burkitt Leukemia following Allografting for Aplastic Anaemia

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Case Report
The patient presented at the age of 17 with a one year history of lethargy and was subsequently found to have aplastic anaemia. Primary treatment was with sibling allogeneic transplant, completed in October 2000. Conditioning was with cyclophosphamide and ATG and GVH prophylaxis was with cyclosporine and methotrexate. She remained on cyclosporin A until May 2003 for GvHD. Whilst remaining asymptomatic, routine blood tests for an outpatient follow-up in November 2003 showed a leukoerythroblastic blood film. The ensuing bone marrow biopsy showed a population of cells expressing CD10, CD19 and CD20 with Kappa light chain restriction and the characteristic morphology of Burkitt leukaemia. Cytogenetics identified the t(2;8)(p12;q24) abnormality which is rare, but consistent with Burkitt type acute lymphoblastic leukaemia. This clone was donor-derived with the XY karyotype. The patient completed six cycles of Hyper CVAD and has remained in remission for over four years.
Post-transplant lymphoproliferative diseases occur most commonly in the setting of ongoing immunosuppression and the majority are B cell non-Hodgkin lymphomas attributable EBV infection. Burkitt leukaemia has rarely been reported in the post-transplant setting and only twice in the donor clone following allogeneic stem cell transplantation. Such cases have been associated with concurrent EBV infection.

At the time of diagnosis with Burkitt leukaemia the patient was found to have no serological evidence of EBV (with both IgG and IgM undetectable) and EBV could not be detected by PCR at that time. The donor had serological evidence of previous EBV infection with IgG but not IgM at the time of donation and in November 2003 did not have detectable EBV by PCR.

In summary, this case represents a rare post-transplant lymphoproliferative disease that occurred in the donor clone following an allogeneic stem cells transplant in the absence of significant immunosuppression and without evidence of overt EBV infection.

No conflict of interest to disclose

P085

Air Filtration in Stem Cell Transplant Units: Is it Always Necessary?

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Infections are a significant cause of morbidity and mortality in recipients of allogeneic haematopoietic stem cell transplants. International guidelines exist regarding prevention and treatment of bacterial, viral and fungal infections in this setting. The use of HEPA filtered air is often mandated by best practise guidelines to reduce infection, especially Aspergillus and other spore born fungi. The evidence basis for this is debatable and implementation of these recommendations is often expensive and they take little account of geographical variance in the incidence of such organisms.

The Wellington Transplant Unit has historically had a very low incidence of fungal infections. Recently the unit has been exposed to major dust contamination from building demolition and rebuilding on the hospital campus. To obviate this threat the transplant unit has nursed patients in newly commissioned HEPA filtered rooms. This project has been prospectively monitored with environmental surveillance cultures and recording of patient infections. Data will be presented showing a very low environmental contamination and a low patient infection rate. This challenges the universal recommendation for the need for protected rooms. This conclusion is further supported by a care model that emphasises early patient discharge to a day care unit which does not have protected rooms.

No conflict of interest to disclose

P086

Extramedullary Relapse of Acute Myeloid Leukemia Post-Allograft

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Aims
The incidence; characteristics and prognosis of extramedullary (EM) relapse of acute myeloid leukemia (AML) post-allogenic transplant is investigated, and we evaluate the potential of PET as a tool in diagnosis.
Methods
Cases were retrospectively reviewed from a single transplant centre of patients having EM relapse of AML post allogenic transplantation from 1997-2007.
Results
Out of 72 cases of AML who had allogenic transplant, 6 cases (8%) had EM relapse. Initial diagnosis age ranged between 19 and 48 years. Five patients were in disease relapse at the time of transplant and one in complete remission. After myeloablative conditioning all received peripheral blood stem cell transplant. Five patients had GVHD prophylaxis with methotrexate and cyclosporine and one had tacrolimus. Half the patients (3) had acute GVHD grade 2-4. Five had extensive chronic GVHD and one had no chronic GVHD. Time to relapse at extramedullary sites ranged from 15 months to six years. All had normal bone marrow at the time of relapse. Sites of extramedullary relapse included testis, axillary soft tissue, epidural space, CNS, lymph nodes, retro-orbital space, bone, chest wall and stomach. PET showed high uptake in areas of EM relapse. All PET positive cases were corroborated histologically. Only one patient had subsequent bone marrow relapse. Currently, two patients are alive (11 and 20 months post EM relapse), four died after 5, 17,20 and 28 months post EM relapse.
Conclusions
EM relapse post allograft is occurring in 8% of AML allografts and with a very poor long term prognosis. EM relapse has widespread sites of presentation with a wide range in time to relapse. It appears more common in patients who had transplant in relapse and occurs despite the development of GVHD. This study also shows that PET can aid in monitoring relapse and treatment response.

No conflict of interest to disclose

P087

Post-Transplant Thrombotic Microangiopathy: Impact of New Diagnostic Criteria on Diagnosis

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Aims
To determine the sensitivity of the newly proposed Bone Marrow Transplant Clinical Trials Network (BMT-CTN) and International Working Group (IWG) diagnostic criteria for transplantation-associated thrombocytopenic microangiopathy (TA-TMA).

Methods
Patients with TA-TMA occurring post-allogeneic transplantation between December 2001 and June 2008 were identified from an institutional data-base. Haemoglobin and platelet levels, changes in blood and platelet transfusion requirements, LDH level, % fragmented cells, Coombs test results, serum haptoglobin, serum creatinine and neurological complications at time of TA-TMA diagnosis were determined retrospectively from review of patient records. Patients were then classified as per BMT-CTN and IWG TA-TMA criteria as previously published (BBMT 2005; 11: 571; Haematologica 2007; 92: 95).

Results
In total, 15 patients with TA-TMA were identified, representing 3% of all allogeneic transplants performed at our institution during the review period. A significant proportion of patients failed to meet both newly proposed criteria. The main limitation of the BMT-CTN criteria was the need for concurrent renal and / or neurological dysfunction to be manifest at TA-TMA diagnosis, which was present in only 73% of our patient cohort. For the IWG criteria, the main limitation to TA-TMA diagnosis was the requirement for a level of schistocytosis >4%, which was definitively present in only 27% of our patients. Median overall survival for the whole cohort was 79 days (range 5-1845 days), and was similar between patients who met or failed to meet either the BMT-CTN or IWG criteria (p=0.5 and p=0.78 respectively).

Conclusions
Our experience suggests that both the BMT-CTN and IWG diagnostic criteria are relatively insensitive, with both potentially misdiagnosing a significant percentage of TA-TMA. Given this diagnostic insensitivity, we caution against widespread adaptation of either the BMT-CTN or IWG criteria as they currently stand.

No conflict of interest to disclose

P088

Is a Second Transplant Worthwhile in Patients with Haematological Malignancies Relapsing After a Previous Autologous Transplant?

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Introduction
Autologous stem cell transplantation (ASCT) is intended as a single treatment procedure. Where significant remission is achieved, the question arises as to the benefit of a second ASCT at subsequent relapse.

Method
Between 1995 and 2007 inclusive, our institution carried out a total of 243 stem cell transplants (counting "tandem transplants" as a single procedure). Of the 243, 7 were true second transplants. The interval between first and second transplants ranged from 1.9-7.6 years. Patients' ages at first transplant ranged from 41-51 and at second transplant 42-55 years. Three had multiple myeloma and 4 had non-Hodgkin lymphoma (NHL). Six were males. Two patients were given transplants with blood stem cells that had been collected and cryopreserved prior to the first transplant; in the other cases the cells were collected prior to the second procedure.

Results
Comparison of stem cells reinfused for the first and second transplants showed median CD34+ cells of 2.9 and 2.0 x 10^6/kg respectively. Median neutrophil recovery (0.5 x 10^9/l) was 9.9 days and 10.4 respectively. However platelet engraftment (≥20 x 10^9litre) had a tendency to be delayed after second transplant (10.1 and 19.3 days respectively) with one patient remaining platelet transfusion dependent at 255+ days following his second reinfusion. Overall survival from first transplant ranged from 1250 to 4178 days and from the second transplant 214 to 2031 days, with six of the seven patients still alive on 30 June 2008.

Conclusion
In cases where long-term remission has been achieved by initial ASCT, a second ASCT can be considered of value for management of patients with relapsed haematological malignancies.

There is no conflict of interest to disclose

P089

Should Immunoglobulin Therapy (IVIg) be used as Prophylaxis against Infection in Multiple Myeloma (MM) Patients undergoing Haemopoietic Stem Cell Transplantation (HSCT)?

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Aim
We assessed the effectiveness of IVIg use in the peritransplant period in preventing sepsis in MM patients who had undergone HSCT.
Method
A retrospective review of consecutive patients with MM who underwent HSCT between January 2000 and December 2007. We looked at the timing of IVIg administration, febrile episodes and microbiological isolates from Day -7 to Day 30 post HSCT.
Results
208 HSCT were performed on 195 patients (137 male), median age 59 (range 34 to 79 years). 94% had associated hypogammaglobulinaemia at HSCT. 177 patients (91%) had ≥ 1 febrile episode requiring blood cultures. IVIg administration was based on clinical decisions.
The patients were allocated into 4 groups based on IVIg administration:
PRE Patients received IVIg at any time pretransplant.
PERI Patients received peritransplant IVIg (Day -7 to Day 30 post HSCT)
PREPERI Patients received IVIg pre and peritransplant.
NIL Patients did not receive IVIg.

Group	Patients	Positive Blood Cultures	Gram + organisms	Gram –ve	Fungal +
PRE	19	9 (47%)	10	4	0
PERI	69	40 (58%)	28	11	1
PREPERI	52	21 (40%)	12	6	2
NIL	55	23 (42%)	15	11	0

Culture positivity for bacterial or fungal infections was not increased in those patients not receiving IVIg replacement at any time before or during their HSCT.
Conclusion
Despite the influence of possible selection bias in this population this review suggests that IVIg in the pretransplant, peritransplant, or in both periods does not reduce the number of infective episodes for HSCT in MM.
No conflict of interest to disclose

P090

Pegfilgrastim Without Chemotherapy Effectively Mobilises PBPC Cells – Early Results of a Single Centre Study

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Aim

Pegfilgrastim (PEG-f) has proven an effective Peripheral Blood Progenitor Cell (PBPC) mobilising agent when administered the day after chemotherapy for patients with myeloma and lymphoma. We have explored the use of PEG-f without chemotherapy to mobilise PBPCs. We compared the mobilisation kinetics of PEG-f to an historical group receiving traditional filgrastim without chemotherapy.

Method

To date, 13 patients who would otherwise be eligible for filgrastim-alone mobilisation were mobilised with PEG-f and compared to 22 patients mobilised with filgrastim. All patients were not actively receiving chemotherapy and were collected for the purpose of long-term PBPC storage ('rainy day' collection). The 13 patients received a 12ug dose of PEG-f. The 22 patients mobilized with filgrastim received daily subcutaneous injections of 10µg/kg filgrastim. For all patients, monitoring commenced on Day 4, three days following initial dose of filgrastim or PEG-f.

Result

We found the median peripheral blood (PB) CD34 count on the first day of monitoring (day 4) was greater for PEG-f mobilisation (p=0.052). On days 5, 6 & 7, differences were not statistically significant, although median on each day was greater for Peg-f than filgrastim.

Day of collection	Median PB $CD34^+$ (cells/ul)	
	PEG-f	Filgrastim
4	36.15	15.75
5	33.9	28.85
6	20.75	17.95

The apheresis collection data demonstrated a pattern similar to the PB $CD34^+$ counts. The median $CD34^+$ cells collected on any given day was 1.48 $x10^6$/Kg for patients mobilised with PEG-f and 1.24 $x10^6$/Kg for patients mobilised with filgrastim. All 13 patients mobilised with PEG-f harvested a total ≥ $2x10^6CD34^+$/Kg, 10 of whom (77%) harvested a total ≥$4x10^6CD34^+$/Kg. Of the patients mobilised with filgrastim, 16 of 22 (72%) harvested a total ≥$2x10^6CD34^+$/Kg and 13 of 22 (59%) harvested a total ≥$4x10^6CD34^+$/Kg.

Conclusion

We conclude that PEG-f without chemotherapy effectively mobilises PBPCs. The study is ongoing.

No conflict of interest to disclose

P092

Bilateral Humeral Head Osteonecrosis Following Cladribine Therapy for Hairy Cell Leukaemia (HCL)

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Background/Aim
Osteonecrosis of the femoral or humoral heads is a recognised complication of chemotherapy for lymphoma with corticosteroid containing regimens and following radiotherapy. Its occurrence in hairy cell leukaemia and in the absence of corticosteroids is rare. We report a case of bilateral humeral head avascular necrosis following cladribine chemotherapy for hairy cell leukaemia and review the literature for further cases.

Result
A previously healthy 45 year old man presented with pancytopenia and was subsequently diagnosed with HCL on bone marrow biopsy. He was treated with a seven day course of cladribine, however at six months a repeat bone marrow biopsy showed a small focus of persistent disease. He received a second, seven day course of cladribine, which was uneventful until the final day when he developed right sided shoulder pain. A shoulder X-ray did not reveal any abnormalities, however a bone scan showed striking increase in radiotracer uptake in both humeral heads consistent with osteonecrosis. He was managed conservatively and made a full recovery. He did not receive steroids or radiation and there was no history of trauma, excess alcohol ingestion, hyperlipidemia or smoking. A PubMed search identified no previous reports of osteonecrosis associated with cladribine and a single case of osteonecrosis occurring several years after therapeutic splenectomy for HCL.

Conclusion
The pathogenesis of osteonecrosis in HCL and other lymphoproliferative disorders is uncertain with current opinion suggesting a contribution from drugs, metabolic factors, altered blood supply, increased intraosseous pressure, mechanical stresses and the presence of tumour. Ninety percent of cases are caused by corticosteroid therapy and excess alcohol ingestion. It has been suggested that osteonecrosis may occur at sites of tumour involvement; whether the humeral heads were involved with HCL in this patient is unknown. The close temporal relationship of cladribine therapy and the development of symptoms suggest a causal relationship.

No conflict of interest to disclose

P093

Haemophagocytic Lymphohistiocytosis (HLH) Following Elective Percutaneous Coronary Intervention (PCI), A Case Report and Literature Review

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Background
HLH is a rare, often fatal disorder characterised by dysregulation of natural killer and T-cell function, resulting in cytokine overproduction and activation of macrophages with haemophagocytosis. We report a case of HLH occurring shortly after elective PCI, possibly implicating it as a trigger for this episode.
Aim
To examine the association of HLH with PCI and clopidogrel.
Methods
Case report and systematic literature review.
Results
A 74 year old woman with a history of ANCA positive vasculitis, well controlled on cyclophosphamide and prednisolone, presented for elective PCI for stable angina. FBE was normal on admission. A loading dose of clopidogrel was administered pre-procedure then 75mg daily was commenced. The following day, the patient became febrile, developed abnormal liver function and pancytopenia. Over the next week, progressive pancytopenia continued, with a nadir in her counts day 12 post-procedure: haemoglobin 81g/L, white cell count 0.6 x 10^9/L and platelets 29 x 10^9/L. In addition, elevated ferritin (21980 ug/L, N<200), triglycerides (5.1 g/L, N<2.0) and LDH (438 U/L, N<200) were reported.
She developed mouth ulcers (which subsequently cultured HSV1). *Herpesvirus* PCR on blood was positive however subtyping was unsuccessful.
Bone marrow biopsy demonstrated marked haemophagocytosis, consistent with HLH. Treatment with dexamethasone, G-CSF, IVIG and cyclosporine was initiated. Over the next fortnight neutrophil and platelet counts returned to normal. One month later haemoglobin and ferritin had also normalised.
Conclusion
While HLH has been noted to occur following *herpesvirus* infection and autoimmune disease, the temporal relationship of this episode to PCI and clopidogrel exposure in a previously stable patient raises the possibility of causality. Clopidogrel is associated with fever, abnormal LFTs, pancytopenia and hypersensivity syndrome, however haemophagocytosis has not been previously reported. Two other cases of HLH following cardiac surgery have been reported. Whether this association is causal or purely coincidental remains to be determined.

No conflict of interest to disclose

P094

The Haemophagocytic Syndrome – Recent Experience With Diverse Aetiologies

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The haemophagocytic syndrome is a rare and life-threatening condition characterised by excessive cytokine driven activation of histiocytes and T-lymphocytes in various organs. The aetiologies of this condition are diverse, including primary or familial forms of disease in addition to secondary haemophagocytic syndromes associated with infection, autoimmune disorders or malignancy. Given the rarity of the disorder and often non specific manifestations, diagnosis can be difficult. Clinical and laboratory criteria for diagnosis are defined, with histologic confirmation of tissue haemophagocytosis being critical. Treatment involves the use of immunosuppressive therapies to interrupt underlying cytokine dysregulation, or specific treatment directed at the underlying causative process. Allogeneic bone marrow transplantation may be indicated in familial forms of the disease.

We describe our recent experience of four patients with haemophagocytic syndrome managed at the Royal Brisbane and Womens Hospital within the last 12 months. Case 1 involves a 15 year old female with a true malignant histiocytosis with associated haemophagocytosis, whose disease was refractory to escalating chemotherapeutic and immunosuppressive therapies, resulting in a rapidly fatal outcome. Case 2 describes a 38 year old female with panniculitis like T-cell lymphoma who developed fulminant hepatic failure associated with haemophagocytic syndrome. Her disease has completely responded to a combination of alkylator therapy, dexamethasone, and cyclosporine. Case 3 is a 20 year old male presenting with an advanced mediastinal non-seminomatous germ cell tumour with severe pancytopenia due to haemophagocytosis, which has been partially responsive to BEP chemotherapy. The final case involves a 22 year old female with non-familial recurrent haemophagocytic lymphohistiocytosis complicated by recurrent life threatening infections who has undergone unrelated allogeneic bone marrow transplantation.

No conflict of interest to disclose

P095

Complicated Haemolytic Anaemia in an Infant

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Aim
To describe a complicated case of congenital haemolytic anaemia highlighting the need to consider dual aetiologies.
Background
Haemolytic anaemia in a newborn may result from congenital or acquired causes. Causes of haemolysis may be obscured by combinations of such events. Anti-B is a well known cause of neonatal haemolysis generally resolving in the first month of life. Phosphoglycerate kinase (PGK) deficiency is a rare inherited enzymopathy causing haemolysis with or without mental retardation.
Case study
Baby P (male) was born at 38 weeks. There was a maternal history of one hydropic male baby followed by two miscarriages. At delivery widespread petechiae, cardiomegaly and hepatosplenomegaly were present. Initial blood count showed haemoglobin 66g/L, reticulocytes 493x x10^9/L, white cell count 19.3 x10^9/L, platelets 40x10^9/L. The blood film showed polychromasia, nucleated red cells but no spherocytosis. Baby P's blood group was B Rh(D) positive, direct antiglobulin test (DAT) negative, bilirubin 266µmol/L (0-85). The maternal group was O Rh(D) positive with a markedly elevated IgG anti-B titre of 32768. Haemolysis persisted with hyperbilirubinaemia. Baby P required frequent transfusions. Further investigations included several negative DATs and a non-diagnostic bone marrow biopsy. At three months of age he fulfilled the diagnostic criteria for Juvenile Myelomonocytic Leukaemia (JMML). By six months the JMML picture had begun to resolve spontaneously. At ten months enzyme studies showed PGK level of 16IU/gHb (195-275). Further testing revealed a new PGK gene mutation.
Conclusion
The possible causes of haemolysis in this child were anti-B and PGK deficiency. Diagnosis was complicated by the negative DATs, the JMML picture and the family history. This case illustrates that the investigation of haemolysis in a seriously ill infant is not always simple and rare causes may need to be considered.

No conflict of interest to disclose

P096

Partial Clinical But Limited Laboratory Response to Prolonged Dasatinib Therapy in Systemic Mastocytosis. A Case Report

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Aim
Systemic mastocytosis (SM) is associated with the D816V mutation on the proto-oncogene c-KIT. Dasatinib (BMS-354825) has been found to have significant in vitro activity against the KIT^{D816V} tyrosine kinase. It is now being trialled in patients with SM. We describe a single patient treated with dasatinib at our institution for twelve months.

Method
Dasatinib was obtained from Bristol Myers Squibb on compassionate grounds. Progress was assessed by regular clinical review, serial bone marrow samples, blood samples and sequential skin photographs.

Result
A 20 year old Aboriginal female presented with a widespread pruritic skin rash, anorexia, diarrhoea and weight loss. Skin and bone marrow biopsy demonstrated systemic mastocytosis. Molecular testing revealed the D816V mutation. Mast cell tryptase was 33.4mcg/L.
Treatment with dasatinib 50mg daily commenced as initial therapy, with steroid and anti-histamine 'pre-medication'. Initiation of treatment was associated with moderate self-limiting exacerbation of skin rash, pruritus and nausea. Dasatinib dose was increased to 50mg twice daily at one month.
The patient reported significant improvement in pruritis, although this was minor on clinical assessment. The weight remained stable. Sequential bone marrow samples showed a small reduction in mast cell infiltrate. The serum tryptase level remained stable. After ten months on dasatinib, the patient reported nausea requiring regular antiemetics. The patient suffered no pleural effusion or cytopenia. Dasatinib therapy ceased at twelve months because of patient preference to pursue traditional remedies.

Conclusion
The use of dasatinib for this uncommon condition appears safe. A transient flare of disease occurred with commencement of treatment and nausea was problematic after prolonged treatment. As has been previously documented, dasatinib may have a limited role in treating SM. In this case, symptoms from cutaneous disease improved on dasatinib. Other parameters, including mast cell tryptase and bone marrow histology, did not show response.
No conflict of interest to disclose

P097

Successful Rituximab Therapy in the Treatment of Refractory Cold Haemagglutinin Disease

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Background

Cold agglutinin disease (CAD)/cryoglobulinaemia is understood to be a B-cell disorder usually resistant to treatment with immunosuppressants including corticosteroids, immunoglobulin and alkylating agents, with splenectomy employed, usually ineffectively, as a subsequent line therapy.

We describe two patients with resistant and refractory severe CAD (nadir Hb of 52 and 43 g/L) and one patient with chronic refractory moderate CAD (nadir Hb 90 g/L). All patients presented to the Launceston General Hospital, Tasmania with CAD associated severe haemolytic anaemia with high LDH and bilirubin, and positive direct antiglobulin test and undetectable haptoglobin, while CAD-titre was ranging between 1:256 to > 1:2048. Bone marrow examination in all patients showed marked erythroid hyperplasia, consistent with haemolysis, with no evidence of infiltrative marrow pathology or other haematological disorders. Initial therapy with either Prednisolone in all patients, or combined with immunosupressive chemotherapy (Vincristine, Cyclophophamide, Imuran) failed to control haemolysis. Subsequent treatment with Rituximab 375mg/m2 IV weekly for four doses in two cases, and extended to 8 doses in one patient resulted in a significant improvement in both CAD and haemolysis. Splenectomy was not performed in any of the cases. All patients showed continuous remission of the disease after a median follow up of 24 months (range 14 to 26) with a current Hb range between 124-145 g/L. Rituximab (Mabthera; Roche, Basel, Switzerland) has demonstrated activity in the treatment of B cell lymphocyte disorders. This antibody, which binds specifically to the CD20 antigen expressed by B lymphocytes, can deplete B cells through complement- and antibody-dependent cellular cytotoxicity. Therefore, Rituximab is presumably active in immunoglobulin-mediated diseases of B lymphocytes, such as CAD.

Conclusion

Rituximab played a significant role in the successful treatment and long-term control of refractory CAD, avoiding the need to consider the uncertain benefit and the morbidity of splenectomy in this cohort of patients.

The authors confirm that there is no conflict of interest in relation to this clinical study

P098

Prospective Study of Abbreviated Course Rituximab for Autoimmune Disease Refractory to Standard Immunosuppressive Therapy – An Interim Report

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Background

Profound B cell depletion by rituximab has led to its use for antibody-mediated autoimmune diseases (AID) adopting standard anti-lymphoma dosing (375mg/m^2 weekly ×4). There are minimal data, however, on the efficacy of abbreviated schedules which may be more cost effective.

Aim

To investigate prospectively the efficacy of abbreviated dose rituximab in a variety of AID refractory or intolerant to conventional immunosuppression (IS).

Methods

All eligible adult patients with AID received one dose of rituximab (375mg/m^2) as part of salvage therapy. A repeat dose was indicated if no (NR) or minimal response (MR) was seen 4-8 weeks after one infusion, *or* relapse after achieving complete (CR) or partial response (PR). Follow-up (FU) was 36 months and disease-specific autoantibodies, lymphocyte subsets and serum rituximab levels were serially analysed. Concomitant IS was tapered at physician's discretion after two weeks according to response using standard criteria.

Results

29 evaluable patients (age 22-83 years) were enrolled from March 2007 to July 2008 with 66% receiving two doses. Median FU was 24 weeks (6-45). 17 were treated for haematological conditions predominantly including immune thrombocytopenia (ITP), thrombotic thrombocytopenic purpura (TTP) and autoimmune haemolysis. Responses (CR/PR/MR) were seen in 11/1/1 in this subgroup. 7 CRs occurred after a single infusion and persisted for a median of 33 weeks (range 4+ to 42+) with one late relapse. One patient died from refractory TTP. Non-haematological conditions (n=12) consisted of mostly motor neuropathy, lupus and vasculitis with responses (CR/PR/MR) seen in 1/7/1 patient(s). In the whole study population, no grade 2-4 toxicity was observed. Following a single infusion, peripheral blood B cells were undetectable from week 2 in all patients and the earliest recovery (≥0.05x10^9/L) was observed at week 24. Serum rituximab levels are in progress and will be presented.

Conclusion

The preliminary data suggest abbreviated rituximab is safe and effective in refractory AID with apparent better efficacy in haematological conditions.

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P099

Anaemia in Heart Failure: A Prospective Evaluation of Clinical Outcome in a Community Population

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Introduction
Although anaemia is well recognised to be associated with an adverse prognosis in patients with congestive heart failure (HF), the cause of the anaemia in HF and its relationship to non-cardiac as well as cardiac complications needs to be better defined, particularly in a general community population.
Methods
We followed the clinical course of 959 patients hospitalised with HF in 2004-2005 in Northern Sydney, Australia. Clinical data were collected prospectively.
Results
38% (n=369) of the patients had anaemia (Hb<120g/L). A normochromic normocytic anaemia was present in 87.8%. Of the 2/3 of patients who had more formal haematinic studies, 15.4% had a confirmed haematinic deficiency. Anaemic and non-anaemic patients were similar in age (79 vs 77 years), however anaemic patients were more likely to have elevated creatinine (48 vs 29%, p<0.001), hyponatremia (20 vs 15%, p=0.05), and LVEF >40% (49 vs 39%, p=0.004), and less likely to receive ACE inhibitors (72 vs 78%, p=0.04). Patients with anaemia had higher 12 month HF readmission rates (22.4 vs 15.7%, p=0.01) and tended to have more multiple (≥2) HF readmissions (6.3 vs 3.9%, p=0.11). Anaemic patients also had more multiple non-HF readmissions (12.4 vs 6.3%, p=0.001). The 12 month mortality was also higher for the anaemic patients (16.4% versus 10.5%, p=0.01).
Conclusion
Anaemia is common (38%) in community patients hospitalised with HF, and is associated with increased HF and non-HF readmissions, as well as increased mortality. Although most patients had normal red cell parameters, a haematinic deficiency was identified in 15.4% of patients, and is important to exclude. In conclusion, anaemia is a common, multifactorial, but potentially treatable cause of adverse cardiac and non-cardiac outcomes in patients with HF.

No conflict of interest to disclose

P100

Immunotactoid Glomerulopathy and Nephrotic Syndrome Complicating Asymptomatic Chronic Lymphocytic Leukaemia

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We report the case of a 39-year-old carpenter with Rai stage 0 chronic lymphocytic leukaemia (CLL), not requiring cytoreductive treatment for four years, who developed severe peripheral oedema four years from the time of diagnosis. Despite stable peripheral blood lymphocytosis he required monthly intravenous immunoglobulin infusion for recurrent respiratory infections associated with hypogammaglobulinaemia. Peripheral oedema was exacerbated by the infusions and was associated with hypoalbuminaemia and nephrotic-range proteinuria. A renal biopsy was performed and demonstrated an immunotactoid glomerulopathy on electron microscopy, causing a clinico-pathological picture of mesangiocapillary glomerulonephritis. Immunotactoid glomerulopathy is an uncommon entity which has a recognised association with B cell lymphoproliferative disorders and dysproteinaemias. The pathogenetic mechanism is uncertain. In some case series, improvement in the glomerulonephritis is seen with treatment of CLL. Treatments described are predominantly alkylator based. Our patient's response to fludarabine, cyclophosphamide and rituximab is described.

No conflict of interest to disclose

P101

Rituximab as a Novel Therapeutic Option for Orbital Benign Lymphoid Hyperplasia

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Introduction

Ocular adnexal lymphoid tumours include a number of histological entities including benign diffuse and follicular lymphoid hyperplasia, and malignant lymphoma. We report the first case of bilateral ocular benign lymphoid hyperplasia successfully treated with Rituximab monotherapy.

Method

Review of clinical, laboratory and radiological investigations was performed.

Results

A 60 year old lady with an initial presentation of left supra-orbital mass (in 2004) was diagnosed on tissue biopsy to have benign lymphoid hyperplasia of the lacrimal gland and surrounding tissues. Flow cytometry and molecular studies showed no evidence of clonality. MRI showed extensive lymphangiomatous infiltrates in retro bulbar and peri-orbital soft tissues bilaterally as well as extra-ocular facial and oral infiltrates. Repeat biopsy from a palatal lesion in 2007 showed similar histological features with negative flow cytometry; this time, clonal rearrangement of the immunoglobulin heavy chain (IgH) gene was demonstrated. There was no evidence of lymphoma elsewhere on radiology or bone marrow biopsy. She was treated with 8 doses of Rituximab (375 mg/m^2) at weekly intervals. A good partial response was noted with clinical improvement in proptosis, and confirmed on objective ophthalmologic measurements including exophthalmetry and visual field assessment. MRI showed complete resolution of extra-ocular infiltrates and significant reduction in size of orbital infiltrates.

Conclusion

Rituximab monotherapy may be a safe and effective treatment for ocular adnexal lymphoid tumours.

There is no conflict of interest to disclose

P102

Rituximab Therapy In Patients with Extra Nodular Manifestations of Chronic Lymphocytic Leukaemia

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Chronic lymphocytic leukaemia (CLL) is a common adult leukaemia, occurring in patients over 50 years of age with median age at diagnosis of around 65 years. While it remains an incurable disease, its indolent nature results in a varied prognosis with a median survival of greater than 10 years reported in early stage disease.

Treatment is typically initiated when patients become symptomatic including alkylating agents (chlorambucil or cyclophosphamide) but combination chemotherapy with vincristine, prednislone or fludarabine may be required in some patients. Rituximab, a chimeric anti-CD20 monoclonal antibody, has shown significant activity in a variety of B-cell lymphoproliferative disorders. The CD20 antigen is also found on B-cells in CLL and rituximab therapy has shown clinical benefit in these patients.

Extra nodular infiltration by small lymphocytes in patients with CLL is an uncommon manifestation. There is limited data on specific therapeutic strategies for treating these patients. The following case reports highlight the usefulness of rituximab in CLL patients with extranodular infiltration, with minimal additional use of chemotherapy.

We present seven patients with various extranodular manifestations including 3 patients with cutaneous lesions with one of them showing amyloid deposits; 2 patients with renal involvement including minimal change glomerulonephritis(GN) and mesangial GN; 2 patients with pulmonary nodular infiltration. All these patients received a short course of chlorambucil therapy followed by rituximab therapy with significant improvement. The therapy consisted of weekly infusions at 375 mg/m2 followed by monthly infusions for 4 months. Some of the patients have required maintenance rituximab therapy. Follow up period range: 2-8 years (median 4 years). 5 patients are alive and well and 2 patients died of unrelated causes. The surviving patients have not required any additional therapy to treat CLL or extranodular manifestations. Hence, rituximab can be used as a single agent therapy in these patients.

No conflict of interest to disclose

P103

Autoimmune Haemolytic Anaemia in Chronic Lymphocytic Leukaemia. Analysis of Patients Presenting in Sydney Northern Metropolitan Area

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Aim
Autoimmune Haemolytic Anaemia (AIHA) is a well recognised complication of Chronic Lymphocytic Leukaemia (CLL). Recent data suggest that both AIHA and a positive Direct Antiglobulin Test (DAT) may be independent risk factors for disease progression. There is debate regarding risk of haemolysis with purine analogue therapy. We analysed the patient cohort presenting to our institution providing haematology services to the northern half of the Sydney metropolitan region.

Methods
We reviewed clinical records and pathology results of patients presenting with CLL to our institution recently with AIHA. 15 patients were identified from a current cohort of 365 patients with CLL (4.1%). These cases are summarised with respect to demographics, CLL clinical stage, treatment, and complications.

Results
There were 15 patients, with a median age of 71 (range 53-86) years, with 8 males and 7 females. The clinical stage of these patients was Binet Stage A - 6, Stage B - 3, and Binet stage C - 6. All had a positive DAT. The CLL was previously treated in 6 patients prior to the onset of AIHA. All were treated for their AIHA with steroids, 4 patients required second line treatment in addition for control of the AIHA including splenectomy, intravenous gammaglobulin, cyclosporin and rituximab. 9 patients (60%) required therapy for progression of their CLL. 3 patients are deceased, 1 due to haemolysis complicated by massive pulmonary embolus, 2 due to progressive CLL.

Conclusion
AIHA was identified in 4.1% of patients with CLL. All were treated with and most responded to steroids. Most presented with early stage disease, but 60% had subsequent progression of their CLL requiring therapy. Although AIHA is a well recognised complication of CLL, data in the literature may be skewed by ascertainment bias to clinical trials and particular institutions. This series reflects patients with AIHA and CLL presenting to the principal haematology service for Sydney northern metropolitan area.

No conflict of interest to disclose

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P104

Correlation of IgVH Mutation Frequency with Zap 70 and CD38 expression in a Cohort of South Australian CLL Patients

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Aim
The degree of mutation frequency (MF) of the IgVH gene is a good determinant of prognosis in CLL patients. Zap70 and CD38 expression have also been reported as surrogate markers for CLL prognosis, however disparate results have been widely reported. This maybe, in part, due to selection of patients presenting to tertiary medical institutions. Therefore, the aims of this study were to examine IgVH Somatic Hyper-Mutation (SHM) status and correlate Zap70 and CD38 expression in a cohort of patients representing CLL in the normal population.
Method
76 CLL patients were examined for IgVH SHM status, Zap70 and CD38 expression. The IgVH gene of each patient was amplified using standard PCR, then sequenced, and the MF determined using the IMGT database. Zap70 (2F3.2-FITC UpState) and CD38 (AT13/5-A647 Serotec) were examined by Flow Cytometry with a calibrated FACS Canto (BD Bioscience). Zap 70 expression was calculated in a ratiometric analysis of MFI of $CD19^+/CD5^+$ cells/MFI of $CD5^+/CD19^-$ cells. CD38 expression was reported as MFI. Statistical analysis was by correlation coefficient.
Results
Zap70 expression and IgVH mutation frequency showed low Zap70 expression (25% percentile) correlating with high MF. High Zap70 expression (75% percentile) correlated with low MF. CD38 expression was inversely correlated with IgVH SHM status ($p<0.05$). A direct comparison between Zap70 and CD38 showed a correlation between low Zap70 and low CD38, however no trend was evident between high Zap70 and high CD38.
Conclusion
In this study there was limited correlation between Zap70 expression and IgVH SHM frequency with a relationship only evident at the upper and lower 25% percentiles, with the majority of patients falling into a grey area. CD38 expression has been proposed as an independent prognosticator in CLL and showed an inverse relationship with IgVH SHM frequency.

No conflict of interest to disclose

P105

High Frequency of Homozygous Deletion on 13q Associated with Deletion of p53 in CLL in a Cohort of 65 Consecutive Cases

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Background
The most common genetic aberrations that determine prognosis in chronic lymphocytic leukemia (CLL) include deletions of regions within chromosomes 13 [del(13q)], 11 [del(11q)] and 17 [del(17p)] with increasing risk respectively. Detection of these aberrations by routine karyotyping has been limited due to the low proliferative rate of the malignant B cells. Fluorescent in-situ Hybridization (FISH) is widely used as it can be applied to either blood or bone marrow in CLL and does not require metaphase spreads.

Methods
65 consecutive CLL cases seen at Royal North Shore Hospital were evaluated by both karyotyping and FISH using the Vysis CLL panel of probes.

Results
57% of results were abnormal by FISH vs 17% by karyotyping. 26 cases (40%) showed del(13q); 7 of which had homozygous interstitial deletions in a proportion of the cells analysed. Interestingly, 5 of these 7 cases showed deletion of 17p, compared with 4 of the 19 cases in which only heterozygous 13q loss was detected. 2 cases with del(17p) were shown to have evolved from heterozygous to homozygous loss of 13q. The karyotype was normal in the 3 cases where metaphase spreads were established. No correlation with disease stage or CD38 expression was seen.

Conclusion
Genomic instability conferred by del(17p) may account for the increased incidence of homozygous 13q loss in this series, however this was not reflected in the karyotypic analyses performed. This high incidence of del(17p) in homozygous del(13q) cases has not been previously documented. Clinical follow-up is ongoing with this patient cohort.

No conflicts of interest to disclose

P106

ATM Inactivation as a Consequence of *TP53* Mutation in B-CLL

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Background
ATM phosphorylation of TP53 is an integral step in limiting cell cycle progression and initiating apoptosis following double stranded DNA damage. Recent evidence suggests that certain 'gain-of-function' *TP53* mutations may interfere with ATM activation.
Methods
Using a novel flow cytometric assay we studied the response of B-CLL cells to DNA damage. We exposed patient samples to etoposide and measured the up-regulation of TP53 and p21 and increase in ATM phosphorylation on serine 1981 (ATM^{S1981}). ATM and TP53-related dysfunction were distinguished by incubation with etoposide and the Mdm-2 inhibitor nutlin-3a. In a separate study we demonstrate that ATM or TP53-related dysfunction is consistent with detection of mutations in the corresponding gene.
Results
We analyzed samples from 93 patients using our functional assay. We identified 54 cases as functionally normal, with 20 ATM dysfunctional and 19 TP53 dysfunctional cases. The mean percentage increase above baseline in ATM^{S1981} in the functionally normal cases was 34%. As expected 19 of the 20 (95%) cases with ATM dysfunction had low or completely attenuated ATM^{S1981} with a mean increase of only 6%. Surprisingly, 9 of the 19 (47%) cases with TP53 dysfunction had low ATM^{S1981} with a mean increase of only 15%. This was significantly lower than observed in the functionally normal cases ($p<0.005$).
Conclusions
Our data are the first evidence of an ATM feedback mechanism in B-CLL. Studies into the mechanism underlying the data, including whether this phenomenon is dependent on the site of *TP53* mutation, are on-going. Clinically FISH stratifies prognosis in CLL with deletion of 11q (ATM) and 17p (TP53) associated with worse prognosis. We suggest that this data represents further rationale for the worse outcome of patients with 17p compared to 11q loss.

No conflicts of interest to disclose

P107

8p11 FGFR1 Gene Rearranged Myeloproliferative Disorder Treated with the Investigational Tyrosine Kinase Inhibitor (TKI) Midostaurin

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Introduction

Rearrangement of FGFR1, located at 8p11-p12, with various fusion partner genes is associated with a rare stem cell myeloproliferative disorder. It usually presents in chronic phase with marked eosinophilia but progresses rapidly with a very poor prognosis to acute leukaemic transformation of myeloid, T or B lymphoblastic lineage. It is resistant to imatinib but other TKIs may be candidate therapies. We describe a case who received treatment with PKC412 (midostaurin), an investigational oral multi-TKI with activity against FLT3, PDGFRA, PKC alpha, beta and gamma, FGFR1, FGFR3 and KIT receptor tyrosine kinases, currently being studied in a phase 3 trial in FLT3-AML.

Case Report

A 63 year old female presented incidentally with prominent neutrophilia and eosinophilia. She later developed constitutional symptoms, nausea, diarrhoea, cervical lymphadenopathy, hepatosplenomegaly, low level circulating blasts, and deteriorating anaemia and thrombocytopenia. Bone marrow biopsy showed marked myeloid hyperplasia with eosinophilia with blasts < 5%. Cytogenetics revealed rare t(8;9)(p12;q33) and FGFR1 rearrangement was confirmed by BAC-FISH. The patient was considered ineligible for stem cell transplantation and after initial hydroxyurea the patient was commenced on single agent midostaurin. This produced beneficial early clinical and biological effects (improved bone pain, stabilized weight, decreased hepatosplenomegaly, reduced white cell count, and resolution of circulating peripheral blood blasts) but after 5 months of therapy the patient developed abnormal LFTs and increasing anaemia without other clinical features of disease progression and the drug was suspended. Within one week the patient showed obvious features of disease progression and CT scan showed periportal and other widespread lymphadenopathy suggesting that the initial LFT disturbance was related to disease progression.

Conclusion

FGFR1 rearranged MPD is a rare poor prognosis disease without established therapy. Midostaurin shows evidence of efficacy and in combination with other strategies may further control initial disease in patients eligible for consideration of stem cell transplantation.

No conflict of interest to disclose

P108

The Intracellular Transport Mechanisms of Nilotinib (AMN107) Are Different to Imatinib, and May Indicate a Strong Association of This Drug with ATP Dependent Efflux

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Aim
We previously demonstrated transport of nilotinib is different to imatinib, however transport kinetics remain unclear. Knowledge of proteins and processes involved is essential to the understanding of drug:drug interactions. Hence our aim was to investigate intracellular uptake and retention (IUR) kinetics of nilotinib.
Method
IUR assays were performed as previously using ^{14}C-nilotinib and imatinib. Time and temperature dependence were investigated in cell lines, and CML mononuclear cells.
Results
After 15 mins, there was no significant difference in the IUR of nilotinib between 4°C and 37°C (*p=0.649*), however after 2 hours the IUR at 37°C was significantly less than that at 4°C (*p=0.003*). In contrast the IUR of imatinib was significantly higher at 37°C than 4°C (*p=0.003 and 0.018*) at both timepoints. This suggests the uptake (first 15 mins) of nilotinib is efficient and predominantly passive, however a temperature dependent (37°C-ATP related) efflux mechanism subsequently dominates, resulting in reduction in IUR at 37°C, but not 4°C. This contrasts with the OCT-1-dependent active transport of imatinib. Investigation of nilotinib efflux using ABCG2 and ABCB1 over-expressing cell lines, revealed no difference (*p>0.05*) in nilotinib IUR between these and parental lines. Treatment with known inhibitors of ABCB1 and ABCG2 did not reverse efflux (*p>0.05*). Investigating the effect in primary cells (n=10), of proton pump inhibitors known to inhibit ATP dependent efflux, revealed a significant increase in IUR at 37°C after 2 hours (*p<0.002*) indicating reversal of efflux, not seen with imatinib.
Conclusion
While on initial exposure nilotinib is primarily taken into the cells in an ATP independent manner, longer exposure results in altered transport kinetics with ATP dependent efflux predominating. Other efflux transporters besides ABCB1 and ABCG2 are likely involved. The significant impact of drugs which inhibit ATP related efflux, may be of clinical relevance in the nilotinib setting, in contrast to the findings with imatinib.
This research was supported by Novartis Pharmaceuticals. The company had no role in analysing the data or preparing the abstract.

P109

Preliminary Testing of a Rapid Quantitative PCR for BCR-ABL Detection: The Cepheid GeneXpert BCR-ABL Monitor System

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Introduction

Real-time quantitative reverse-transcriptase PCR (RQ-PCR) detection of BCR-ABL mRNA is the gold standard for monitoring response to treatment of CML patients. The novel Cepheid GeneXpert BCR-ABL Monitor (GX) is a self-contained cartridge-based system, which utilises pre-loaded software on a personal computer to perform RQ-PCR detection of p210 transcripts in peripheral blood, in less than 2.5 hours.

Aim

To undertake a preliminary evaluation of the GX, within a routine haematological cytogenetics (non-molecular) laboratory.

Method

54 peripheral blood (EDTA) samples (1 newly diagnosed CML; 53 follow-up CMLs) were tested according to procedures and conditions provided with the GX kits. The samples were also tested in parallel at one of 4 reference sites routinely performing BCR-ABL RQ-PCR testing.

Results **QUALITATIVE PCR**

	Reference NEG	Reference POS
GX NEG	8	2
GX POS	0	1

QUANTITATIVE PCR

	Reference NEG	Reference POS
GX NEG	17	3
GX POS	0	23

- Sensitivity = 83% (24/29)

5 false negatives: 2 were p190 transcripts by qualitative PCR (not detected by GX)
2 samples were stored overnight before testing – 0.006% & 0.03% levels detected by reference lab. 5th false negative found to have a transcript level of 0.001% by reference lab (cut-off level of detection by the GX).

- Specificity = 100%
- As expected due to different methodologies, absolute transcript levels detected by the GX were different to those of the reference laboratory, but comparable when normalised to the same baseline.
- 4 Imatinib patients tested serially over 6 months showed consistent results with reference testing and GX. 3 patients showed a 1-log reduction in BCR-ABL transcripts over 6 months; 1 patient showed complete lack of molecular response.

Conclusion

Preliminary testing of the GX has yielded very promising results, prompting us to continue quantitative precision testing of the system. However discrepancies between results for samples with low-level transcript numbers require further investigation. Lack of p190 and p230 transcript detection is a disadvantage but is likely to be addressed with the second generation of instrument.

No conflict of interest to disclose

P110

DNA-based Monitoring of Minimal Residual Disease (MRD) in Chronic Myeloid Leukaemia (CML)

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Aim
To develop a DNA-based method for monitoring MRD in CML and compare it to the conventional RNA-based method.
Methods
For DNA-based monitoring, the previously-determined sequence of the *BCR-ABL* breakpoint was used to synthesise PCR primers and MRD was quantified in a three round nested PCR. For RNA-based monitoring, conventional reverse transcriptase PCR (RT-PCR) was used.
Results
Preliminary results using mixing experiments showed that DNA-PCR could accurately quantify MRD down to 10^{-6} without confounding by nonspecificity
To date, 26 samples from 20 patients have been studied by both methods during treatment. In 13 patients MRD down to approximately 10^{-4} was measured by both methods, the correlation coefficient between them being 0.85; in 5 patients disease could not be detected by either method; in 7 patients disease was quantified only by DNA-PCR; and in one patient disease was quantified only by RT-PCR. The level of detection of RT-PCR was approximately 10^{-4} whereas that of DNA- PCR was approximately 10^{-6}. For 19 samples MRD was quantified by DNA-PCR in 2 independent experiments. The SD of these replicates was 0.38 log units. Some difficulty in primer design and/or some non-specific amplification in the PCR were observed in three patients in whom *Alu* sequences flanked the *BCR-ABL* breakpoint
Conclusions
DNA-based monitoring of MRD in CML has several advantages compared to RNA-based monitoring. Collection, processing and transport of samples is simpler and more robust, reverse transcription is not required and quantification is more sensitive. The disadvantages are that extra resources are required for the initial steps of isolation and sequencing of the breakpoint and synthesis of patient-specific primers. However the cost of these initial steps may be able to be amortised over the multiple tests which are performed during monitoring of treatment.

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P111

Heterogeneity of Genomic BCR-ABL Fusion Sequences in Chronic Myeloid Leukaemia Patients

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Translocation of ABL1 to fuse with BCR is an initiating event in chronic myeloid leukaemia (CML), yet our understanding of this process is limited. It is technically difficult to sequence the genomic breakpoints of CML patients and only small series have been published.

Aim

To examine the distribution and nucleotide structure of BCR-ABL breakpoints and recombination events.

Methods

DNA samples were obtained from 37 chronic phase CML patients and 5 BCR-ABL cell lines, all expressing b2a2 or b3a2 BCR-ABL mRNA. PCR products containing the BCR-ABL fusion were identified by long PCR with a single forward primer in BCR and multiple reverse primers distributed from ABL1 exon 1b to exon 2. In a subset of 8 samples primers were used to amplify the predicted reciprocal ABL-BCR fusion on der(9).

Results

BCR breakpoints occurred in intron 13, exon 14, and intron 14 (3kb). ABL1 breakpoints occurred in intronic regions from upstream of exon 1b to intron 1a (140kb). No obvious clustering of breakpoints was identified. 62% of ABL1 breaks were within genomic repeat regions, versus 14% of BCR breaks. 62% of BCR-ABL fusions involved microhomology with 1-5 bases of identity in the BCR and ABL1 ends. The ABL-BCR reciprocal translocation was 'unbalanced' at a nucleotide level in 3/8 with duplication of normal ABL1 and/or BCR sequences adjacent to the breakpoint (median length 100bp).

Conclusions

Deletion and microhomology at the recombination site is evidence of non-homologous end-joining with 'micro'single strand annealing. Duplication of BCR or ABL1 sequences in both BCR-ABL and ABL-BCR was common in the small number analysed so far. Duplication might arise from staggered DNA breakpoints in one gene, with duplication of the sequence between the two breaks. The molecular anatomy of BCR-ABL fusions is heterogeneous. The biological and prognostic significance of this diversity warrants further investigation.

No conflict of interest to disclose

P112

Initial Experience of Alemtuzumab Used as Salvage Therapy for Etanercept-refractory Acute GVHD

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Aims
To review the outcome of etanercept-refractory acute graft versus host disease (GVHD) salvaged with alemtuzumab-based therapy at our institution.
Methods
Retrospective review of post-allogeneic stem cell transplant patients treated with alemtuzumab for refractory GVHD. Alemtuzumab was used in all cases as 3rd line therapy after failing initial therapy with methylprednisolone then 2nd line therapy with ATGAM and etanercept +/- mycophenolate. Alemtuzumab dose was 10mg subcutaneously for 5 consecutive days (BBMT 2008; 14: 10). Response was assessed at 28 days following commencement of alemtuzumab.
Results
To date, 4 patients have received alemtuzumab for refractory acute GVHD. All 4 patients had grade IV GVHD, with the predominant organ involved the gastrointestinal tract in 3 and skin in 1. At 28 days post-alemtuzumab, 2/4 patients achieved complete resolution of their GVHD, including 1 patient with gastrointestinal GVHD and the 1 patient with skin GVHD. With short follow-up (<6 months), 2/4 patients have died, including 1 responding patient and 1 non-responder. Causes of death included sepsis / invasive fungal infection (n=1) and progressive GVHD (n=1). Other significant infections post-alemtuzumab have included multifocal bacterial pneumonia complicated by abscess formation (n=1) and CMV reactivation (n=2).
Conclusions
Alemtuzumab appears to induce promising responses in GVHD refractory to multiple previous lines of therapy. Infectious complications appear significant, though not out of keeping with that expected in such a heavily immunosuppressed patient population. Further study of alemtuzumab as therapy of refractory GVHD is warranted.

Authors do not know of any conflict of interest

P113

Acute Graft-versus-host Disease after Liver Transplantation

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Aim and Method
Acute Graft-versus-host Disease (GVHD) is a very rare complication of liver transplantation. Its presenting features include pancytopenia, rash and diarrhoea. It has a high mortality and optimal management is unknown. We reported two male patients from our institution who developed acute GVHD after orthotopic liver transplantation. Their clinical features, investigations, treatments and progress, with a review of literature, were presented.

Result
Patient 1, age 57, had hepatitis C complicated by hepatocellular carcinoma (HCC). He received a liver transplant from a 27 year old female cadaveric donor.
Patient 2, age 65, had hepatitis B and HCC. He received a cadaveric liver transplantation from a 74 year old female.
Their operations were uneventful. Both patients developed pancytopenia, rash and diarrhoea one month post transplantation. Bone marrow biopsies showed marked hypocellularity with evidence of mixed chimerism. Skin biopsies were consistent with acute GVHD. Patient 1 was treated with methylprednisolone, cyclosporin and basiliximab and died six days later from multiorgan failure. Patient 2 was treated with methylprednisolone, cyclosporin, mycophenolate mofetil and etanercept. He recovered from acute GVHD, however aspergillus infection supervened, which responded to voriconazole and posaconazole. He is currently stable and well eight months after his liver transplant.

Conclusion
Acute GVHD post liver transplantation is a rare complication with high mortality, usually from bone marrow failure. Its presenting features may mimic common transplant complications such as drug side effects and infections. Hence a high index of suspicion is necessary. Risk factors include age, and in contrast to allogeneic bone marrow transplant (BMT), close HLA matching. The diagnosis remains clinical with appropriate histology and evidence of chimerism.
There is no evidenced based treatment at present and management is guided by experience in the more common acute GVHD seen after allogeneic BMT. There is evidence for etanercept use as first line therapy with steroids in acute GVHD following allogeneic BMT. There is no prior published literature on its use in the liver transplantation setting.
No conflict of interest to disclose

P114

Infusion of Mesenchymal Stromal Cells for Later Graft-versus-host Disease after Allogeneic Haemopoietic Stem Cell Transplantation

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Aims
Infusion of mesenchymal stromal cells (MSC) for acute graft-versus-host-disease (GVHD), refractory to steroid therapy, has received increasing attention and is currently the subject of an international phase III study. Local ethics approval was obtained for a phase I study of infusion of MSC in patients not eligible for the phase III study, with the aim of this study being to demonstrate safety and efficacy of the treatment.

Methods
To date, four patients have been treated. Two patients were very late after transplantation, namely seven and eight years, both had obliterative bronchiolitis together with conjunctival GVHD and the sicca syndrome. The other two patients were five and six months post transplant and predominantly had cutaneous GVHD of extensive degree, one with hepatic GVHD and one with severe oral mucosal GVHD.
MSC were culture expanded from marrow taken from the original sibling donor or, in the case of the unrelated donor transplants, one received MSC from haploidentical family donors whilst the other received MSC from an unrelated donor. MSC were characterised by morphology and phenotype. The median dose of MSC was 1.7×10^6 MSC/kg (range 0.3 - 2.1), infused fresh or cryopreserved, twice weekly for four weeks or given as required.

Results
Two patients with obliterative bronchiolitis did not improve subjectively or objectively in relation to the obliterative bronchiolitis. One of these patients had significant improvement in his conjunctival GVHD. The other two patients with predominantly cutaneous GVHD both had complete responses. One patient required repeated intermittent infusions over a period of ten months for relapses of the cutaneous GVHD, on each occasion achieving a complete response. No adverse effects were seen from infusion of MSC.

Conclusions
Infusion of MSC can be effective in later GVHD in selected cases. With the really late cases with obliterative bronchiolitis the long term scarring associated with this condition precluded a response. Further studies in early chronic GVHD are indicated.

No conflict of interest to disclose

P115

Chronic Graft-versus-host Disease and Remission Status Are Significant Factors for Survival Following HLA-matched Stem Cell Transplantation for Acute Myeloid Leukaemia

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Background and Aims

Allogeneic stem cell transplantation (SCT) for acute myeloid leukaemia (AML) is a well-established curative treatment modality. Improving survival outcomes relies on identifying factors, which limit toxicity and maximise the graft-versus-leukaemia (GVL) effect responsible for long-term disease control. This study aimed to determine factors associated with overall and disease free survival in a continuous cohort of patients with AML who underwent HLA-matched myeloablative or non-myeloablative SCT.

Methods

Eighty-six (n=86) patients underwent HLA-matched myeloablative or non-myeloablative SCT for AML between 1996 and 2008 at this institution. Conditioning regimens included cyclophosphamide/TBI, busulphan/cyclophosphamide or fludarabine/melphalan. Cord blood transplants were excluded. Combination GVHD prophylaxis was used (cyclosporin, methotrexate, prednisone) with GVHD scored using standardised CIBMTR guidelines. Data sets were analysed using multi-variate Cox regression models, Kaplan-Meier analysis & the log-rank test.

Results

Forty-three of 86 patients (50%) have survived with a median follow-up of 33.4 months. Median age was 42 years (range 15-67). Thirty patients (35%) received unrelated transplants. Sixteen patients (19%) received non-myeloablative conditioning. The only significant factor for overall survival was patients in any remission (CR1 or greater) compared with relapsed or refractory disease (Relative Risk(RR) 2.2, p=0.01). Significant factors for disease free survival were the presence of chronic graft-versus-host disease (RR of relapse/death 0.3, p=0.04) and patients in first CR (RR 0.281, p=0.005). No other factor including age, CMV status, conditioning intensity or donor characteristics (including related/unrelated) were significant factors in multi-variate analysis.

Conclusions

This analysis confirms the importance of disease stage and chronic graft versus host disease as significant factors in survival for patients transplanted with AML. The presence of chronic GVHD could be used as a surrogate marker for the graft-versus-leukaemia effect and underlines its importance in disease control. Donor source and conditioning regimens are not significant factors in survival consistent with recently published data.

No conflict of interest to disclose

P116

Detailed Profiling of Allogeneic T Cell Phenotype in HLA-Matched and -Mismatched Donor/Recipient Pairs

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Aim
Allogeneic bone marrow transplantation is commonly utilised for the definitive treatment of leukaemias and lymphomas. Graft-versus-host disease (GVHD) is the major risk of such an approach, resulting in considerable morbidity even in sibling HLA-matched donor/recipient pairs. In vitro analysis of allogeneic T cell behaviour may predict the onset of acute GVHD in bone marrow transplant (BMT) recipients.
Method
In order to define parameters for subsequent HLA-matched BMT, we utilised the mixed lymphocyte reaction (MLR) of 20 healthy HLA-mismatched responder/stimulator pairs to monitor the emergence of alloreactive effector $CD4^+$ and $CD8^+$ T cells by multiparameter flow cytometry, including central memory and effector T cell subsets, and $CD4+CD25^+CD127^-FoxP3^+$ regulatory T cells (Tregs). Additionally, Th1/Th2 cytokine secretion profiles were measured on days 2 and 7 of MLR by cytokine bead array. Several HLA-matched siblings were tested prior to BMT to examine their alloreactive responses in comparison with HLA-mismatched MLR.
Result
Allogeneic T cells stimulated in HLA-mismatched MLR gained an effector phenotype ($CD3^+CD8^+CD27^-CCR7^-CD45RA^+perforin^+$) after 7 days, and secreted the Th1 and Th2 cytokines IL-2, IL-4, IL-5, IL-10, IL-13, IFNγ and TNFα. Upregulation of IFNγ, IL-13 and IL-2 secretion correlated with alloreactive T cell proliferation. In four HLA-matched MLR, allogeneic T cell proliferation and cytokine release was minimal, although TNFα secretion by donor T cells was elevated in comparison to HLA-mismatched MLR levels. Interestingly, Tregs were present at baseline and day 7 of both HLA-matched and -mismatched MLR.
Conclusion
Our studies have defined the emergence of alloreactive effector $CD4^+$ and $CD8^+$ T cells, in addition to the typical range of cytokine production in HLA-mismatched pairs. These findings have provided parameters for predicting GVHD in potential BMT donor/recipient pairs. We are currently testing further HLA-matched sibling pairs to identify the secretion of key cytokines that may influence post-transplantation complications.

No conflict of interest to disclose

P117

GSTM1 and GSTT1 Gene Polymorphism in Patients with Bone Marrow Failure Syndromes in North India

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Introduction
Bone marrow failure syndromes (BMFS) comprise of aplastic anemia and myelodysplastic syndromes (MDS). BMFS are classified as constitutional, acquired secondary and acquired primary subtypes. The definition of exact etiology is important for the obvious reasons of proper management, family screening and genetic counseling etc. Constitutional factors reportedly contribute to BMFS in nearly 1/3 of young patients. The acquired factors include drugs, chemicals, toxins, infections (mainly viral) etc. The exact frequency of multiple etiological factors, especially constitutional, is not well documented. Most of the genetic predisposition syndromes (chromosomal instability syndromes, constitutional chromosomal disorders and toxin modifying enzymes variants etc.) require specific diagnostic tests and many (like Fanconi anemia) require modified treatment strategy. Data on etiological factors is largely not available from Indian subcontinent.

Materials and methods
The PCR genotyping for GSTM1 and GSTT1 (toxin modifying enzymes variants) was undertaken in 112 aplastic anemia patients, 50 MDS patients and 106 normal healthy controls, as a part of Department of Biotechnology sponsored project.

Results
Normal GST genotype was detected in 57 aplastic anemia patients, 23 MDS patients and 88 control individuals. GSTT1 null genotype was found in 12 aplastic anemia patients, 10 MDS patients and 8 control individuals ($p>0.05$). Prevalence of GSTM1 null genotype was found to be significantly more in BMFS (26 aplastic anemia and 21 MDS patients) as compared to 8 control individuals ($p<0.01$). Composite GSTM1 and GSTT1 null genotype was found in 4 aplastic anemia patients.

Conclusions
GSTM1 null genotype most likely contributes to the causation of BMFS in our patient population.

No conflict of interest to disclose

P118

Compartmentalization of Allogeneic-T cell Responses in the Bone Marrow and Spleen of Humanized NOD/SCID Mice Containing Activated Human Resident Myeloid Dendritic Cells

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The human allogeneic (allo)-T cell responses within recipient lymphoid tissues and the degree to which they are altered by activated tissue-resident dendritic cells (DC) remains unknown. This study examined human allo-T cell recruitment and the early allo-T cell responses within the bone marrow (BM) and spleen (SP) of humanized (hu)NODSCID recipients containing activated human tissue-resident myeloid (M)DC.
Human naïve allo-T cells were transferred into poly(I:C) treated or untreated huNOD/SCID recipients containing human tissue-resident DC derived from transplanted $CD34^+$ cells. The activation of human tissue-resident MDC by poly(I:C) treatment, and the recruitment, proliferation and effector differentiation of allo-T cells in the BM and SP of huNOD/SCID recipients were analysed *in vivo* by flow cytometry.
Poly(I:C) treatment induced transient activation of human MDC within a maximum of 8 hours, evident in the BM as an increased proportion of MDC expressing CD86 and in the SP by MDC expressing CD86 and producing IL-12. Poly(I:C) pretreated huNOD/SCID recipients recruited allo-T cells into both the BM and SP but developed different allo-T cell responses within the BM and SP compartments. In the BM, allo-T cells underwent multiple divisions and increased numbers of IFN-γ^+ and TNF-α^+ effector cells, whereas the majority of splenic allo-T cells underwent a single division and had fewer effector allo-T cells. This experimental transplantation model demonstrates that early human allo-T cell responses are regulated by compartmentalization in the BM and secondary lymphoid tissues such as spleen; events that provide potential insights into clinical transplant reactions.

No conflict of interest to disclose

P119

Mechanisms of Histone Deacetylase Inhibitor induced Thrombocytopenia

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Aims
Histone deacetylase inhibitors (HDACi) are novel anti-cancer agents which have undergone rapid clinical development in phase I/II trials. These drugs have single agent efficacy in relapsed/refractory acute myeloid leukaemia, hodgkin lymphoma, myeloma and cutaneous T-cell lymphoma. Reversible thrombocytopenia is a consistent dose limiting toxicity for this class of drugs, and does not appear to be due to a direct cytotoxic myelosuppressive effect, and may limit future combination treatment strategies. We aim to dissect the mechanisms of thrombocytopenia, to see if it may ultimately be circumvented.

Methods
More than 150 BALB/c and C57BL/6 mice, in several groups, have been dosed with daily intra-peritoneal panabinostat (LBH-589) and controls at a range of concentrations (10-20mg/kg) on weekly and alternate weekly schedules. Full blood examinations were performed daily in consecutive groups throughout treatment. Serum thrombopoetin (TPO) levels were measured by ELISA. The age profile of platelets was measured using thiazole orange (TO) to stain younger, reticulated platelets prior to quantitive flow cytometry.

Results
Both C57BL/6 and BALB/c mice treated with panobinostat have predictable, progressive reduction in platelet counts compared to controls, from the normal range of 900-1100x10^9/L to a nadir of 200-400x10^9/L at five days (P=<0.001). Further dosing only results in a plateau in levels rather than further reduction. On cessation, there is a rapid rebound thrombocytosis (1300-1500x10^9/L) prior to normalisation over a week. Re-challenge with panobinostat results in an identical pattern of thrombocytopenia. TPO levels appeared to be unaffected by administration of panobinostat. TO staining and bone marrow are underway and will be presented.

Conclusions
Panobinostat induced thrombocytopenia is predictable, and appears to be self limiting regardless of dose. Endogenous TPO levels do not alter in response to the thrombocytopenia. Further investigations will focus on whether effects are intrinsic to effects on platelets themselves or production by megakaryocytes and pro-platelet formation.

No conflict of interest to disclose

P120

Acute Myeloid Leukaemia (AML) as a Model to Dissect MicroRNA Transcription Networks During Myelopoiesis

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Aim
To investigate the role of miRNAs in the characteristic differentiation block observed in AML using microRNA (miRNA) and mRNA microarray analysis of normal karyotype AML
Methods
28 diagnostic bone marrow samples were obtained after informed consent. Mononuclear cell fractions averaging 75% blasts were isolated and subjected to miRNA microarray analysis using the Exiqon platform. Computational mRNA targets of differentially expressed miRNAs were identified and correlated with mRNA array data published by Valk, *et al.* N Engl J Med. 2004.
Results
69 human miRNAs were found to have a significant change (< or > 1.5 fold) in AML samples when compared to normal bone marrow. Clustering of miRNA expression profile showed poor clustering of all FAB subtypes present, except, 9 miRNAs were able to segregate the M1 (n=7) and M5 (n=7) FAB subtypes. mRNAs that were differentially expressed between these two FAB subtypes were identified in the microarray database. Computational mRNA targets of the 9 miRNAs were then correlated with this data to identify putative miRNA:miRNAs pairings. Two sets of pairings were found to link with myeloid and monocytic differentiation respectively. These differentially expressed miRNAs may explain the respective monocytic and myelocytic differentiation block seen in the M5 and M1 subtypes respectively.
Conclusions
miRNAs have been shown to be extremely important in haematopoiesis and the development of cancer. We show the potential of miRNA profiling of subtypes of AML as a model to investigate haematopoiesis, particularly in the differentiation block observed in these AML subtypes.

No conflict of interest to disclose

P121

Anti-IgD Antibody is a Novel Modulator of T-Bet in Human Peripheral Blood Mononuclear Cells

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Aims

The transcription factor, T-Box expressed in T-cells (T-Bet), is a regulator of T-helper 1 (Th1) immune responses, which are enhanced in several autoimmune and inflammatory conditions. Experiments with murine models have demonstrated that treatment with anti-IgD antibody induces a bias towards a Th2 response. However, the effects of anti-IgD antibody on human Th1/Th2 immunity are unknown. The aims of this pilot study were to determine:

1. effects of anti-IgD antibody treatment on T-Bet expression in human peripheral blood mononuclear cells (PBMCs)
2. expression levels of surface membrane IgD (smIgD) on PBMCs.

Methods

Peripheral blood (45 mL) was collected from 13 healthy males (n=6) and females. PBMCs, B-cells and monocytes were isolated using standard techniques. The effects of anti-IgD antibody (10υgm/υL to 100υg/υL) on T-Bet were determined by RT-PCR and western blot. Expression of smIgD on each cell population was examined by dual-immuno-fluorescent labelling flow cytometry, using PE and FITC conjugated antibodies specific for cell markers and smIgD respectively.

Results

Using RT-PCR, anti-IgD had a dose-dependent modulating effect on T-Bet expression when cells were stimulated with PMA and ionomycin. Using flow cytometry and immunocytochemistry, B-cells and monocytes appeared to express IgD, or an IgD-like molecule, on the cell surface.

Conclusions

This pilot study is the first to describe the effect of anti-IgD antibody on T-Bet expression levels in human PBMCs. Results indicate a dose-dependent effect of anti-IgD on T-Bet expression. Successful control of the expression of T-Bet, and hence down-regulation of the Th1 immune response, may have important implications for future treatment of autoimmune and inflammatory conditions.

No conflict of interest to disclose

P122

The Th1 Regulatory Molecule, T-Bet, is Increased *In Vitro* by Recombinant Human Osteopontin in Peripheral Blood Mononuclear Cells

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Aims
T-Box expressed in T-cells (T-Bet) is a transcription factor that has an essential regulatory role in T-helper 1 (Th1) immune responses, such as those seen in Rheumatoid Arthritis (RA). Osteopontin, a phosphoprotein, is highly expressed by fibroblast-like synoviocytes in RA synovium. While osteopontin is associated with bone resorption and tissue remodelling, its role in RA is unknown. The aim of this project was to determine whether recombinant human osteopontin (rhOPN) alters the gene expression of pro-inflammatory cytokines or molecules associated with regulatory function in unstimulated peripheral blood mononuclear cells (PBMCs) *in vitro.*
Methods
Peripheral blood (40mL) was collected from 12 healthy adults. PBMCs were isolated and cultured for 24 hr at 37°C with and without rhOPN (1ug/mL). Cytokine gene expression was determined by RT-PCR and PCR, using primers for IL-1β, IL-2, IL-6, IL-12, TNF-α, IFN-γ, T-Bet, CD25, Foxp3, TGF-β, and IL-10.
Results: The gene expression of T-Bet was consistently and significantly increased by exposure to rhOPN ($p<0.01$). Densitometric analysis of gels revealed an approximate 4-fold increase in T-bet gene expression in response to rhOPN. The other molecules tested showed no significant alteration in gene expression in response to rhOPN.
Conclusions
Previous research has indicated that T-Bet is associated with T-cell activation, induction of a Th1 cellular immune response and trafficking of immune cells into inflamed tissue. Our results demonstrate an increased expression of T-Bet gene expression in response to osteopontin and suggest that rhOPN may have a pro-inflammatory effect on PBMCs. Osteopontin may thus enhance the infiltration of immune cells into the rheumatoid synovium.

No conflict of interest to disclose

P123

The Right Test for the Wrong Person at the Wrong Time - Haemoglobinopathy Testing in the Hunter Area

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Aim
Review indications for haemoglobinopathy testing, as performed by Hunter Area Pathology Service.
Method
A cross-sectional audit was performed of all haemoglobinopathy testing requests received by Hunter Area Pathology Service during 2007. Laboratory workflow sheets, Laboratory Information System (LIS) and area-wide patient database (Clinical Access Portal, CAP) for all haemoglobinopathy requests were reviewed.
Results
339 haemoglobinopathy requests were received in 2007. Stated indications were: microcytosis (15%, n=52); anaemia (14%, n=46); provisional diagnosis of haemoglobinopathy (36%, n=121); African refugee status (23%, n=78); no clinical information (6.5%, n=22). 71% of assays performed to confirm a provisional diagnosis of haemoglobinopathy were normal (87 out of 122). Twenty two of 31 patients (71%) who had haemoglobinopathy testing for microcytosis were found to have thalassaemia. Two patients with microcytosis were actually identified as sickle cell traits. One out of thirteen patients investigated for polycythaemia was found to have Haemoglobin Kempsey. 32% of patients investigated for microcytosis and/or anaemia had not had iron studies performed. Only one patient had haemoglobinopathy testing performed in the first trimester of pregnancy. Pre-anaesthetic assessment was not listed on any request forms.
Conclusion
Published guidelines outline indications for haemoglobinopathy testing. This audit highlights differences between such “best practice” and current practice. Census-based statistics demonstrate likely under-diagnosis of patients within the Hunter region. Important indications such as pre-natal counselling and pre-operative risk assessment are under-represented. Education of health professionals in regards to guidelines for haemoglobinopathy testing is a current and future priority aiming to minimise morbidity and mortality associated with Haemoglobinopathies.

No conflict of interest to declare

P124

A Study of HFE Gene Mutations in Iron Deficient Females

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Aim
Since HFE gene mutations in hereditary haemochromatosis are associated with increased iron absorption, this study was carried out to determine whether these mutations and correspondingly iron deficiency and anaemia would be less frequently observed in iron deficient patients presenting for intravenous iron therapy.

Method
Following ethics committee approval and informed consent, EDTA blood samples were obtained from 196 cases of females (ages 16 – 70 years) with iron deficiency (ferritin at referral <30ug/L), (4 cases were excluded, patient questionnaire not completed). Tests were carried out for Hb, ferritin and HFE, the latter by standard PCR methods using sequence specific primers capable of identifying 3 HFE mutations (C282Y, H63D, S63C).

Results
Heterozygous C282Y with mean Hb 112.6g/L (range 69-137g/L), ferritin 33ug/L (range 2-35ug/L) was identified in 6.8% of the group (expected gene frequency 14.1%). 80/132 (60.6%) patients with the normal allele and 5/13 (38.5%) C282Y carriers had a normal Hb.
Heterozygous H63D with mean Hb 140g/L (range 70-144g/L) mean ferritin 16.4ug/L (range 3-53ug/L) was identified in 22.4% (expected 23.6%).
66.8% of patients with wild-type HFE who acted as controls had a mean Hb of 114.95g/L (range 66-140g/L) and a mean ferritin 16.66ug/L (range 3-76ug/L). Other data to be supplied.
Using Fisher's exact test, no statistical difference was present when the Hb and ferritin of the iron deficient control group was compared with those heterozygous for C282Y and H63D.

Conclusion
The presence of the HFE gene mutations in iron deficiency does not appear to confer a superior advantage in terms of iron stores or Hb when compared to iron deficient females with a normal HFE genotype. However while not statistically significant there was a trend towards a higher Hb in the C282Y carrier iron deficient group. It is possible that this study was not powered to detect small changes and a larger survey may be necessary.

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

P125

Serum Prohepcidin and Ferritin in Myelodysplasia

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Aim
Hepcidin formed from its precursor, Prohepcidin is a key regulator of iron metabolism.Dysregulation of the latter and transfusion dependent anaemias together with iron overload are features of the Myelodysplastic Syndromes (MDS), in particular Refractory Anaemia with Ring Sideroblasts (RARS).The present study was organised to understand the relationship between hepcidin and ferritin and determine the value of measuring hepcidin in MDS.
Method
EDTA and serum samples were collected from 19 patients with MDS (RA 8,RARS 8,RCMD 3),7 patients were transfusion dependent,65 patients with miscellaneous haematological diseases and 58 female patients with iron deficiency (total,142).Serum ferritin was measured using a chemiluminescence immunoassay ECLIA (Roche Modular E170).Prohepcidin was measured by an ELISA assay (DRG International Inc,Mountainside,NJ).
Results
23/33 patients with a raised se prohepcidin (NR: 58-158ng/ml) had a high ferritin (>450ug/l), in contrast to 18/39 who showed a low ferritin (<30ug/l). A 3 way table comparison of low, normal and high ferritins and prohepcidin was statistically significant. Scatterplot analysis revealed a Pearson r coefficient value of 0.19 in keeping with a non-linear relationship between prohepcidin and ferritin in the studied groups.
Conclusion
This study while demonstrating an association between hepcidin and ferritin failed to show a significant correlation between the two in MDS and may reflect the heterogeneity of the latter with its varying degrees of anaemia and iron overload.

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

P127

Innovations in the Haematology Outpatient Service at Christchurch Hospital, Christchurch, New Zealand

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Introduction
I took over the position of Charge Nurse Manager in July 2006. One of my challenges in this new position was to address the issues of the Haematology Outpatient Service and implement change to improve patient flow and to develop patient centred care.

Problems
The role of the outpatient nurse who had worked in isolation for many years and had little opportunity for professional development. The service needed reviewing to accommodate the increasing number of patients being seen in Haematology Outpatients.

Solutions:
The nurse underwent a performance review. Based on the outcome of this review a plan was implemented to address the issues that were identified. The nurse was booked in and has attended a number of professional development courses. This is an expectation of the nurse to fulfil the competencies of practice outlined by the Nursing Council of New Zealand. The nurse was also was mentored by both the Clinical Nurse Specialist in the Bone Marrow Transplant Unit and the Haematology Day ward.
A business proposal was developed by me and the Clinical Nurse Specialist in the Haematology Day Ward and this is currently being implemented to initiate change. This business proposal includes addressing the lack of space, introducing Nurse led education session and improving patient flow between the Haematology Outpatient Service and the Haematology Dayward.

Conclusion
This is an exciting time for the service however, as with any change there have been challenges. There has been compromise made to ensure that this area continues to develop and meet changing needs of the service.

P128

Role of Flowcytometry in the Diagnosis of Chronic Lymphoproliferative Disorders: An Indian Perspective

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Objective
The diagnosis of chronic lymphoproliferative disorders (CLPD) requires analysis of multiple parameters: cell morphology, tissue histopathology, immunological markers, molecular/cytogenetic investigations. There is paucity of information regarding diagnostic and prognostic parameters of CLPDs from Indian sub-continent.
Study Design
A prospective study was carried out to analyze the peripheral blood, bone marrow aspirate morphology, trephine biopsy infiltration pattern and lymphoid cell flowcytometry (FCM) findings in CLPD patients. These findings were compared with few other disease parameters.
Results
CLPDs comprised of 11.03 % of hematological malignancies diagnosed between January 2005 and December 2006 at our institute. Chronic lymphocytic leukemia (CLL) and hairy cell leukemia (HCL) were the common CLPDs out of a total of 91 patients. According to morphology and flowcytometry, 70 cases were diagnosed as CLL, 14 as HCL and rest of the 7 cases included in 'miscellaneous' category. Sixty nine CLL cases showed typical CLL immunophenotype. All 14 but one HCL case revealed typical HCL immunophenotype. The CLL cases were divided according to Rai and Binet staging systems, and correlation of various stages with other prognostic parameters like trephine biopsy patterns, CD 38 positivity, serum lactic dehydrogenase (LDH) level, lymphocyte doubling time (LDT) and Coomb's test was analyzed. The trephine biopsy pattern showed significant statistical association with staging systems (p=0.016 and p=0.005). The other prognostic markers did not show significant statistical association with staging systems hence their role as independent markers cannot be excluded.
Conclusion
The advantages of FCM are being realized increasingly in India, therefore this communication is likely to serve as a baseline data in our setup.

No conflict of interest to disclose

P129

Chronic Falciparum Malaria

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Infection with *Plasmodium falciparum* is well known as a cause of acute malaria among travellers from endemic areas, such as Africa and South-East Asia.
Chronic infection may occur in endemic areas among those with a degree of partial immunity, and cases have been reported in people who left an endemic area up to 5 years previously. We report a case of chronic falciparum malaria in a 15 year-old boy who had not been out of Australia for 5 years, had not been near an airport nor had overseas visitors. Our patient had lived in Africa some years ago and had not returned since.

Many immigrants are asymptomatic *P*. *falciparum* carriers . Their immunity probably prevents clinical symptoms . In the absence of reinfections their immunity would decrease and symptoms would occur. The patient is HIV positive. In patients with HIV, susceptibility to malaria and parasitaemia increase as immune responses fail. The patient's "apparent" neutropenia is also discussed. Neutropenia in African patients should not be assessed according to Caucasian reference ranges. The neutrophil count is significantly lower in black Africans than in Caucasians and other ethnic groups.

Neutropenia due to increased neutrophil margination can be seen in malaria. The neutropenia here may be multifactorial including ethnic neutropenia, increased neutrophil margination and marrow suppression by HIV +/- HIV therapy.

In conclusion, the patient reported is a HIV positive African man who presents with chronic falciparum malaria and ethnic neutropenia.

No conflict of interest

P130

Unsuspected Histopathological Lesions in Bone Allografts

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Background and Aim

Bone donors are predominantly elderly patients undergoing elective hip surgery. Most blood donor selection criteria apply. Other evaluation, including histopathological examination of bone, is also done. These were reviewed to determine occult lesions in bone in these subjects.

Methods

Retrospective analysis of bone allograft histopathology reports at Christchurch Bone Bank between 2006 and 2007. Donors had no obvious/known haematological disease. Decalcified, paraffin-embedded bone core biopsies and synovial specimens stained with H and E were examined.

Results

There were 489 specimens from 465 patients including 24 from 12 subjects who had bilateral surgery. Donor age ranged from 24 – 92 years (mean 66 y). 54.2% were females and 45.8% males. 448 (91.6%) specimens showed no abnormalities (other than osteoarthritis) while 29 (5.7%) did. None showing an abnormality was from a patient who had bilateral surgery. 13 (2.6%) specimens failed for technical reasons. Overall, the mean age of both those with and without abnormalities was 66 years. 12/29 (41.3%) abnormalities were in subjects > 70 years. The youngest donor with an abnormality was 42 years. 20 (4.0%) of specimens showed increased lymphocytes / plasma cells such that a lymphoproliferative or autoimmune disorder could not be excluded (mean age 65 y). In 5/20 there were paratrabecular lymphocyte collections. 3 (0.6%) had features suggestive of myeloproliferative / dysplastic disorders (mean age 64 y). 6 (1.2%) had other abnormalities (mean age 71 y) - 2 had crystal aggregates consistent with pseudogout or chondrocalcinosis, 2, non-necrotising granulomas and 1, an acute and chronic inflammatory infiltrate. No specimen showed avascular necrosis (though 7 had radiological evidence), tumour or metabolic bone disease.

Discussion and Conclusions

As expected and similar to the few reports on the subject, our results show that unsuspected histopathological abnormalities in bone occur in older subjects some of whom may have unrecognised, possibly pre-clinical haematological disease.

No conflict of intereest

P131

A Case of EDTA and Temperature Dependent Pseudo-neutropenia

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We present a case of pseudo-neutropenia due to EDTA-dependent neutrophil aggregation in a 47-year-old man with chronic renal failure. The patient first presented in 2002 with idiopathic end stage renal failure. A haematology referral was made in 2008 for investigation of long standing neutropenia. The patient's neutrophil count over this time had ranged from 0.2 to 0.8 x 10^9/L, as assessed by an automated full blood analyser (Abbott Cell-Dyn 4000). There was no history of recurrent infections. The neutropenia had been previously investigated in 2005, including autoimmune studies and a bone marrow biopsy, with no cause identified.

In the most current investigation, the patient's peripheral blood film revealed the presence of numerous small neutrophil aggregates. Retrospective review of the available previous films showed this to be a consistent unreported finding. To further investigate the phenomenon, we performed a fresh 37°C blood collection in both EDTA and citrate collection tubes. The immediate automated neutrophil counts at 37°C were 2.34 x 10^9/L in EDTA, and 2.52 x 10^9/L in citrate. Allowing the samples to cool to room temperature for 1 hour, decreased the neutrophil count to 0.71 x 10^9/L in EDTA, with no change in the citrated specimen. There was incomplete restoration of the neutrophil count on rewarming the EDTA sample to 37°C. Patient serum was further investigated using the Granulocyte Immunofluorescence Test (GIFT) and the Granulocyte Agglutination Test (GAT). Both the GIFT and GAT were positive using a granulocyte panel from four random blood donors.

In this case we have demonstrated EDTA- and temperature-dependent neutrophil aggregation, associated with the presence of anti-neutrophil antibodies in patient serum. Although pseudo-neutropenia is rare, the case reinforces the importance of careful blood film examination in preventing unnecessary and invasive investigations due to spurious automated counts.

No conflict of interest to disclose

P132

Disease Characteristics and Flowcytometric Analysis of Paroxysmal Nocturnal Hemoglobinuria (PNH) cases from North India

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Introduction
Classical Paroxysmal nocturnal hemoglobinuria (PNH) is diagnosed in the setting of strong clinical suspicion, using certain conventional and newer tests. Conventional tests include Ham's acidified serum test (HAST), sucrose lysis test (SLT) and urine hemosiderin. In addition flowcytometric (FCM) analysis of RBCs and WBCs is performed using anti – CD 55 and anti – CD 59 monoclonal antibodies (moab).

Aim
To analyze the disease characteristics and flowcytometric results of classical PNH cases.

Method
Between January 98 and December 2006, tests for PNH were conducted for 198 adult patients in our institute. Clinical details, blood counts and bone marrow findings were noted, in addition to the results of conventional and FCM tests.

Result
Thirteen cases fulfilled the diagnostic criteria of classical PNH. FCM, HAST and SLT were positive in all. Presenting complaints mostly related to cytopenia/s and intravascular hemolysis; only one patient suffered thrombotic event. Median duration of onset of symptoms to diagnosis and to last follow up was 3 years (range 6 months – 10 years) and 4.5 years (range 12 months- 11.4 years) respectively. Bone marrow aspiration and trephine biopsies were hypercellular in 9, normocellular with variable cellularity in 3 and hypocellular in 1 patient. Erythroid hyperplasia was observed in all. Using FCM, three PNH cell populations were detected in 5 patients (38.46%), rest 8 showed two PNH cell populations. PNH III populations in the RBCs and neutrophils ranged between 7-60% and 13-61% respectively.

Conclusion
Different composition of PNH cell types (a mixture of type I, II and III cell populations in 38.46% patients in our study) and rarity of thrombotic events emerge as important features of PNH patients diagnosed at our institute. It is important to make a distinction between classical PNH and 'PNH defect' detected in other hematological disorders.

No conflict of interest to disclose

P133

Interpretation of Full Blood Count (FBC) – Results of a Survey at the Australian National University (ANU) Medical School

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Introduction
With the introduction of problem-based learning (PBL) in medical curricula, students are called upon to interpret investigations such as Full Blood Count (FBC) early on. This study aimed to assess situations in which students at ANU Medical School were required to interpret FBC, and their ability and level of confidence.

Methods
Separate student and staff e-surveys were used; student surveys were designed to assess situations in which students required to interpret FBC, determine ability using an optional problem solving exercise, and perceived level of confidence. Staff surveys aimed to assess perceptions on students' abilities in using this skill.

Results
Of 72 student respondents, more [70 (97.2%)] reported using FBC interpretation within PBL sessions than within clinical situations [35 (48.6%)]. Some degree of difficulty in interpretation was reported by 52 (76.4%). Of 66 (91.7%) students who answered the optional interpretative question, depth of interpretation varied with 65 (98.5%) identifying anaemia, 38 (57.6%) microcytic anaemia, and 17 (25.8%) microcytic hypochromic anaemia. Forty three (65.2%) provided one, and 31 (47%) two correct differentials. Using a 1-5 scale confidence scale (5 most confident), 24/68 (35.3%) respondents scored 3, 26 (38.2%) scored <3 and 18 (26.5%) >3. Academic staff surveyed (n=25) gave non-committal (9), categorical positive (4), categorical negative (4) and conditional negative responses (8) regarding student skills in FBC interpretation.

Conclusions
Students are required to interpret FBC early on within new medical curricula. A large proportion of students report difficulties with this skill. Although simple cases are solved with ease and confidence by students, greater effort is required to improve depth of interpretation, and level of confidence.

No conflict of interest to disclose

P134

A Study in the Use of Recombinant Human Haemopoietic Growth Factors to Improve Myeloid Cytogenetic Cultures: A Western Australian Experience

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The proliferation, maturation and survival of bone marrow precursor cells in vivo require the presence of specific growth factors. The aim of this study was to improve the mitotic index and chromosome quality of our current bone marrow cultures using growth factors added in vitro.
To achieve this a combination of recombinant human haemopoietic growth factors (GF) were added to the culture medium. The growth factors used were granulocyte colony stimulating factor, granulocyte-macrophage colony stimulating factor, stem cell factor and interleukin-3. This combination was selected based on previous published studies.
Twenty four-hour short-term synchronised cultures were performed in RPMI supplemented with 20% bovine serum. Forty four paired parallel bone marrow cultures were analysed in a blinded fashion and the GF cultures compared with the current (reference) cultures.
Cultures containing the growth factors had an improved banding score and higher mitotic index, whilst maintaining the same percentage of abnormal cells when compared to the reference cultures. This improvement in quality should result in optimisation of time taken for slide making, metaphase selection and analysis of both normal and abnormal cells. This should have efficiency and cost savings for the laboratory. The improved chromosome quality should enable the detection of additional chromosome abnormalities over time.

No conflict of interest to disclose

P135

Response of E459K BCRabl Mutation to Tyrosine Kinase Inhibitors

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Aim
The first generation Tyrosine Kinase Inhibitor (TKI) Imatinib is now the treatment of choice in Chronic Myeloid Leukaemia (CML). However many patient's develop resistance, most commonly through mutation of the ABL kinase domain that disrupts drug binding or favours the active conformation of the kinase. Second generation TKI's such as Dasatinib and Nilotinib have been developed to overcome resistance but have varied activity on the different mutations. Extensive in-vitro and in-vivo studies have been performed to ascertain the drug sensitivity pattern of each mutation to aid treatment, but many mutations remain uncharacterised. We present two case reports as evidence of the in-vivo resistance of mutation E459K to Imatinib and subsequent sensitivity to Dasatinib.
Method
Q-PCR using Taqman technology on the Applied Biosystem 7500 Sequence detection System was used to monitor BCR-abl levels in two CML patients at various time intervals to monitor disease response to treatment. Loss of response to treatment was investigated by performing mutation studies on the ABL kinase domain using nested PCR and sequencing with Applied Biosystem BigDye V3.1 chemistry and 3130 PRISM instrument.
Results
Two patients with varied clinical histories developed resistance to Imatinib therapy and entered molecular relapse. BCRabl mutation studies revealed E459K as the sole mechanism of resistance. Both underwent a change of treatment to Dasatinib whereby they demonstrated a rapid decrease in disease, achieving a major molecular response.
Conclusion
We present two case reports as evidence of the in-vivo sensitivity pattern of BCRabl mutation E459K, aiding clinical use of first and second generation TKI's against this mechanism of disease resistance in CML.

No conflict of interest to disclose

P136

Pauci-immune Crescentic Glomerulonephritis Associated with Bortezomib for Myeloma and Amyloidosis

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A 66-year-old man was diagnosed with renal and cardiac AL amyloidosis, without evidence of myeloma, in 2004, and treated with 12 cycles of monthly melphalan followed by autologous stem cell transplantation. He relapsed in April 2007, after a plateau phase of 8 months, and was treated with thalidomide, to which he responded well. In March 2008 he was noted to have worsening paraproteinaemia, with a monoclonal IgG kappa band rising from 1 to 34 g/L over two months. This was associated with hypercalcaemia and acute renal failure. Investigations demonstrated myeloma, with a bone marrow plasmacytosis without evidence of amyloidosis. He was commenced on bortezomib (1.3 mg/m^2 on days 1, 4, 8 and 11, of a 21-day cycle) and dexamethasone 20 mg weekly. After two cycles, his serum calcium and renal function had normalised and his monoclonal IgG kappa band had decreased to 19 g/L. The patient was hospitalised after cycle 2 with abdominal pain and found to have microscopic glomerular haematuria. Although the serum creatinine was normal, renal biopsy demonstrated a pauci-immune crescentic glomerulonephritis. The patient's renal function progressively worsened despite cessation of the bortezomib, a pulse of intravenous methylprednisolone (500 mg daily for 3 days) and oral cyclophosphamide-prednisolone therapy. Six weeks later he became anuric and haemodialysis-dependent. Renal amyloidosis has a rare association with pauci-immune crescentic glomerulonephritis, but almost all reported cases have occurred in the context of rheumatoid arthritis. However, in Phase I trials of bortezomib, one report of proliferative glomerulonephritis with acute renal failure was described.

No conflict of interest to disclose

P137

Comparison of Serum Free Light Chain Assessment to Urinary Protein Electrophoresis in the Diagnosis of Monoclonal Gammopathies – an Updated Report from Sir Charles Gairdner Hospital, Nedlands, Western Australia

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Aim
The serum free light chain (SFLC) assay is reported to have increased sensitivity to electrophoretic techniques in detecting light chains. It is currently used to diagnose and monitor response to therapy in light chain monoclonal Gammopathies, with the potential to replace urine electrophoresis (UPEP). We undertook a comparison of serum protein electrophoresis (SPEP) and SFLC in combination, to our current institutional practice of SPEP and urine protein electrophoresis ± immunofixation (IFE) for diagnosing monoclonal gammopathies. Here we present an analysis of data collected over 6 months.
Method
Patients from our institution previously undiagnosed but having a history suggestive of a monoclonal gammopathy were included. Samples without a direct request for SFLC were identified by review of routine requests for SPEP or UPEP. Quantitation of SFLC was by nephelometry on a Dade Behring BNII Nephelometer. Serum and urine electrophoresis was performed on the SPIFE 3000™ (Helena Laboratories) with scanning densitometry using QuickScan 2000. Inpatient notes were obtained and reviewed to determine eventual diagnosis where possible.
Results
Between 1 March and 31 August, 2007, 215 cases were identified. Fourteen percent (31/215) had paraprotein by SPEP and 15% (33/215) an abnormal κ to λ ratio (κ/λ) (N=0.26 – 1.65). In 63 cases (29%) a urine specimen was obtained of which 22% (14/63) had an M-protein. In 12 cases, the κ/λ was abnormal without an M-protein by SPEP – none were monoclonal gammopathies, 1 had CLL, 2 NHL, 4 renal dysfunction and the remainder were non-haematologic disorders. In 6 cases, SPEP identified an M-protein without the κ/λ being abnormal – all were cases of MGUS. The SFLC alone was able to identify all 9 new cases of MM and 6/12 cases of MGUS. There were no cases in which the UPEP detected an abnormality without either the SPEP or SFLC assay being abnormal.
Conclusion
SPEP + SFLC is a promising alternative in the initial work-up for monoclonal gammopathies. Our results are consistent with those reported in the literature but are limited by small numbers and limited follow-up from diagnosis. Additionally, all tests were from one test-kit batch and an assessment of batch to batch variation cannot be made.

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P138

Decreased Frequency or Cessation of Zolendronate Does Not Impact on C-Telopeptide Levels in Multiple Myeloma Patients

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Aim
Intravenous Zolendronate (a bisphosphonate therapy) significantly reduces osteolytic progression and improves quality of life in multiple myeloma patients. It is usually administered monthly based on trial data. There is no set duration of treatment. C-Telopeptide is a useful marker of bone resorption. Our aim was to determine if C-Telopeptide levels were altered in patients whose zolendronate treatment was changed from monthly to three monthly administration or ceased altogether.

Method
We conducted a retrospective analysis over a period of 24 months on a group of nine patients. We measured their C-Telopeptide levels after patients had zolendronate ceased or changed to 3-monthly infusions. Five patients had zolendronate ceased and four patients had zolendronate changed to 3-monthly administration over this time period. C-Telopeptide levels were correlated with paraprotein measurements and the presence of lytic lesions was noted.

Results
The median age of the patients was 61. Four patients were male and five were female. Eight out of nine of the patients had lytic disease at diagnosis. Seven patients had IgG Kappa; one had IgG Lambda and one patient had Kappa free light chain multiple myeloma. The median C-Telopeptide level in patients changed to 3-monthly zolendronate was 68.4pg/ml. This was similar to patients who had zolendronate ceased where the median C-Telopeptide level was 105.5pg/ml. (Normal C-telopeptide levels are < 700 for men and women aged 60). No patient had worsening paraprotein levels or new lytic lesions.

Conclusion
Our results suggest that three monthly administration of zolendronate or cessation in stable patients does not impact upon bone resorption based on measurements of C-Telopeptide. A larger randomised trial measuring C-Telopeptide levels and comparing monthly with three monthly infusions would be useful to further prove these observations.

No conflict of interest to disclose

P139

Lymphoid Subsets and Regulatory T Cell Profiles in Patients with Relapsed Multiple Myeloma in a Subset of Patients Enrolled in the REVLITE Trial

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Aim

The profile and impact of altered lymphocyte subset (LSS) and regulatory T-cells (Treg), in multiple myeloma (MM) is controversial. Moreover, how they change with immunomodulatory drug (IMiDs) treatment is unclear. We aim to correlate LSS and strictly defined Treg ($CD4^+CD127^{dim}FOXP3^+CD25^+$) profiles of patients with relapsed/refractory MM with disease response during lenalidomide (len) treatment.

Method

Sixteen out of 40 patients have been recruited into the phase II single-arm trial (REVLITE) of low-dose len (15mg d1-21q28) and dexamethasone (20mg/d, d1-4,9-12, 7-10). Baseline LSS and Tregs were measured by flow cytometry, and compared to 10 age-matched healthy donors. Subsequent serial assessment with each treatment cycle was done. The international uniform response criteria were used.

Results

After a median of 4 cycles (range 2-9), 13 patients had a response (1 IF-negative CR, 12 PR). At baseline, total $CD3^+$ T-cell (6.1 vs. 11.2 $x10^5$/ml; p=0.007), $CD4^+$ T-cell (2.0 vs. 9.7 $x10^5$/ml; p<0.00005), Treg (0.39 vs. 4.3 $x10^4$/ml; p=0.0003), and B-cell (0.76 vs. 3.2 $x10^5$/ml; p=0.05) numbers were lower in patients compared to healthy controls. Conversely, $CD8^+$ T-cell numbers were conserved (3.2 vs. 2.7 x 10^5/ml; p=0.28) and activated cytotoxic T-cells ($CD8^+CD57^+$) were higher in MM patients (2.1 vs. 0.68$x10^5$/ml; p=0.01). In 10 patients completing at least 4 treatment cycles, no recovery in total T or B-cell numbers were observed despite all achieving at least a PR. Treg numbers increased after 4 cycles (0.12$x10^4$/ml at baseline vs. 1.16$x10^4$/ml, p=0.005), but still remained below normal range. Correlation with final clinical outcome is yet to be assessed.

Conclusion

Patients with relapsed/refractory MM have low CD4:CD8 ratios and depressed Treg and B-cell numbers which, despite clinical responses, have not normalised after 4 len-dex cycles. Treg recovery in responding patients is seen, albeit incomplete, suggesting that Tregs play an important role in the modulation of tumour micro-environment. Longer-term follow-up in larger patient numbers will be revealing.

The REVLITE trial is an investigator initiated study but was partially supported by funding from Celgene. The company had NO role in analysing the data or preparing the abstract.

P140

Trogocytosis in Multiple Myeloma

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Aim

Trogocytosis is the transfer of cell membrane material from one cell to another during cell-cell contact. Recent studies have suggested that the transfer of material across the immunological synapse can alter the function of T cells and even produce adaptive T regulatory cells Tregs. We sought to identify the extent of trogocytosis in patients with multiple myeloma and to determine whether this process is at least partly responsible for immune tolerance.

Methods

An in vitro model of trogocytosis was established in which plasma cells were biotinylated and then cultured with patient mononuclear cells. Flow cytometry was used to determine cell surface expression of membrane proteins.

Results

Plasma cells (CD38++) were easily biotinylated and trogocytosis occurred with 36% of T cells but not B cells in the in vitro culture model. Of the antigen presenting cell molecules at the immunological synapse (CD80, CD86, B7-H1, B7-H3 and PD-L2) only CD80 and CD86 showed significant transfer to T cells. HLA-G expression on T cells was less than 1% on 69/70 different samples. CD80 expression was found on the T cells of 9% of patients and CD86 was found on 13% of patients (n= 95). Both CD4 and CD8 memory (CD45RO+) cells were involved but not naïve T cells. In vitro stimulation and mRNA studies showed that T cells acquire cell surface antigen but do not produce CD80 and CD86 mRNA. No trogocytosis was evident on age-matched controls.

Conclusions

T cell trogocytosis is common in patients with multiple myeloma. Transfer of proteins from tumour cells to T cells may alter T cell function and provide a mechanism of immune tolerance for the tumour.

No conflict of interest to disclose

P141

Outcome of High-Dose Chemotherapy and Autologous Peripheral Blood Stem Cell Transplantation for Multiple Myeloma at Waikato Hospital

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High-dose chemotherapy and autologous peripheral blood stem cell transplantation (SCT) is considered the standard of care for newly diagnosed patients with multiple myeloma (MM) up to 70yo. A retrospective audit was undertaken to assess the efficacy and outcome of a cohort treated on a uniform Mel200 protocol at a single institution over 11 years.

All patients who received their first SCT for MM at Waikato Hospital between 1996 and June 2007 were included; second SCTs were not. 56 pts were identified, 30 females / 26 males, median age at SCT 55.8 (range 32.5-68.1). Paraprotein subtypes were 28 (50%) IgG, 10 (18%) IgA, 14 (25%) light chain and 4 (7%) IgD or multiple plasmacytomata. Induction chemotherapy was CVAMP in 43 (77%), with CVAD, Cy-Dex and VBMCP in the remainder. Stem cell mobilisation was cyclo-GCSF; conditioning Mel200 +/- methyl-prednisolone.

All patients engrafted; neutrophils >0.5 median 11d (9-18), platelets >20 median 13d (8-29). Median length of stay was 19d (13-39). All-cause mortality at 100d was 1pt (2%). Assessment of response post-SCT was incomplete in many cases; however 98% (49/50 informative pts) achieved PR or better.

Median follow-up was 36 months and 13pts have died; overall survival (OS) was median 109mo (9yrs). Event free survival (EFS, relapse or death) was median 58mo. Subsequent second malignancy was seen in 2pts.

These data confirm excellent outcomes from SCT for MM at Waikato Hospital, with low mortality, brisk engraftment and highly satisfactory EFS and OS.

No conflict of interest to disclose

P142

Autologous Stem Cell Transplant in AL Amyloidosis: Data on 4 Cases from a Haematology Unit in Western Sydney

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Introduction
AL amyloidosis is characterized by tissue deposition of light or heavy chain with associated organ dysfunction. Recent data on the use of autologous stem cell transplant has shown limited benefits with respect to disease outcome[1]. We present data on 4 cases treated since January 2007.

Methods
At diagnosis, all patients had a blood count, biochemistry, bone marrow, fat pad biopsy (stained with Congo Red) and 2Dechocardiography for interventricular septal thickening. Stem cell harvest was done after cyclophosphamide and GCSF mobilization. Melphalan 200mg/ m2 conditioning was used. Pegylated GCSF for neutrophil recovery. Disease monitoring was with free light chain assay.

Results
The age range was between 43 – 61 years. 3 are male and one is a female. 2 patients had myeloma with amyloidosis and 2 had primary AL amyloid All patients had cardiac and renal involvement. The range of interventricular septal thickness was between 14 - 17 mm. Post stem cell infusion the median period of engraftment was 14.5 days (10 – 17 days).
Peri transplant period in one patient was complicated by spontaneous splenic rupture[2] and CMV reactivation. All patients had febrile neutropenia as a complication. All patients are surviving at present, median of 6 months (range 1.5 – 18 months). Immunological remission was documented with a normal free light chain ratio in all of them.

Conclusion
Optimal therapy of AL amyloidosis is not clear. Our 4 patients with cardiac amyloid and preserved performance status, tolerated the procedure and had rapid reduction in amyloid light chain .In our opinion autologous stem cell transplant still remains a therapeutic option for patients with amyloid.

No conflict of interest to disclose

P143

Assessment of Outcome of Tandem Autologous Stem Cell Transplantation for Patients with Multiple Myeloma at a Single Institution

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Autologous Peripheral Blood Stem Cell Transplantation (APBSCT) after a conditioning regimen with Melphalan is considered the standard consolidation therapy for Multiple Myeloma (MM). Tandem APBSCT has been increasingly used to treat MM with the goal of prolonging disease-free survival (DFS), especially in patients who achieved only a partial remission.

We prospectively analysed the outcome and prognostic factors of 17 patients with MM treated with tandem ABSCT during the period from March 2006 to July 2008 at the Launceston General Hospital, Australia. The male-to-female ratio was 15:2 , and the median age was 57 years (range:37-70). There were 12 patients with IgG myeloma, 1 with IgA myeloma and 4 with light chain disease. Median time from diagnosis to the first transplant was 6 months (range: 4-36). Two of these patients had relapsed disease prior to the tandem therapy.

All patients were anaylsed for unfavorable cytogenetic risk using PCR and FISH at our labaroatory in UTAS. Sixteen of 17 patients underwent a tandem transplant while 1 had only one transplant, due to a generalised herpes zoster infection after the first transplant. Most of patients (11) received VAD induction chemotherapy, 1 patient received Velcade containing regimen and 5 patients received Thalidomide therapy. All patients underwent stem cell mobilisation with Cyclophosphamide and G-CSF.

After a median follow up period of 16 months (range:4-28), all patients were alive and 16 remained in remission (2 CR, 14 PR), while one of the relapsed MM patients showed further disease progression and is currently being treated successfully with Lenalidomide.

In summary, our analysis shows that a significant number of patients with MM derived DFS benefit from upfront treatment with tandem autologous PBSCT, which was well tolerated. Further randomised studies to compare the effect of tandem or single APBSCT with the novel chemotherapeutic agents for MM are warranted.

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

P144

Spontaneous Rupture of Spleen Following Autologous Stem Cell Transplant for Myeloma with Amyloidosis: A Case Report

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Introduction
Myeloma can be associated with amyloidosis. Amyloidosis can complicate stem cell transplant recovery. We present a unique complication, which involved a spontaneous splenic rupture coinciding with neutrophil recovery after a stem cell transplant**.**

Case
A 62-year-old man with a history of myeloma diagnosed in August 2007, which was treated with Thalidomide and dexamethasone for 5 months, which gave a good partial response, was autografted for his myeloma in Feb 2008. Blood film since diagnosis always had features consistent with hyposplenic state, with an intact spleen documented on imaging which was consistent with amyloid. He had evidence of amyloid as documented on a bone marrow biopsy, abdominal fat pad biopsy stained for Congo red. He was harvested after cyclophosphamide and GCSF mobilization and the procedure was uneventful. A cell dose of 12.6 x 10^6 /kg of CD34 cells were obtained. He was autografted with melphalan 200-mg/ m^2 and he received pegylated GCSF for his neutrophil recovery on day +1 post stem cell infusion. His ensuing neutropenia was complicated by febrile episode and was managed by broad-spectrum antibiotics.
On the day+10 there was evidence of neutrophil recovery with ANC of 1.0x 10^9/L. On day+14 he developed acute abdomen with hypovolemic shock. His FBC at that time had revealed Hb of 70g/L, decreased by 20 g/l from previous days results. CT scan at that time revealed hemoperitoneum with a ruptured spleen. An urgent laparotomy and splenectomy was performed. He was managed in ICU with blood product support. He recovered uneventfully from his surgery. Histologically amyloidosis was confirmed in the spleen. He subsequently had mucositis possibly attributed to CMV reactivation, which was managed by ganciclovir.
The patient was subsequently discharged on day +37. He continues to be well; he has had a complete response from his autograft as documented by normal FLC ratio and a bone marrow plasma cell percentage of 2%.

Conclusion:
Spontaneous splenic rupture is a well documented complication described in literature as a complication of amyloid infiltration[1]. It can happen during GCSF mobilization or during neutrophil recovery and should be monitored for in a transplant setting.

No conflict of interest to declare

P145

Severe Factor V Deficiency Associated with AL Amyloidosis: Good Response to Therapy With Cyclophosphamide, Thalidomide and Dexamethasone (CTD)

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Clinical Summary
A 39 yr old man underwent elective cholecystectomy at which gross hepatomegaly, abnormal LFTs and perioperative bruising was noted. Liver and bone marrow biopsies confirmed amyloid deposits. Serum and urine paraprotein assays were negative, but serum κ free light chains (FLC) were marginally elevated (78 mg/L) and the marrow biopsy showed elevated clonal plasma cells (9%). INR was prolonged (3.2) and individual factor assays confirmed severe isolated Factor V deficiency (0.03U/ml; Factor X 1.27 U/ml) and no evidence of a circulating Factor V inhibitor. Further organ assessment indicated predominant extensive hepatic and renal involvement, but cardiac echo and biomarkers were normal (ALP 2515 U/L; albumin 14 g/l; urinary protein 13.6g/24h; creatinine 138μmol/L). There was no family history of similar presentations and genetic testing for familial amyloidosis was negative; these findings, along with the phenotype, confirmed a diagnosis of systemic AL amyloidosis. He was treated with 1 cycle of VAD (Vincristine, Adriamycin and Dexamethasone) and then 5 cycles of CTD which he tolerated well. Rapid, marked improvement in organ function has resulted, as illustrated by current selected laboratory parameter values (INR 1.2; Factor V 0.58 U/ml; ALP 255 U/L; albumin 33 g/l; urinary protein 3.93g/24h; creatinine 104 μmol/L; serum κ FLC 15 mg/L; BM plasma cells 2%).

Conclusion
Isolated coagulation factor deficiencies have been described infrequently in patients with AL Amyloidosis and, when present, are usually of factor X. We describe a rare case of systemic AL amyloidosis associated with severe Factor V deficiency. The patient has shown an excellent early response to therapy with CTD, a recently described and well tolerated regimen. The marked improvement in organ function makes further therapy, for example with high dose melphalan, a safer proposition.

No conflict of interest to disclose

P146

Light Chain Deposition Disease – A Rare Presentation with Predominant Hepatic Involvement

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Background
Light chain deposition disease (LCDD) is an uncommon clonal plasma cell disorder, with predominant kidney involvement. We present a case of LCDD associated with chronic lymphocytic leukaemia, manifesting as liver involvement with unexplained bleeding tendency.

Case Report
A 68 year old man was referred in June 2007 with transient lymphocytosis (4.3x10^9/L) of classic CLL immunophenotype. Examination revealed no lymphadenopathy or splenomegaly but gross hepatomegaly which was homogenously enlarged on CT. Serum showed 2g/L IgG kappa, immuneparesis and mildly abnormal liver function (GGT 164U/L, ALP137U/L). He received no treatment and failed further follow up. Ten months later he experienced persistent bleeding post TURP requiring 8 units of packed cells with no surgical cause found. Liver function showed biochemical deterioration but eGFR, coagulation profile and platelet aggregation were normal. Collagen binding to von Willebrand antigen ratio was persistently reduced. Bone marrow biopsy demonstrated extensive non-congophilic deposits, nodular lymphoid aggregates and a plasmacytosis. The deposits were histochemically consistent with light chains. Serum paraprotein was unchanged, free light chain assay demonstrated a κ:λ ratio of 13.4 (RR 0.3 - 1.7) and he was nephrotic (1121mg/L) with 5% intact IgG κ and 10% free kappa light chains. Skeletal survey, sestamibi and MRI scans showed no bony disease.

Discussion
LCCD is distinguished from amyloid on biochemical, histological and clinical characteristics. Presentation is most commonly nephrotic, but heart and liver may be affected. In comparison to amyloid, only one report of concurrent bleeding diathesis was identified in the literature. Given two prior reports of poor outcome with treatment of hepatic LCDD with infusional VAD (rapid liver failure and liver rupture) the patient has been commenced on lenalidomide.

No conflict of interest to disclose

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P147

Successful Therapy for Intravascular Large B Cell Lymphoma with Immunochemotherapy

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Aim
Intravascular Large B cell lymphoma (IVLBCL) is a rare extra nodal lymphoma characterised by selective growth of lymphoma cells in the lumina of small vessels. Given the rarity of this entity, the optimal therapeutic strategy is unknown.
Method
We report two cases of IVLBCL successfully treated with rituximab based chemotherapy and review the literature for further information regarding treatment.
Results
Case I – A 59 year old caucasian male presented with generalized seizures and worsening mentation. He was initially diagnosed with acute demyelinating encephalomyelitis and treated with methylprednisolone, however failed to improve. CT scanning performed subsequently revealed bilateral enlarged kidneys with biopsy showing features of IVLBCL. Despite a poor performance status, he was treated with high dose methotrexate and R-CHOP and has had a major response (CRu) with minor residual abnormalities on MR scan. There has been no evidence of disease progression at 18 months.
Case II- A 51 year old asian female presented with pyrexia of unknown origin. She had no lymphadenopathy or organomegaly however CT scan of the chest revealed pulmonary infiltrates which upon biopsy revealed IVLBCL. She received 6 cycles of R-CHOP chemotherapy and remains in remission 10 months post treatment.
These cases illustrate the two variants of IVLBCL; the western variant commonly presents with CNS and skin involvement; the asian variant presents with fever, bone marrow involvement, hepatosplenomegaly and haemophagocytosis. Most literature on IVLBCL is in the form of case reports and small series. Earlier series have suggested anthracycline based chemotherapy to be standard treatment (3- year OS 30%) while experience with ASCT remains limited. Recently the IVL Study group (Japan) reported a significantly higher 2 year PFS (56% vs. 27%) and OS (66% vs 46%) in patients who received R-chemotherapy compared with chemotherapy alone**.**
Conclusion
These data suggest R-chemotherapy should be considered standard of care.

No conflict of interest to disclose

P148

Follicular Lymphoma of the Gastrointestinal Tract – A Case Series of Seven Patients

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Aim and Method
Follicular lymphoma is a common indolent non-Hodgkin's lymphoma. However, primary follicular lymphoma (FL) arising from the gastrointestinal tract (GIT) is rare. We reviewed the characteristics of seven patients with GIT follicular lymphoma encountered at our institution from May 2005 to July 2008. The patients' presenting symptoms, investigations, treatment and progress are presented. A review of other studies is performed.

Results
PRESENTING SYMPTOMS: included dyspepsia (n=4), nausea (n=1), abdominal pain (n=1) and bowel obstruction (n=1). Sites of involvement included duodenum (n=6) and ileum (n=1). On endoscopy, the appearances were of a patchy or confluent nodular infiltrate of the mucosa. Biopsy revealed the typical histological and immunohistochemical features of follicular lymphoma, primarily grade 1.
STAGING: included CT scan, PET scan and bone marrow biopsy. The stage of disease at presentation was: Stage IE (n=4), stage II (n=1;abdominal lymphdenopathy) and stage IV (n=2; BM, BM+abdominal lymphadenopathy). On PET scan, only two patients had FDG uptake at the site of GIT involvement.
INITIAL TREATMENT: differed depending upon the stage (and therefore aim of treatment) and symptoms. These included: observation (n=2), R-CHOP+rituximab maintenance (n=1), R-chlorambucil/prednisolone (n=1), R-CHOP+radiotherapy (n=1), R-chlorambucil/prednisolone+radiotherapy (n=1) and to be decided (n=1).
OUTCOME: Three patients treated with chemotherapy **+/-** radiotherapy achieved remission. The two patients who were observed have progressed. One received radiotherapy alone and entered remission while the other elected for ongoing observation. The remaining two patients are either receiving treatment or awaiting a treatment decision. All patients are alive with a follow-up of 1-38 months with the following status: remission (n=3), slowly progressive disease under surveillance (n=2), receiving treatment (n=1) and awaiting treatment decision (n=1)

Conclusion
FL of GIT is rare. The duodenum was the most commonly involved site in our series. Presenting symptoms and endoscopic appearances are non-specific and biopsy reveals the unexpected diagnosis. Chemotherapy and radiotherapy are effective treatments. Optimal management is unknown but the unique location with attendant risk of perforation or obstruction often requires consideration of early treatment.
No conflict of interest to disclose

P149

Durable Spontaneous Remissions in Lymphoproliferative Disorders: Two Case Reports

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There are few reported cases of lymphoproliferative diseases spontaneously remitting. Our first case is a 59 year old woman with stage IIIA marginal zone lymphoma (MZL), diagnosed in 1980 (initially diagnosed “malignant lymphoma; nodular pattern”; this was later revised). A staging lymphogram demonstrated widespread enlargement of inguinal and abdominal nodes; LDH not tested. She was monitored and during follow-up, her lesions resolved clinically. A lymphogram showed reduction in disease bulk. Repeat nodal biopsy showed reactive changes without any evidence of malignancy. Despite follow-up, she presented in 1997 with a supraclavicular node. CT revealed pulmonary nodes and bulky pelvic disease, which was biopsied and classified as nodular lymphocyte predominant Hodgkin’s disease (HD). Original slides re-classified as HD. Six cycles of ABVD achieved CR, but she relapsed in 2005 with stage IIIA disease in a similar distribution. Repeat histology in 2005 showed MZL (1980 slides were reviewed and re-classified as MZL). The patient received six cycles of CVP-R and remains in CR.

Our second case is a 75 year old woman, diagnosed in 1988 with stage IV follicular lymphoma (FL); normal LDH and B2M. Bone marrow was involved, with a large cell component in both marrow and blood (confirmed monoclonality on flow cytometry of blood). No lymphadenopathy detected clinically. Marrow biopsy in 1992 showed spontaneous remission. In 2005 she presented with abdominal bloating and stage IIIA disease, with moderate intra-abdominal lymphadenopathy. Nodal biopsy confirmed grade 3a FL. Rituximab was commenced this year for progressive disease causing hydronephrosis, with significant reduction in lymphadenopathy. The patient remains on maintenance rituximab.

Spontaneous remissions can occur with a range of non-Hodgkin lymphomas and can be durable, as demonstrated above. The mechanism underlying this is poorly understood. These phenomena may be due to immune modulation, possibly secondary to infection. Understanding the processes underlying spontaneous remissions may help to better elucidate lymphoma biology.

No conflict of interest reported

P150

Treatment Outcomes in Elderly Patients (75 years old and older) With Diffuse Large B-Cell Lymphoma (DLBCL)

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Aim
Aggressive non-Hodgkin lymphoma is a common presentation in elderly patients. Treatment decisions are difficult, as most large randomised studies have excluded patients aged more than 80 years. This study aimed to identify prognostic factors for survival and to develop treatment recommendations for the elderly.
Method
We undertook a retrospective (14 years) clinical audit of 48 elderly patients treated for DLBCL at our institution.
Result
Overall survival (OS) was correlated with age adjusted International Prognostic Index (aaIPI). Four other features had a significant correlation with survival: number of days spent as an in-patient, complete remission rate (CR), delay of dose administration during treatment, and administration of Granulocyte Colony Stimulating Factor (G-CSF). Survival was similar in patients treated with intensive chemotherapy (R/CHOP) and those who received reduced intensity chemotherapy. Addition of rituximab to chemotherapy did not alter outcomes in this cohort. Consolidation radiotherapy in bulky disease improved CR. Localized radiotherapy alone provided excellent response rates in localised lymphoma.
Conclusion
We confirm that good CR rates can be achieved with both standard intensity (CHOP) and reduced-intensity chemotherapy, in the very elderly with DLBCL. G-CSF support during chemotherapy improved rates of CR and overall survival.
The role of rituximab requires further study in this group of patients.

No conflict of interest to disclose

P151

2 Cases of Hepatosplenic T-Cell Lymphoma in the Setting of Underlying Autoimmune Disease and Immunosuppression

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Background
Two cases of Hepatosplenic T cell lymphoma in immunosuppressed patients are reported.
Case 1
A 30yo man presented with bruising, lethargy, fever, weight loss and splenomegaly. He was found to be cytopenic, (Platelet $49x10^9$/L, Neutrophils $1.7x10^9$/L, Haemoglobin 118gm/L), with normal cell counts 2 months prior. There was a history of immunosuppressive use for severe psoriasis, initially with weekly methotrexate (12months), followed by Cyclosporin (4 years), punctuated by 3 months of etanercept use 2 years prior to presentation.
Bone marrow exam revealed infiltration of marrow sinuses by intermediate-sized lymphocytes in an intra-sinusoidal distribution. Immunophenotype by immunoperoxidase staining and flow cytometry was consistent with T cell lymphoma (CD2/3/7/16/56/TIA-1 POS, CD4/8/38/57/HLA-DR NEG); cytogenetic analysis found a malignant clone with add(1p), loss of (14) & (20). A diagnosis of hepatosplenic T-cell lymphoma was made on the distinct histological appearance and the immunophenotype.
Case2
A 39yo man presented with a flare in Crohn's disease, hepatomegaly and incidental neutropenia, later developing splenomegaly. (Platelet $281x10^9$/L, Neutrophils $0.5x10^9$/L, Haemoglobin 140gm/L) Crohn's disease was initially diagnosed 13 years prior, necessitating a bowel resection and intermittent mesalazine. A combination of azathioprine and prednisone was used for 4 years prior to presentation. Liver biopsy demonstrated an infiltrate of intermediate to large T cells in liver sinusoids. Immunophenotyping of marrow and liver tissue showed a T cell monoclonal population (CD2/3/7/16/TCRαβ POS, Vβ12 POS, CD4/8/38/57/HLA-DR NEG). Karyotyping revealed isochromosomes (7q), (8q) and (Y) loss. A diagnosis of hepatosplenic T-cell lymphoma was made on the distinct histological appearance and the karyotype.
Discussion
Hepatosplenic T-cell Lymphoma is a rare entity first described in γδ T cells, with the αβ variant later recognised. Patients tend to be adolescent and young males. Auto-immune disease and treatment with immunosuppression appears to be risk factors, with a number of series describing patients post solid organ transplants and anti-biologicals for autoimmune disease. The disease is aggressive with poor median survival of 1-2 years. There had been anecdotal reports of long term survival with intensive chemotherapy followed by autologous or allogeneic stem cell transplants.
No conflict of interest to disclose

P152

A Population Based Study of the Effect of Rituximab Immunotherapy on the Survival of Patients with Diffuse Large B-cell Lymphoma (DLBCL) in Queensland

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Aim

Rituximab has improved the survival of patients with DLBCL in randomized studies. We performed a population-based analysis to see if this effect was generalisable to adults with DLBCL in Queensland.

Methods

We compared survival outcomes during a six year period from Jan 2000 to Dec 2005. Data was abstracted from a single consolidated statewide cancer database called the Queensland Oncology Repository. We defined the "pre-rituximab" and "post-rituximab" eras according to the dates of availability of rituximab on the Australian Pharmaceutical Benefits Scheme (PBS) schedule (1 July 2003 for patients ≥ 60yrs and 1 April 2005 for patients < 60yrs). However, since younger patients may have received rituximab before April 2005, we conducted a sensitivity analysis using a single cutoff date of 1 July 2003 to demarcate between pre- and post-rituximab eras. The effect of "rituximab era" on overall survival (OS) was assessed by the log-rank test, and through multivariate proportional hazards regression controlling for demographic variables and comorbidities.

Results

Of 1210 patients with DLBCL there were 641 patients in the pre-rituximab group and 569 in the post-rituximab group. The 2yr OS was 60% and 69% in pre-rituximab and post-rituximab eras, respectively. The difference in survival between eras remained significant (*p<0.001*) even after controlling for comorbidities and other demographic variables such as age and sex; adjusted hazard ratio (HR) for the post-rituximab era was 0.69 (95%CI: 0.57-0.82). The effect of rituximab era on survival was still significant (HR 0.74, 95%CI: 0.62-0.89) even when the pre- and post-rituximab eras were defined using a single demarcation date of 1 July 2003. The rate of stem cell transplantation also declined at the same (11% vs 4%, pre- vs post-rituximab respectively, *p<0.001*). Age and comorbidities also significantly impacted survival, but not residence (urban vs rural) or socioeconomic status.

Conclusion

In this population based study, the introduction of rituximab has resulted in a 9% absolute improvement in 2yr OS among Queensland patients with DLBCL.

Authors declare that there are no conflict of interests