

Wednesday 21 October
HSANZ Symposium: Targeted Therapy

0830-1000
Hall C
0830

Acute Leukaemia

Daniel Tenen

Abstract not received at time of going to print

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0900

JAK2 Mutation Positive Myeloproliferative Diseases

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The majority of JAK2 mutations in patients with myeloproliferative disorders (MPD) are located in the “pseudo-kinase” domain and they activate the kinase domain through a poorly understood allosteric mechanism. Therefore, attempts to derive inhibitors have focused on targeting the ATP binding site and so far only a few attempts have been made to search for drugs that would specifically bind the mutated JAK2. There appear to be some differences in the way the different JAK2 mutations activate signaling. The most frequent mutation, JAK2-V617F located in exon 14, is found in all 3 MPD entities polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF), whereas mutations in exon 12 are associated solely with PV and mutations in exon 16 are found in patients with B cell ALL and Down syndrome. However, it can be expected that the JAK2 inhibitors that act as ATP analogues will be effective in all three classes of JAK2 mutations and will also be effective in mutations activating the thrombopoietin receptor, MPL. The experience with JAK2 inhibitors in clinical phase I and II studies with PMF patients so far showed beneficial effects on spleen size and constitutional symptoms, but little impact on the mutant allele burden. Some of these JAK2 inhibitors are now entering phase III trials. An update on the current state of these studies will be given and some results from pre-clinical animal models will be presented.

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Molecular Targeted Therapy of CML

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The clinical features of chronic myeloid leukemia (CML) are caused by a functionally overactive tyrosine kinase, Bcr-Abl, which is the product of the *BCR-ABL* fusion gene, consequent to a reciprocal t(9;22)(q43;q11) chromosomal translocation. The tyrosine kinase inhibitor (TKI) imatinib has become the first-line treatment for CML, and has improved survival and may alter the natural course of the disease in many patients. Despite these remarkable results, the emergence of resistance to this TKI has become a significant problem. Much progress has been recently made in elucidating the mechanisms which underlie imatinib resistance. The most common cause of such drug resistance is the selection of leukaemic clones with point mutations in the Abl kinase domain leading to amino acid substitutions which prevent the appropriate binding of the drug. Genomic amplification of *BCR-ABL*, modulation of drug efflux or influx transporters, and Bcr-Abl independent mechanisms also play important roles in the development of resistance. Persistent disease is another therapeutic challenge and may, in part, be due to the inability of imatinib to eliminate primitive stem cell progenitors. There is a pressing need, therefore, to develop and test novel drugs and strategies for the effective treatment of CML. A multitude of agents targeting signal transduction pathways downstream of Bcr-Abl have been developed, and have shown *in vitro* and *in vivo* efficacy in overcoming imatinib resistance. However, the Bcr-Abl tyrosine kinase activity of the oncoprotein still remains the most promising therapeutic target, and the focus has been directed in more recent years to developing TKIs with greater potency and improved 'fitting' in the kinase pocket. There are currently three such 'second generation' TKIs being explored in clinical trials: nilotinib, dasatinib and bosutinib. Furthermore, the emerging role of immunotherapy and exploitation of tyrosine kinase-independent pathways are promising aspects of translational research.

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An Age-dependent ABO Discrepancy Between Mother And Baby Reveals a Novel A^{weak} Allele

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Background

A and B antigens are synthesized on glycoproteins and glycolipids of erythrocytes (RBCs) and other tissues by glycosyltransferases encoded by the *ABO* gene. A^1 alleles differ from *B* alleles by 7 nucleotides of which 4 encode amino acid differences. The O^1 allele differs from A^1 by a nucleotide deletion that creates a frameshift. However, 48 different *A* subgroup alleles are known to encode A_{weak} expression. Cord blood from an infant born to an A_2B mother typed group O, H+. Results were confirmed on a heel prick sample. All samples were investigated to determine the reason for the discrepancy.

Methods

Genomic DNA was analysed by *ABO* genotyping using in-house PCR-RFLP/PCR-ASP assays and by sequence analysis. RBCs were characterised by flow cytometry using monoclonal anti-A.

Results

Surprisingly, routine analysis showed the mother's genotype as A^1B and the infant's as A^1O^1 . PCR-ASP screening for known mutations causing weakened A/B expression and rare *O* alleles were negative. DNA sequencing revealed a novel mutation, 311T>A in the A^1 -like allele of both subjects, which predicts an amino acid change, Ile104Asn. Flow cytometry demonstrated A antigen on the mother's RBCs equivalent to the A_2 phenotype. A antigen was barely detectable on the cord RBCs, however, a sample drawn at 11 months demonstrated increased A expression.

Discussion

Amino acid 104 resides in the α_2 -helix of the stem domain but is not involved directly with catalysis. Possibly, Asn104 destabilises the helix and/or changes the subcellular localization. Based on structural data and analogous glycosyltransferases, we hypothesised that Ile104 is involved in dimer formation and that the mother's normal B-transferase stabilises the altered A-transferase (so-called allelic enhancement). However, the altered A-transferase alone may be too unstable to function effectively. Speculation that the baby's A antigen might strengthen as more complex/branched carbohydrate chains are produced was confirmed by the later sample.

No conflict of interest to disclose

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PUPPY Underpins Platelet Planning: Results from the Prospective Ututilisation of Platelets and Plasma (Puppy) Study

Neil Waters, Mary Comande, Mark Polizzotto, George Grigoriadis, Marija Borosak, Damien Jolley, Erica Wood
Australian Red Cross Blood Service, ²Monash Institute of Health Services Research

Background

In order to plan for platelet demand and meet clinical need in an emergency an understanding of platelet use is required. Restricting platelet use to clinically urgent cases and/or deferring elective surgery may be required, however few data exist to inform planning.

Aims

To determine indications for, and urgency of, platelet transfusions in Victoria.

Methods

Random sample survey, adapted from Bloodhound study of red cell utilisation. 1252 platelet units (752 pooled, 500 apheresis, including 7x4 Paediatric) were randomly tagged with case report form (CRF). CRFs were completed by issuing hospital scientist.

Results

1158 units were issued to hospitals. Analysis of 1149 (99.2% response) returned CRFs shows 830 (72.2%) issued for transfusion, 300 (26.1%) expired in hospitals, 19 (1.6%) recalled or other disposal.

Clinical conditions requiring transfusion included malignant haematology 413 (49.8%), benign haematology 49 (5.9%), Oncology 68 (8.2%), other medical 52 (6.2%), cardiothoracic surgery 91 (11.0%), urological surgery 30 (3.6%), other surgery 87(10.4%) and other/unknown 40 (4.8%)

Clinical urgency of transfusion was acute (<1 hour) in 126 (15.2%); urgent (<24 hours) in 527 (63.5%); semi-urgent (<1 week) in 130 (15.7%) and unknown in 45 (5.4%) cases; 2 (0.2%) transfusions were deferrable >1 week. Support for elective procedures used 66 (7.9%).

Where sufficient information was supplied (540 [65.1%]), appropriateness was assessed against NHMRC guidelines, with 454 (84.1%) were assessed as appropriate.

Median platelet count prior to transfusion was $19 \times 10^9/L$ (IQR 11 - 55); for bleeding patients $25 \times 10^9/L$, for prophylaxis (no risks) $11 \times 10^9/L$, and prophylaxis (with risks): $15 \times 10^9/L$.

Conclusions

Platelet usage is concentrated in treating haematological and malignant disorders, and supporting major surgery. High levels of urgent transfusion and low numbers of transfusions supporting elective surgery demonstrate that in a shortage conventional triage strategies would have little impact on requirements and additional strategies are required to ensure continued adequacy of supply.

No conflict of interest to disclose

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Bacterial Screening of Platelets in Australia: First 12 month experience

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*Australian Red Cross Blood Service,*¹*Melbourne, Victoria,*²*Sydney, NSW,*³*Brisbane, QLD,*⁴*Perth, WA,*⁵*Adelaide, SA, Australia.*

Aims

To describe clinical and logistical aspects of introduction of routine bacterial contamination surveillance screening of platelets in Australia.

Methods

Seven ARCBS testing laboratories (5 new facilities) use the BacT/ALERT® 3D automated microbial detection system. Closed system sampling (15-20mL) from each platelet component occurs at 24h; samples are inoculated for aerobic and anaerobic culture. Platelets are released 'negative to date' while culture continues over component shelf-life. Initial machine positive (IMP) and all follow up results are notified immediately to transfusing laboratories. Communication and education was undertaken to inform clinicians and laboratory personnel about introduction of bacterial screening. In the 12 months following implementation (late April 2008 – end April 2009) ARCBS transfusion medicine staff provided clinical follow up of cases where transfusion had occurred prior to IMP notification.

Results

Feedback from clinicians showed understanding of the need to undertake bacterial screening and follow up. Initial concerns related to management of different clinical scenarios and potential workload for laboratories and clinical staff. Of 116 594 platelet components screened there were 1364 (1.17%) IMP notifications, of which 372 (27.3%) platelets or their associated components (red cells, plasma) were transfused. Follow up of these cases found three possible reactions related to transfusion. Many patients (39%) were already on antibiotic and no confirmed or high probability bacterial cases were reported. Of all screened platelets, 205 were confirmed positive/indeterminate (0.18%). In 59 (29%) of these the organisms were deemed clinically significant. Transfusion was prevented in 59% (35/59) of these cases due to early notification; no transfusion reactions were reported.

Conclusion

ARCBS has successfully implemented bacterial contamination screening of platelets in Australia, contributing to a small but important further improvement in transfusion safety. ARCBS transfusion medicine staff work with hospital and laboratory personnel to manage the clinical follow up of bacterial screening.

No conflict of interest to disclose

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Combined Prion and Leukocyte (PR*LR) Filters Vary with respect to Vesicle Formation During Storage

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Aim

Prior work (in press) showed that platelet-derived microparticle (PDMP) levels can increase by 2 log or more during cold storage of unfiltered whole blood, but leukofiltration with current technology reduces platelet (PLT) and PDMP levels significantly; these levels remain low during storage. Leukoreduction (LR) filters can be surface-modified to attract and retain prions and leukocytes (PR*LR filters). We compared three prototype PR*LR filters with existing LR technology for vesicle formation during storage.

Methods

Following institutional ethics committee guidance, healthy adult males were recruited and consented to donate 450 mL of whole blood (WB). Pools of ABO-identical donations were redistributed into standard collection sets, each equipped with a different filter. Non-filtered WB was retained as a control. Filtered WB was separated into RBC and plasma (FFP) components. Samples taken at 0, 7, 14, and 21 days were centrifuged at 2000g for 20 minutes. Supernatant aliquots were incubated with PE-conjugated anti-CD42b or mouse IgG1 (control), and fixed with paraformaldehyde. PE fluorescence, forward scatter, and side scatter were used to gate PDMP and PLT events.

Results

Pre-storage filtration with current LR and prototype PR*LR filters gave comparable results with respect to PDMP-gated events, but RBCs filtered with two of the three PR*LR prototypes developed many PLT-gated events during storage, on par with non-filtered WB. One PR*LR prototype gave comparable results to existing LR technology in all measured respects.

Conclusions

LR filtration technology is relatively mature, but new designs, such as those intended to reduce prions, should be evaluated not only for their intended effects (e.g., leukoreduction and prion reduction), but also for unintended effects, such as the formation of vesicles with thrombogenic potential (e.g., those with platelet antigens).

This research was supported by Asahi Kasei Medical Co., Ltd., which provided materials and financial support for an independent investigation of filtration technology.

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FFP Puppy Digs Up Some Surprising Results - Prospective Utilisation of Platelets and Plasma (Puppy) Study

Mary Comande¹, Neil Waters¹, Mark Polizzotto¹, George Grigoriadis¹, Marija Borosak¹, Damien Jolley², Erica Wood¹

¹ Australian Red Cross Blood Service, ² Monash Institute of Health Services Research

Aim

Determine clinical indications for, and urgency of, Fresh Frozen Plasma (FFP) use to inform supply and contingency planning.

Methods

Random sample survey adapted from Bloodhound red cell utilisation study, performed August 2008 to February 2009. FFP units (n=1993) were randomly tagged with case report form (CRF) at production and distributed to Victorian transfusion laboratories. At time of issue for transfusion, CRFs were completed by laboratory scientists.

Results

Interim analysis of the first 1343 (67.4%) returned CRFs shows 1243 FFP units (92.6%) issued for transfusion, 96 (7.1%) discarded and 4(0.3%) recalled.

Major clinical requirements for FFP were: cardiothoracic surgery 215 cases (17.3%); gastroenterology 172 (13.8%); solid organ transplant, including plasma exchange for renal transplant, 161 (13.0%); haematology/oncology 147 (11.8%) and trauma 78 (6.3%). In 636 cases (51.2%) FFP was used to support an interventional procedure, of these, 112 (19.2%) were elective, 316 (49.7%) non-elective and 198 (31.1%) urgency was unknown to the laboratory. Use for warfarin reversal was often in conjunction with other clinical indications.

Clinical urgency of transfusion was acute (required <1 hour) in 471 cases (35.1%); urgent (1-24 hours) in 661 (49.2%); semi-urgent (24 hours -1 week) in 58 (4.3%) and non-urgent (>1 week) in 9 (0.7%). In 44 cases (3.3%) urgency was not known. AB plasma is in demand for trauma, other emergencies and ABO-incompatible renal transplantation.

Conclusions

Analysis of interim data shows 84% FFP transfusions required within 24 hours with significant use in cardiothoracic surgery and organ transplantation. Surprisingly, solid organ transplantation, including use of AB FFP for ABO-mismatched renal transplants, was third largest use. High use in critical illness and complicated coagulopathy has implications for contingency planning, as alternatives to FFP in those contexts are typically unavailable (unlike warfarin reversal). Information about changes in clinical use of FFP will inform future blood supply planning.

No conflict of interest to disclose

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Routine Autologous Blood Collection and Subsequent Transfusion for Donors of Bone Marrow (BM) is Unnecessary

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Background

Australian Bone Marrow Donor Registry and World Marrow Donor Association guidelines stipulate harvest centres should collect ≥ 1 autologous red cell units from allogeneic donors for transfusion during or after a marrow harvest. However, the necessity for routine autologous collection has not been established. This practice increases cost and inconvenience and does not eliminate the most significant transfusion associated risks, including bacterial contamination and administrative error.

Aim

To perform a single centre retrospective analysis of the utility of autologous blood collection pre-allogeneic BM donation and review transfusion practices of stored autologous units.

Methods

Fifty-nine consecutive BM donors presenting between January 2004 and December 2009 were identified. Sibling and volunteer unrelated donors were included. Haemoglobin (Hb) measurements pre-autologous blood collection, pre-harvest and post-harvest (prior to transfusion), where available, were retrospectively analysed. Transfusion of autologous units was audited.

Results

The donors comprised 34 males (mean age 40yrs–range:16-52) and 25 females (mean age 39yrs–range:24-66). Forty-seven donors had autologous blood collected (33:1unit and 14:2units). The mean Hb pre-autologous donation for males ($n=27$) was 156g/L (range:139-179g/L) and for females ($n=20$) was 137g/L (range:116-157g/L). The mean reduction in Hb post-autologous collection was 12g/L (-31g/L to -1g/L) and 7g/L(-30g/L to +4g/L) for males ($n=23$) and females ($n=17$) respectively, despite routine iron supplementation. The mean BM harvest volume was 1068ml (range:200-1725ml). The mean post-harvest Hb pre-transfusion in males ($n=19$) was 122g/L (range:92-151g/L) with a mean drop of 23g/L (range:5-43g/L), for females ($n=12$) the respective results were 108g/L (range:84-145g/L) and 26g/L (range:10-49g/L). No donor who had a post-harvest Hb measured met NHMRC minimum criteria for transfusion ($Hb < 70g/L$). Twenty-seven of the 47 donors who had autologous blood collected, were transfused, although in 56% their Hb pre-transfusion was not checked. There was no significant difference in post-harvest Hbs of transfused (pre-transfusion 115g/L range:84-151g/L) versus non-transfused donors (118g/L range:88-145g/L).

Conclusion

Routine autologous blood collection prior to BM harvest leads to a drop in Hb pre-harvest, wasting of blood and unnecessary transfusions. Post-harvest Hb did not decrease to levels considered detrimental to healthy persons in any donor. We conclude routine autologous blood collection from healthy BM donors is unnecessary.

No conflict of interest to disclose

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Haemovigilance In New Zealand – Four Years and Counting ...

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New Zealand Blood Service, ¹Christchurch, ²Wellington, ³Hamilton, ⁴Auckland, New Zealand

Aim

To collect data on transfusion- and blood donation-related hazards.

Methods

For transfusion-related events reporting is voluntary. European Haemovigilance Network definitions are used. Specialists review reports to determine if the stated category and 'imputability' to transfusion are appropriate. Data for 2005 – 2008 is presented. Donor incidents are collected using an incident reporting system. A central office collates data and prepares an annual report.

Results

Adverse events in recipients and blood donors are summarised below.

Table 1. Adverse events in recipients receiving blood components, 2005 - 2008 – number (frequency/ 10,000 components transfused):

	Febrile non-haemolytic transfusion reactions	Allergic reactions (all grades)	Transfusion-associated circulatory overload	Transfusion-related acute lung injury	Acute haemolytic transfusion reaction	Delayed haemolytic transfusion reaction	Incorrect blood component transfused	Other [@]	Total
2005*	131 (12)	89 (8)	8 (1)	10 (1)	0	6 (1)	10 (1)	17 (2)	271 (26)
2006	190 (12)	148 (10)	7 (<1)	10 (1)	2 (<1)	10 (1)	22 (1)	31 (2)	420 (28)
2007	193 (13)	155 (10)	17 (1)	9 (1)	4 (<1)	12 (1)	21 (1)	44 (3)	455 (30)
2008	207 (12)	160 (9)	20 (1)	4 (<1)	1 (<1)	19 (1)	39 (2)	70 (4)	520 (30)

* data for 8 months only.

@ events not fitting clearly-defined categories and previously-unrecognised complications of transfusion.

In 2008 there were 185,738 whole blood & apheresis donations and 1416 adverse events involving 1306 donors (762 events / 100,000 donations).

Conclusion

Greater awareness and ease and standardization of reporting are the keys to a successful HV programme. This can promote rational transfusion use and the introduction of measures to improve blood safety.

No conflict of interest to disclose

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Hospital Influences Risk of Transfusion in Cardiac Surgery: An Analysis of a Large Patient Cohort

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Background

The international literature reports significant variation in transfusion practice in cardiac surgery; however there are very few Australian data on transfusion in this setting. We aim to investigate the variation in transfusion practice between the major cardiac surgery units in Victoria.

Methods

Data were prospectively collected on 9363 cardiac surgery patients at six major Victorian hospitals between January 2005 and December 2008 through the Australasian Society of Cardiac and Thoracic Surgeons Cardiac Surgery Database. There included patient demographics, co-morbidities and medication use, surgery type, peri-operative complications, clinical outcome and peri-operative transfusion.

Results

Procedure types were: coronary artery bypass graft (CABG) surgery in 60%, valve surgery 14%, CABG + valve 10% and other procedure 16%. There was significant variation in transfusion of all blood components between the six hospitals and this variation was not accounted for by patient or surgery related factors. The adjusted odds ratio for risk of transfusion for red cells varies at each hospital from 0.22 to 1.98, for platelets from 0.39 to 3.3 and for plasma from 0.27 to 2.0.

Conclusion

There was significant variation in transfusion practice across the major hospitals in Victoria performing cardiac surgery which was not accounted for by patient or surgery related factors. Patient laboratory results and pre-operative transfusion details were not available for inclusion in this study, however a project is currently underway to address this through the linkage of hospital laboratory data with this and other clinical outcome registries. With increasing concern about potential adverse outcomes associated with peri-operative transfusion in cardiac surgery, further studies are required to determine factors contributing to this variability in transfusion practice and how it may influence patient outcomes.

No conflict of interest to disclose

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0900

An Audit of the Safety and Efficacy of Prothrombinex[®]-VF for Acute Reversal of a Prolonged INR, at Two Large City Hospitals

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1. North Shore Hospital, Waitemata District Health Board, Auckland, New Zealand

2. Middlemore Hospital, Counties Manukau District Health Board, Auckland, New Zealand.

Aim

To determine the 30 day risk of thromboembolism (arterial and venous) following administration of Prothrombinex-VF (PTX) to acutely reverse a prolonged INR.

Method

A prospective audit of patients who received PTX between August 2008 and May 2009 at two Auckland hospitals. Patients receiving PTX for reasons other than a prolonged INR were excluded. Eligible patients were followed for 30 days to determine the rate of thromboembolism and mortality. All issues of PTX were authorised by a Clinical Haematologist with the decision to use vitamin K and/or Fresh Frozen Plasma (FFP) made on a case by case basis.

Results

A total of 122 doses of PTX administered to 120 patients for acute reversal of a prolonged INR. PTX (+/- vitamin K and FFP) reversed the INR to <1.5 in 80% of cases. Patients were elderly (71y +/-12), predominantly male (58%) and unwell (45% haemodynamically compromised). Indications for warfarin administration included atrial fibrillation (AF) (70%), mechanical heart valve (MHV) (12%) and venous thromboembolism (VTE) (8.5%). PTX was administered (+/- vitamin K and FFP) to rapidly reverse the INR due to life threatening bleeding (55%), for an acute procedure/surgery (33%) and for medically unwell patients with a raised INR (9%). By 30 days, 21 (18%) patients were dead. There were 16 thrombotic events (1 venous; 15 arterial) of which 9 were fatal. These thromboses occurred in high risk patients (13 with AF or MHV). Only 2 unexpected nonfatal events (1 major cerebrovascular accident; 1 myocardial infarction) occurred in patients treated for VTE. There was no observed difference in the rate of thrombosis if patients did or did not receive FFP with PTX (12% versus 14% p=NS).

Conclusion

There is a high rate of thrombosis and death in our elderly patients receiving PTX used to rapidly reverse an elevated INR. Most events occurred predictably in patients with pre-existing AF or MHV and in those patients treated for an acute haemorrhage. Our data suggest that PTX is effective in reversing a prolonged INR, and the frequent thrombotic events associated with its use reflect the patient population and co-morbidities. The high rates of thrombosis were not attenuated by concomitant administration of FFP.

Conflict of Interest Statement

This research was supported by CSL. The company had no role in the design, data analysis or preparing the abstract.

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How is a Tool Developed for the Aerospace Industry Being Used to Prevent a Leading Cause of ABO Incompatible Transfusions?

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Introduction

Errors in sample collection and labelling represent a leading cause of transfusion related patient morbidity and mortality. Accurate specimen labelling is a critical step in pre-transfusion compatibility testing.

A shift in the thinking about how errors occur has provided new ways to approach possible solutions in specimen labelling using Failure Mode and Effect Analysis (FMEA). FMEA is a systematic method for identifying potential process failures before they occur, with the intent to eliminate or minimise the risks associated with them.

Aim

To reduce the number of mislabelled blood grouping specimens in the Outpatient Pathology Department (OPD) at the Peter MacCallum Cancer Centre.

Method

We employed FMEA on the labelling of blood grouping specimens in the OPD to:

- Map the *process* of labelling of a specimen tube
- Identify the *potential* failure modes
- Identify the *causes and effects* of the failure modes
- Evaluate the *risks* associated with the failure modes
- Identify the current controls

Results

The following failure modes were identified:

- Specimen tubes – poorly designed tube labelling
- Physical environment – privacy and workflow issues
- Human factors – errors in positive patient identification

These were prioritised according to their detectability, frequency and the seriousness of their consequences. Proposed corrective actions were assigned to the high risk failures and the process was redesigned to address these failures. The FMEA tool was then used to determine the effectiveness of the redesigned process before the implementation of the proposed corrective actions.

Conclusion

The next phase is to fully implement the proposed corrective actions in the OPD and then to evaluate the effectiveness of these actions in reducing the number of mislabelled blood grouping specimens.

Our experience has demonstrated that FMEA is a simple and effective method to proactively identify failure modes and prioritise risk reduction strategies.

No conflict of interest to disclose

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Bedside Transfusion Practice Audit in eight New Zealand Hospitals

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New Zealand Blood Service Auckland New Zealand

Aim

To determine the level of adherence to the ANZSBT guidelines with the administration of resuspended red cell transfusions at the patients bedside at eight large public hospitals in New Zealand, and to check the hospital blood policies against the ANZSBT component administration guidelines

Method

Episodes were collected prospectively by the Transfusion Nurse Specialists at each site. Data were recorded by direct observation. Further data were recorded from the patients clinical notes after the Transfusion had been completed.

Results

420 transfusions were audited from a spread of specialities. Identity checks were generally well performed. Notable exceptions were asking patients to state their identity (45% compliance overall), and wearing wristbands in neonates (33%) and daycases (57%). The two-person check of unit against patient and prescription was performed variably with one hospital failing to do this at the bedside in almost a quarter of transfusions audited. Checking patient vital signs revealed confusion over the role of pulse oximetry vs observed respiratory rate. A nurse stayed with the patient for the first 15 minutes of the transfusion in only 86% of cases. Only 60% of adverse reactions were reported to blood bank. Post-transfusion documentation was well performed. Transfusion duration was over 4 hours in up to 10% of transfusions. To identify the extent of overlapping omissions in safety checks, a composite safety check list was compiled. Only 67% of transfusions satisfied this list, with up to five omissions per transfusion

Comment

Direct observation by the auditors may have an effect on compliance. However the audit demonstrated areas of non compliance for recommend best practice for patient safety with the administration. of red blood cells transfusions

No conflict of interest

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Procedural Adverse Events in Transfusion – Lessons from STIR 2006-09

Lisa Stevenson, David Beilby, Karen Botting, Slavica Curcic, James Daly, Bridget Glazebrook, Geoff Magrin, Ellen Maxwell, Richard Rogers, Merrole Cole-Sinclair, Carole Smith, Neil Waters, Deane Wilks, Larry McNicol, Erica Wood.

STIR expert group and advisory committee, Blood Matters-better safer transfusion program, Department of Human Services and Australian Red Cross Blood Service, Melbourne, Victoria, Australia

Aim

STIR (Serious Transfusion Incident Reporting) is the voluntary haemovigilance framework developed by the Blood Matters-better safer transfusion program. Hospitals and laboratories in Victoria, Tasmania, ACT and Northern Territory participate. The program aims to;

- measure and monitor serious transfusion incidents, including near misses
- derive recommendations for better, safer transfusion practice and disseminate these to health services and the Australian Red Cross Blood Service.

Method

STIR provides a central system for reporting events related to administration and handling of fresh blood components and pre-transfusion samples. Health services electronically submit initial reports to STIR. Detailed case report forms relevant to the event type (e.g. incorrect blood component transfused, IBCT) are provided to the reporting institution to complete. Information (de-identified for institution) regarding the case is returned to STIR for data entry and review by an expert group. STIR links with the Victorian sentinel event program.

Result

To date STIR has been notified of **439** adverse events in **432 patients**. Combined procedural adverse events, incorporating incorrect blood component, wrong blood in tube and near miss events, accounted for **40%** of all reports during this period.

Common themes include:

- failure to comply with established patient identification procedures when collecting pre-transfusion samples
- failure to identify patients at the bedside before transfusion administration

STIR has received **38** IBCT events, including **22** where the component did not meet specific patient requirements, **9** incorrect but ABO-compatible events, and **3** ABO incompatible events. Four reports are still to be investigated. No deaths related to transfusion were reported.

Conclusion

Procedural adverse events are preventable events. The common theme through all the procedural reports is failure to identify the patient correctly, either due to incorrect identification procedures or failure to follow local hospital procedures. STIR recommends that **all** staff administering transfusions should be trained in the correct procedure, particularly the importance of patient identification.

No conflict of interest to disclose

Wednesday 21 October
ASTH Free Communications 3

0830-1000
Hall D

O105

0830

Patients with Enhanced Clot Solubility in Acid Conditions Require Differentiation between Raised Plasma Levels of the Aspartic Protease Pepsinogen I and Inhibitory Antibodies to Factor XIII

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Background and Aims

The *in vitro* solubility of plasma clots in 2% glacial acetic acid (GAA) indicates either Factor XIII (FXIII) deficiency, antibodies to FXIII or the presence of an unknown acid-activated protease (AP). The AP was previously postulated to be “pepsin-like activity” or cathepsin D (CD). We describe four subjects with clot solubility in GAA (but not urea) and normal FXIII levels. This study aims to assess their plasma levels of common APs and to explore the possibility of inhibitory antibodies.

Methods and Results

We mixed subject plasma with normal plasma or cryoprecipitate and added calcium to form a clot. The clots lysed in GAA, consistent with presence of an inhibitor. However IgG purified from subjects' plasma did not cause clot lysis and IgG-depleted plasma clots retained the ability to lyse in GAA. Pepstatin, an AP inhibitor, prevented clot lysis in the subject group, confirming lysis was due to an AP. Clots only dissolved at pH <3.2 (acetate/acetic acid) consistent with pepsin activity and lower than expected for CD. In subjects 1- 4 CD levels (Calbiochem ELISA) were 41, 46, 38 and 44 ug/L respectively and within the normal range (n=20, 36-70 ug/L). However pepsinogen I levels (Biohit ELISA) were 570, 780, 490 and 540 ug/L and were markedly elevated compared to 16 normal donors (65-200 ug/L). Pepsinogen II levels (Biohit ELISA) were normal.

Conclusions

An AP, and not a FXIII inhibitor, caused clot lysis in plasma from subjects with acid-soluble clots and normal FXIII. These subjects had high plasma levels of pepsinogen I, the acid-activated precursor to pepsin, and we postulate that pepsin digested the clot. Abnormal clot lysis tests require follow up with a FXIII assay and possibly also a pepsinogen assay, to confirm or exclude a true FXIII deficiency or inhibitor.

No conflict of interest to disclose

Wednesday 21 October
ASTH Free Communications 3

0830-1000
Hall D

O106

0845

Exon 28 Mutation Testing in von Willebrand Disorder

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Aim

To determine the usefulness of exon 28 mutation testing in patients with von Willebrand disorder (VWD).

Method

The size and complexity of the von Willebrand factor (VWF) gene makes full gene mutation testing impractical for most laboratories. Of the 52 exons of this gene, exon 28 is by far the largest, coding for 14% of the mature protein. This exon contains all the mutations for type 2B VWD, approximately 70% of known mutations for type 2A and type 2M, and 20% of type 1 mutations.

We have performed sequencing of exon 28 in 15 families with type 1 and type 2 VWD with ristocetin cofactor levels <40IU/dl.

Results

Mutations in exon 28 of the VWF gene were identified in 9 of the 15 families tested. Type 2B mutations were found in two families thought to have this sub-type, as well as in another family with previously unclassified type 2. A type 2A mutation was found in another patient with unclassified type 2.

In four families with presumed type 2M, mutations were found in all of them, with two having the same mutation and similar phenotype. In one of these families, two of the six affected family members did not have the identified mutation, casting doubt on its causative nature. These three mutations have been previously reported as type 1, 2A or 2M, but bioassay results of the reported index cases seem to be consistent with type 2M.

A type 1 mutation was found in one of four presumed type 1 patients, with no mutations found in the other three. No mutation was found in a further three patients with possible acquired type 2 VWD.

Conclusion

Mutations were detected in exon 28 in all families with type 2. Exon 28 analysis is particularly useful for identification of type 2B.

No conflict of interest to disclose

Wednesday 21 October
ASTH Free Communications 3

0830-1000
Hall D

O107

0900

Gestational Thrombocytopenia in Three Sisters with Type 2B von Willebrand's Disorder

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Aim

To describe three sisters with von Willebrand's disorder (VWD) who developed thrombocytopenia during their first pregnancies.

Method

All three sisters had been previously diagnosed with mild unclassified VWD. BC developed mild thrombocytopenia early in her first pregnancy with normal von Willebrand's studies on presentation. The provisional diagnosis of immune thrombocytopenia (ITP) was made. Her platelet count was $32 \times 10^9/L$ when she was treated with immunoglobulin and steroid, but with no response to either. Her thrombocytopenia resolved after delivery. Her second sister, NT had a similar experience during her first pregnancy. Her eldest sister received desmopressin prior to delivery and was later diagnosed with thrombocytopenic thrombotic purpura (TTP). She was managed with steroids and plasma exchange. Post partum platelet aggregometry did not demonstrate increased aggregation to low concentrations of ristocetin.

Results

Type 2B was suspected on the basis of thrombocytopenia during pregnancy. NT was tested following an injury - FVIII 27 IU/dl; VWF Antigen 39 IU/dl; RiCof 27 IU/dl ; RiCof/Ag 69%; CBA 14 U/dl, CBA/Ag 36%, platelet $145 \times 10^9/L$. Ristocetin-induced platelet aggregation (RIPA) at low concentration is required to diagnose Type 2B VWD. As the sisters were unavailable for platelet aggregometry; exon 28 of VWD gene was sequenced, since all type 2B mutations are detected in this exon. The mutation 3916C>T; Arg1306Trp was detected. This common type 2B mutation is well recognised on the VWD mutation database, confirming the family as type 2B VWD.

Conclusion

All three women were misdiagnosed during their first pregnancies. They demonstrated the increase in VWF during times of stress such as pregnancy, trauma or surgery, leads to increased platelet binding to platelet Glycoprotein 1b in type 2B VWD and hence thrombocytopenia, which resolved after delivery. Type 2B also needs to be differentiated from pseudo (platelet-type) VWD and mutation studies provide a definitive diagnosis for type 2B.

No conflict of interest declared

Wednesday 21 October
ASTH Free Communications 3

0830-1000
Hall D

O108

0915

Vancomycin Induced Thrombocytopenia in Hospitalised Patients

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Aim

Vancomycin is an antibiotic used widely in Australian hospitals to treat Methicillin-resistant *Staphylococcus aureus* (MRSA). Only recently has vancomycin been implicated in causing drug-induced thrombocytopenia (DIT) and as such, there are very limited studies dedicated to establishing the characteristics of this disease. This will be the first prospective study on vancomycin induced thrombocytopenia (VIT). It aims to establish vancomycin as a causative agent in thrombocytopenia through the detection of vancomycin dependant antibodies and describe the clinical presentation of these patients.

Methods

Blood samples were obtained with informed consent from inpatients at St George Hospital and Sutherland Hospital at a mean of 13 days after vancomycin initiation. Vancomycin dependent antibodies were detected using flow cytometry on patient sera and, for any positive results, monoclonal antibody immobilisation of platelet antigens (MAIPA) was used. Patient medical records were reviewed for duration and dose of vancomycin treatment, other concomitant medications and for clinical signs and symptoms of DIT. Platelet counts for the duration of their stay in hospital were also monitored.

Results

Out of 33 enrolled patients (11 females and 22 males; aged between 30 and 89), 6 patients tested positive for vancomycin dependent antibodies (3 females and 3 males; aged between 30 and 80). Of these 6 patients, 4 patients showed an average of 45.5% drop in platelet count within two days of vancomycin commencement. The other 2 patients showed no significant platelet drop within the time of vancomycin administration.

Conclusions

In this preliminary study, the incidence of vancomycin dependent antibodies detected was 18.2% and 66.7% of these patients developed thrombocytopenia due to vancomycin. This also suggests that, like other DITs (e.g. heparin induced thrombocytopenia), pathogenic and non pathogenic antibodies may be associated with vancomycin induced thrombocytopenia.

No conflict of interest to disclose

Wednesday 21 October
ASTH Free Communications 3

0830-1000
Hall D

O109

0930

Is Bone Marrow Examination Necessary To Safely Manage Idiopathic Thrombocytopenic Purpura In Adults?

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Aim

Guidelines for the management of adults below 60 years presenting for the first time with apparent clinical Idiopathic Thrombocytopenic Purpura (ITP) suggest that bone marrow aspirate and trephine (BMAT) is not routinely required. However, in certain settings, it is often clinical practice to perform BMAT for this indication. We retrospectively determined the prevalence of alternative pathologies in patients with a clinical diagnosis of ITP.

Method

This study was conducted at St John's Medical College Hospital, Bangalore, a tertiary centre in southern India. The clinical notes accompanying BMAT request forms between January 2004 and July 2008 (excluding July to December 2008) were reviewed. Cases were excluded if subjects were outside the age 18-60 years, had bi- or pancytopenia (excluding mild-moderate, micro/normocytic anaemia), fever, HIV infection, previous or suspected malignancy, organomegaly, lymphadenopathy, or were on medication known to cause thrombocytopenia. All patients whom had a marrow and did not meet these exclusion criteria had their charts selected for review. Where the clinical presentation was consistent with acute ITP, the patient's BMAT report was selected for review.

Results

Of 4483 BMAT slips reviewed, 374 cases were selected for chart review, and 312 charts obtained. 117 cases had a clinical presentation suggestive of ITP and had BMAT results available. The mean age was 33 years. Females comprised 81/117 (69%). Anaemia was seen in 72/121 (59%). In 116/117 cases there was no alternative pathology on BMAT. In one case, myelofibrosis was identified. Megakaryopoiesis was described as "normal" in 108 and "reduced" in 8 cases.

Conclusion

In this large study investigating BMAT in adults with apparent ITP, it appears that alternative pathology is rare, suggesting that routine BMAT is unnecessary. These findings suggest that in countries with a high burden of anaemia, BMAT may be safely omitted in patients with a clinical diagnosis of ITP.

No conflict of interest to disclose

Wednesday 21 October
ASTH Free Communications 3

0830-1000
Hall D

O110

0945

Antiphospholipid Testing:- Is More Necessarily Better?

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Aim

International Society on Thrombosis and Haemostasis (ISTH) recommendations for lupus anticoagulant testing currently propose that at least two tests utilising different assay principles be used for the diagnosis of the antiphospholipid syndrome. Recent North American and European questionnaires have demonstrated that of the laboratories surveyed, the majority use the dilute Russell's viper venom time (dRVVT) and the activated partial thromboplastin time (APTT) using a lupus anticoagulant sensitive reagent. It has been the practice of our laboratory to perform four different screening tests including the dRVVT and APTT, as well as the kaolin clotting time (KCT) and dilute thromboplastin time (DTT). We aim to determine whether the use of four tests as opposed to the recommended two is more sensitive or specific for the detection of a lupus anticoagulant.

Methods

We undertook a retrospective analysis of the last 18 months of lupus anticoagulant testing undertaken in our laboratory (n=1283). After the application of exclusion criteria, we analysed 1003 results. Sensitivity and specificity of positive mixing studies (i.e failure to correct) for APTT, KCT, dRVVT and dTT and combinations of these assays were calculated.

Results

Sensitivity for APTT, DTT, DRVVT and KCT was 67%, 86%, 86% and 76% respectively. Specificity of all individual tests was similar at ~ 96-97%. Standard combination as used by most laboratories (i.e APTT + DRVVT) revealed 86% sensitivity (confidence interval 83-89%); no better than DTT or DRVVT analysis alone. The most sensitive combination of tests was APTT + DRVVT + DTT (97% sensitivity – 94-100% confidence interval).

Conclusion

Although current ISTH recommendations stipulate a requirement for two screening tests, our data suggests that for maximal sensitivity, analysis of APTT, DRVVT and DDT in combination yields the best results.

No conflict of interest to disclose

Wednesday 21 October
HSANZ Free Communications 9

1100-1230
Hall C

O111

1100

Prognostic Impact of Elevated Pre-Transplant Serum Ferritin in Patients Undergoing Allogeneic Haemopoietic Stem-Cell Transplantation For AML

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Aim

To identify the prognostic impact of pre-transplant iron load in patients with acute myeloid leukaemia (AML) undertaking allogeneic stem cell transplantation (SCT).

Methods

Retrospective audit of consecutive patients with AML undertaking allogeneic SCT at RBWH between January 2000 and May 2007. Patients were identified from an institutional data base. Iron load was measured by serum ferritin concentration prior to commencing conditioning for transplantation.

Results

Data was censored after May 2008 to ensure a minimum post-SCT follow-up of ≥ 12 mths. For the entire cohort, median overall survival (OS) was 7.2 yrs, with 5yr OS 55%. Transplant related mortality (TRM) and relapse probability at 5yrs were 25% and 31% respectively. Pre-SCT ferritin values were available in all patients. Of the entire cohort, only 2 patients (1%) had normal ferritin levels pre-SCT; median pre-SCT ferritin was 2220 $\mu\text{g/L}$ (range 151-9080 $\mu\text{g/L}$; normal range 10-200 $\mu\text{g/L}$). On univariate analysis, increased ferritin $>$ median was significantly associated with reduced OS; 3yr OS 48% vs 68% for pre-SCT ferritin $>$ vs $<$ median respectively ($p=0.014$). When pre-SCT ferritin was divided into quartiles, the reduced OS post-transplantation appeared to be restricted to that subgroup of patients with the highest pre-SCT ferritin values (i.e 4th quartile); 3yr OS 40% vs 65% for 4th quartile vs quartiles 1-3 respectively ($p<0.005$). Patients with the highest pre-SCT ferritin values also experienced significantly greater TRM (3yr TRM 33% vs 16% for 4th quartile vs quartiles 1-3 respectively; $p=0.02$). On multivariate analysis, raised pre-SCT serum was significantly associated with reduced OS ($p<0.01$), whether analyzed by $>$ vs $<$ median values, or 4th quartile vs quartiles 1-3 respectively.

Conclusion

Iron overload, as measured by raised ferritin, is extremely common in AML patients undertaking allogeneic SCT. Pre-SCT serum ferritin levels appear to represent a new prognostic marker for OS post allogeneic SCT in these patients. Prospective studies to limit iron-overload prior to allogeneic SCT are needed.

No conflict of interest to disclose

Wednesday 21 October
HSANZ Free Communications 9

1100-1230
Hall C

O112

1115

Increased Expression of the Chemokine Receptor CCR5 on Human Blood Dendritic Cells is Associated with Acute Graft vs Host Disease post Allogeneic Stem Cell Transplantation

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Dendritic cells(DC) are potent antigen presenting cells, that are central to the development of acute graft-versus-host disease (aGVHD) following allogeneic hematopoietic cell transplantation (alloHCT). DC migration is tightly regulated by the expression of chemokine receptors.

In this study we investigated the patterns of expression of the chemokine receptors CCR5 and CCR7 on circulating blood CD11c⁺myeloid and CD11c⁻plasmacytoid dendritic cells by multiparameter flow cytometry from the peripheral blood of 32 patients post alloHCT, and assessed their correlation with aGVHD.

Peripheral blood was collected twice weekly up to day 120 post transplant. CCR7 receptor expression on both CD11c⁺DC was detected in 60% of patients the mean percentage of CCR7 receptor expression was 12%(range 0.3-39%). In contrast, CCR5 receptor expression on both CD11c⁺DC was detected in all but one patient, the mean percentage of CCR5 receptor expression was 30%(range 0.4-90%). The maximum percentage expression of the two chemokine receptors on DC was correlated with the development of aGVHD and its severity. No correlation was found between CCR7 receptor expression and the incidence or severity of aGVHD($p=1.0$). However, there was a correlation between CCR5 expression on DC and the development of aGVHD. 19 patients who developed aGVHD, all had detectable levels of CCR5 expression on both CD11c⁺DC median 42%(range 5.2-89%) prior to the onset of aGVHD, while patients with no aGVHD CCR5 was expressed at a significantly lower level ($p<0.0001$). Patients with grade 2-4 aGVHD had higher CCR5 expression on both CD11c⁺DC post transplant (median 56%, 73% respectively), compared to patients with grade 0-1 on both CD11c⁺(median 4.3%, 2.7% respectively)($p<0.0001$).

CCR5 receptor expression is higher on DC of patients who develop severe aGVHD. This raises the possibility of monitoring CCR5 expression on DC as a means of predicting the development of aGVHD and of using targeted therapies to block CCR5 interactions to reduce or prevent the severity of aGVHD.

No conflict of interest to disclose

Wednesday 21 October
HSANZ Free Communications 9

1100-1230
Hall C

O113

1130

Fludarabine/Melphalan Allografting in Australia and New Zealand, 1998-2008: Excellent Overall Survival in a High-risk Population

Adam Bryant¹, Anna Kalff², David Ritchie², Biju George³, Mark Hertzberg³, Keith Fay^{1,4}, Paul Cannell⁵, Leanne Berkahn⁶, Leonie Wilcox⁷, John Moore¹.

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Background

Over the last ten years, Reduced Intensity Conditioning (RIC) has increasingly been used in allogeneic Haematopoietic Stem Cell Transplantation (HSCT). RIC has generally been reserved for patients who have been heavily pre-treated, are of advanced age or have significant co-morbidities. The Fludarabine Melphalan (Flu/Mel) combination is the most commonly used regimen in Australia and New Zealand, however its efficacy and relative benefit in myeloid and lymphoid malignancies has not been previously assessed.

Method

This was a retrospective analysis of Flu/Mel allografts performed in nine allogeneic HSCT centres in Australia and New Zealand between January 1998 and December 2008. All centres were sent an electronic Case Report Form which was returned to the coordinating centre at St. Vincent's Hospital, Sydney. This current data set analyses the outcome from six centres that have responded to date. Statistical analysis was performed using Prism 5 software.

Results

There were n=214 patients with a slight male preponderance (M=54%). The median age of recipients was 54 years (18-67) with 88 patients transplanted for lymphoid malignancies (excluding acute lymphoblastic leukaemia) and 126 patients for myeloid malignancies. Transplant related mortality at D100 was 12.6% and similar for both myeloid and lymphoid malignancies (12.5% vs 12.6%, p=0.97). The median overall survival was 51.4 months with no significant difference in overall survival between the myeloid and lymphoid cohorts (p=0.20). Further analysis will be presented with a particular emphasis on variables associated with outcome.

Conclusion

This study is, to our knowledge, the largest analysis of Flu/Mel conditioning ever published. The data demonstrates that Flu/Mel can be delivered to heavily pre-treated patients of advanced age with excellent long term overall survival. In this analysis there did not appear to be an advantage of the Flu/Mel regimen for lymphoid or myeloid malignancies.

No conflicts of interest to disclose

A212

Wednesday 21 October
HSANZ Free Communications 9

1100-1230
Hall C

O114

1145

Factors Predicting the Outcome of the Blood and Marrow Transplant Patients Admitted to Intensive Care Unit.

Nalini Pati¹, Biju George¹, Ian Kerridge¹, Nicole Gilroy², Vineet Nayyar³, Eddie Stachowski³, Mary McGurgan¹, Gillian Huang¹, Ken Bradstock¹, David Gottlieb¹, Mark Hertzberg¹

¹ Blood and Marrow Transplant Unit, ² Department of Infectious Diseases,

³ Intensive Care Unit, Westmead Hospital, Westmead, NSW, Australia, 2145

Aim

To identify factors predicting outcome of patients admitted to intensive care (ICU) following allogeneic haematopoietic stem cell transplantation (allo-HSCT).

Methods

Retrospective audit of all allo-HSCT patients requiring ICU admission.

Results

Between 2000 and 2009, 392 patients underwent an allo-HSCT. 106 (27%) required ICU admission (n = 129). The median age was 47 (range 16-65) with myeloablative transplant in 89 and reduced intensity in 40 patients. Respiratory failure was the main reason for admission (54.6%) followed by sepsis (41.5%). Improvement in organ failures were seen in 29.2% of the patients, 39.2% remained stable while 28.4% deteriorated following admission. Most patients (n = 67, 51.9%) were discharged from ICU but only 48 (37%) were discharged from the hospital (ICU). A higher proportion of patients were admitted after day + 30 post-transplant (40% Vs 29%), with those admitted prior to day +30 having a lower likelihood of survival (17% vs 23%). Univariate analysis identified; number of organ failures at admission, progression of organ failure during ICU admission, APACHE II score at admission, steroid refractory GVHD, and requirement for inotropic support or dialysis as significant predictors for survival in ICU. Patients requiring intubation and mechanical ventilation had a poor outcome than the group did not (p=0.001). While prior ICU bacterial infection did not alter the outcome (p=0.221) but the onset of a new infection in ICU did influence the outcome (p=0.0001). In contrast factors which did not alter the outcome are type of transplant, graft source, presence of neutropenia and mucositis.

Conclusion

While ICU support is justified for HSCT patients leading to improved survival, high APACHE II score, multiorgan failure, progression of organ failure during ICU stay, and the need for ventilation or dialysis, carries a dismal prognosis and is unlikely to be influenced by lengthy ICU admissions. There remains the importance of a good scoring system in regard to prognosticate and decide upon the continuity of the treatment on these critically ill patients.

No conflicts of interest to declare

Wednesday 21 October
HSANZ Free Communications 9

1100-1230
Hall C

O115

1200

The Impact Of An Outpatient Based Autologous Stem Cell Transplant Service (ASCT) On Hospital Bed Stay And Clinical Resources. The Sir Charles Gairdner Hospital Experience

Dejan Radeski, Susan Hyde, David Joske, Bradley Augustson, Steven Ward, Patrick Crawford and Gavin Cull

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Aim

To examine the impact of outpatient based ASCT (OP-ASCT) on hospital bed stay and clinical resources.

Method

A retrospective analysis of 107 consecutive ASCT at SCGH between January 2007 and June 2009. Data was collected from patient notes and computerized hospital information systems.

Results

Patients for ASCT were referred from their consultant to the nurse practitioner/transplant coordinator. All patients were considered candidates for OP-ASCT. High dose melphalan was administered in the day care ward. BEAM was administered using infusional chemotherapy in the day care ward and via CADD pump at home. Review was daily during chemotherapy and every 1-2 days after chemotherapy. The nurse practitioner was responsible for OP management of problems including nausea, mucositis, dehydration and blood products. Febrile neutropenia was not managed as an OP and mandated admission.

Indications for ASCT included myeloma (42%), NHL (30%), Hodgkin disease (17%) and other (11%). Following pre-transplant assessment, 85/107 (79%) were planned for OP-ASCT and 22/107 (21%) for IP-ASCT. Reasons for IP-ASCT included co-morbid medical problems, psychosocial problems and geographical issues. For IP-ASCT who remained until haematological recovery, median length of stay in hospital (LOS) was 18 days. For OP-ASCT, 17/85 (20%) did not require admission at any point. For those requiring admission, the median was day +6 post-transplant and the main indication febrile neutropenia. Median LOS for OP-ASCT was 6 days. For the entire cohort, median time to first post-transplant transfusion with red cells was 6 days and platelets 7 days. Median time to neutrophil count >1.0 was 11 days post-transplant. Transplant related mortality was 2%.

Conclusion

Planned outpatient based ASCT substantially reduces the median length of stay in hospital. Benefits include reduced pressure on in-patient beds and timely administration of conditioning chemotherapy. A suitably resourced day care ward and dedicated nurse transplant coordinator are required to run this service.

No conflict of interest to disclose

A214

Wednesday 21 October
HSANZ Free Communications 9

1100-1230
Hall C

O116

1215

Haplo-identical Transplant Without T Cell Depletion Using a Reduced Intensity Conditioning Protocol in Feasible in Older Patients with Haematological Malignancies

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Blood and Marrow Transplant Unit, Westmead Hospital, Sydney

Aim

To explore the feasibility of haplo-identical transplants in older patients with haematological malignancies lacking a suitable histocompatible donor.

Methods

Between April 2008 and May 2009, 6 patients underwent haploidentical transplant using Fludarabine 30 mg/m²/day (Days – 6 to -2), Cyclophosphamide 14.5 mg/kg daily (Day – 6 and -5) and TBI 200 cGy on day -1. Graft source was unmanipulated bone marrow. Cyclophosphamide (50 mg/kg on Day +3) with tacrolimus and mycophenolate (started on day +4) was used as GVHD prophylaxis.

Results

Four males and 2 females with a median age of 55 years underwent haploidentical transplant for AML (n = 3), high grade MDS (n = 1) and non-Hodgkin's lymphoma (n = 2). Median CD34 cell dose infused was 2.1 x 10⁶ CD34/Kg (range: 1.3 – 2.9). All patients engrafted with a median neutrophil and platelet engraftment of 15 (range: 13 – 55) and 19 days (range: 0 – 66) respectively. Acute GVHD occurred in 2 (33%) [both grade II] with limited chronic GVHD in 3 (60%). Toxicity was minimal with grade 1-2 mucositis in all. None had bacterial or fungal infections but CMV reactivation occurred in 2. One patient with AML had early relapse at day 21 and expired Day 89 post transplant. Four patients achieved complete chimerism by day 30 while 1 patient who had mixed chimerism by day 30 (donor < 90%) rapidly lost donor chimerism and is presently well with autologous reconstitution. Non-relapse mortality at 3 and 6 months is 0%. At median follow up of 7 months (range: 2-12), 5 (83.3%) are alive with a DFS of 66%.

Conclusions

Reduced intensity haplo-identical transplants without T cell depletion is a feasible option with low toxicity in elderly patients who lack a suitable histocompatible donor. Further studies will determine the place of haploidentical transplantation in patients without suitable donor options.

No conflict of interest to disclose

Wednesday 21 October
HSANZ Free Communications 10

1100-1230
Meeting Rooms 1/2

O117

1100

Long-Term Outcomes of 90 Patients with Primary Cutaneous B-Cell Lymphoma: Analysis of the Peter MacCallum Cancer Centre/St Vincent's Hospital Melbourne Cutaneous Lymphoma Database

Suzanne O Arulogun¹, H Miles Prince^{1,5,6}, **Kirsten E Herbert**¹, Gail F Ryan², Stephen Lade³, Sarah Swain³, Peter A Foley^{4,5}, Odette Blewitt¹ and Chris J McCormack⁴
Departments of ¹Haematology, ²Radiation Oncology and ³Pathology, Peter MacCallum Cancer Centre; ⁴Department of Dermatology, St Vincent's Hospital, Melbourne; ⁵University of Melbourne; ⁶Monash University; Victoria, Australia

Background

Primary cutaneous B-cell lymphomas (PCBCL) are a rare group of neoplasms. There have been few long-term follow-up analyses worldwide of large patient cohorts. The WHO/EORTC classification system identifies three main subtypes of PCBCL: primary cutaneous marginal zone lymphoma (PCMZL), primary cutaneous follicle centre lymphoma (PCFCL), and primary cutaneous diffuse large B-cell lymphoma, leg type (PCLBCL, LT). The former two subtypes are typically considered to have an indolent behaviour.

Aims

We aimed to identify long-term outcomes and prognostic indicators in all patients with PCBCL treated at our institutions. Patients whose diseases behaved more aggressively than expected (compared with previous reports of these subtypes) were analysed in particular detail.

Methods

The Victorian Cutaneous Lymphoma Database was interrogated to identify all patients with PCBCL since 1991 (n = 90). All patients had a biopsy-proven diagnosis of B-cell lymphoma of skin with no systemic disease identified on staging investigations at time of diagnosis. Survival data were analysed using Kaplan-Meier survival analysis. Univariate analyses were performed for the following factors: subtype, site/extent of cutaneous involvement, age at diagnosis, initial treatment and time-to-relapse.

Results

The 90 patients with PCBCL had a biopsy-proven diagnosis of PCMZL (n=31, 34.4%), PCFCL (n=39, 43.3%), PCLBCL, LT (n=20, 22.2%) and subtype unknown (n=4). Interestingly, 5 patients (5.5%) had concurrent diagnoses of two different cutaneous B-cell lymphoma subtypes. First-line treatments included: radiotherapy (63.6% of patients), chemotherapy (18.2%), surgical excision (18.2%) and rituximab (12.1%). Relapse rate post-initial treatment was 51.5%. Fifteen patients presented with unusual disease courses: either progression of indolent disease (PCMZL or PCFCL) to systemic involvement, concurrent diagnoses of two B-cell lymphoma subtypes, or development of PCLBCL after initial diagnosis of an indolent subtype.

Conclusions

Progression to more aggressive disease in PCBCL indicates a relatively poor prognosis and our analysis suggests that this may be more common than previously recognised. We describe a subgroup of patients with unusual and most interesting disease courses.

No conflict of interest to disclose

Wednesday 21 October
HSANZ Free Communications 10

1100-1230
Meeting Rooms 1/2

O118

1115

Hyper-CVAD + Rituximab Followed by High-dose Busulfan, Melphalan and Autologous Stem Cell Transplantation in First Response is Well-tolerated and Produces Prolonged Event Free Survival in Patients with Mantle Cell Lymphoma (MCL)

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² Bone Marrow Transplant Service of the Department of Clinical Haematology, Royal Melbourne Hospital, Melbourne.

Introduction

Hyper-CVAD+Rituximab (R) alternating with high dose methotrexate + cytarabine (HDMTX/ARA-C +R) results in a >85% CR rate (cf CHOP (30%) or CHOP+ R (50%)). The role of consolidating the excellent initial Hyper-CVAD+R responses with AuSCT remains controversial. We report on our experience of using Hyper-CVAD+R followed by a consolidative BuMel ASCT as initial therapy for patients (pt) <65 years old with MCL.

Patient characteristics

n=25, female=8, median age=53 (range 29-62), Stage IV n=19, \uparrow \square 2microglobulin n= 9, \uparrow LDH n= 7. Median calculated MIPI score = 5.4 (range 4.4 – 6.1). Twenty pt have completed therapy, one declined AuSCT after achieving a CR. Four pt are yet to undertake planned autografts; of these 2 have completed R-HyperCVAD and are in CR, 1 has completed 3 cycles of R-HyperCVAD and 1 has completed 2 cycles of R-HyperCVAD and has been de-escalated to R-CHOP due to recurrent sepsis.

Treatment outcome

Median follow is 65 Mo (range 2-96 Mo). Hyper-CVAD+R induced CR in 21 of 22 evaluable patients (95%). Median time to AuSCT = 8 Mo (range 4-12 Mo). One patient (age=58) died at day +21 post-AuSCT. Of 20 patients completing therapy, 14 (82%) remain in CR (median follow-up 60m). Five have relapsed (40 Mo, 42 Mo, 49 Mo, 54 Mo and 55 Mo respectively) and 2 have died of progressive disease. On an intention to treat, the 5y EFS and OS for all 25 pt are 58% and 87% respectively, and for those completing AuSCT 63% and 87% respectively. We have observed no cases of MDS or secondary AML.

Conclusions

These data confirm the tolerability and efficacy of Hyper-CVAD+R regimen in MCL. Further, consolidation with BuMel AuSCT may delay the time to disease progression compared to Hyper-CVAD+R alone and improve the OS of 60% at 5 years predicted by the MIPI score.

No conflict of interest to disclose

Wednesday 21 October
HSANZ Free Communications 10

1100-1230
Meeting Rooms 1/2

O119

1130

Protein Kinase C β II Expression in Diffuse Large B Cell Lymphoma Predicts for Inferior Outcome of Anthracycline Based Chemotherapy with and Without Rituximab

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Protein Kinase C β II (PKC β II) expression has been reported to indicate inferior prognosis in diffuse large B-cell lymphoma (DLBCL) treated with anthracycline-based chemotherapy.

Aim

To compare the prognostic significance of immunohistochemically determined PKC β II expression in *de novo* DLBCL treated with CHOP chemotherapy with and without rituximab.

Method

80 consecutive patients treated at St. Vincent's Hospital with *denovo* DLBCL, 48 treated with CHOP, and 32 with rituximab plus CHOP (R-CHOP), were studied using immunohistochemistry for PKC β II on diagnostic tissue samples. Staining results were correlated with patient characteristics and clinical outcome. Overall Survival (OS) and Progression-Free Survival (PFS) were determined by the Kaplan-Meier method, and comparisons were determined by the log-rank test.

Result

PKC β II expression correlated with inferior OS and PFS in CHOP treated patients with low-risk International Prognostic Index (IPI) disease (0-2 adverse factors), but not in the overall patient group unstratified by IPI. PKC β II expression significantly correlated with inferior OS and PFS in R-CHOP treated patients unstratified by IPI status.

Conclusion

PKC β II expression has prognostic significance not only for CHOP therapy in low-risk IPI disease, but also for all patients receiving CHOP plus rituximab. Immunohistochemically-demonstrated PKC β II expression thus identified patient subgroups where alternative treatment strategies may confer superior outcome.

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

A218

Wednesday 21 October
HSANZ Free Communications 10

1100-1230
Meeting Rooms 1/2

O120

1145

Retrospective Analysis of Patients with Primary CNS Lymphoma Treated with Methotrexate and Reduced Dose Whole-brain Radiotherapy

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Aim

Radiotherapy dose is a major risk factor in development of neurotoxicity in patients with primary CNS lymphoma. This study assessed the effect on outcome and toxicity of reducing whole brain radiotherapy to 30Gy when combined with methotrexate chemotherapy.

Methods

Retrospective observational study of patients with histologically proven PCNSL in one tertiary referral centre in Western Australia. The primary endpoint was event-free survival of the patient group. Response rate and therapy toxicity were secondary end points. Results were compared to TROG 92.01 study, where a whole-brain dose of 45.4Gy plus 5.4Gy boost of radiotherapy was combined with methotrexate. A subset of patients receiving rituximab were analysed separately to determine any significant differences in survival or toxicity.

Results

23 patients between 2001 and 2008 with primary CNS B cell lymphoma were included. They were all immunocompetent with no extra-cerebral disease. Median age of the patient group was 62 years (range 42 to 76 years) and ECOG varied from 1-3. All patients received methotrexate (1g/m² day 1 and day 8) and whole-brain radiotherapy of 30G starting day 15. In addition ten patients received rituximab.

Treatment was generally well tolerated with only four patients requiring dose reduction. 14 (61%) patients had complete radiological remission. As of May 2009 16 patients remain alive with a median follow-up of 18 months and overall survival was 70%. Seven patients had relapsed lymphoma with five occurring in the CNS. Four deaths occurred as result of PCNSL relapse. Median event-free survival was 37 months. Six patients had symptoms consistent with neurotoxicity and it contributed to one death. There were no significant differences in the rituximab group.

Conclusion

For PCNSL patients treated with methotrexate and radiotherapy, reducing the dose of radiotherapy to 30Gy does not compromise survival. Severe neurotoxicity may be reduced. Longer-term follow-up is required to confirm these findings.

No conflicts of interests to disclose

Wednesday 21 October
HSANZ Free Communications 10

1100-1230
Meeting Rooms 1/2

O121

1200

Cardiovascular Disease Is A Frequent Late Complication In Survivors Of Hodgkin Lymphoma

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Aims

The primary aim of this study was to define the frequency of and risk factors for late complications of the therapy for Hodgkin's lymphoma (HL). Here, we focus on cardiovascular disease (CVD) coronary and carotid artery disease, and valvular dysfunction.

Methods

The South Australian Cancer Registry was used to identify HL patients within the North Western Area Health Service of Adelaide. Patients older than 18 yrs, free of disease for at least 5 years and diagnosed after 1975 were invited to participate.

Results

At the time of writing 58 patients were fully evaluable. 43 patients received chemotherapy and 36, radiotherapy (RT), (16 RT only and 20 combined), 28/36 patients received mantle field RT. 35/58 (60 %) evaluable patients were alive at a median 18 years post treatment. 12/58 (20%) had a confirmed diagnosis of symptomatic CVD (3 cerebro-, 10 cardiovascular) . Three patients died from complications of CVD. There was an association between the incidence of CVD and radiotherapy with 10/12 CVD patients having received radiotherapy compared to 2/12 having had chemotherapy alone.

Conclusions

The incidence of CVD was similar to that observed in the literature (17%). Not surprisingly a high number of survivors were unaware of this risk and also the interference of life-style choices and personal risk factors. Hence, early detection, counseling and preventative measures are of paramount importance.

This research was supported by Roche. The company had no role in data analysis or abstract preparation.

Wednesday 21 October
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Meeting Room 3

O123

1100

The Tyrosine Kinase Inhibitor Dasatinib Dysregulates Bone Remodelling Through Inhibition of Osteoclasts *In Vivo*

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4. St. Vincent's Institute, Fitzroy, Melbourne, Australia

Aims

Dasatinib is a tyrosine kinase inhibitor that is used to treat chronic myeloid leukaemia in patients resistant or intolerant to imatinib mesylate. We have previously demonstrated that therapeutic concentrations of dasatinib potently inhibit osteoclast formation and activity *in vitro* due, in part, to its specificity for the macrophage colony stimulating factor (M-CSF) receptor, c-fms. In the present study, we examined whether dasatinib could significantly alter bone remodelling *in vivo*.

Methods

Nine-month-old Sprague Dawley rats were administered dasatinib (5 mg/kg/day) or vehicle control (10% DMSO/90% polyethylene glycol 300 [v/v]) by gavage or zoledronic acid (ZOL; 100 µg/kg/6 weeks) sub-cutaneously. Following 4, 8 and 12 weeks of treatment, animals were sacrificed and serum biochemical, bone morphometric and histological analyses were carried out.

Results

Micro-computed tomographic (µ-CT) analysis of cancellous bone at the proximal tibia showed that trabecular bone volume (BV/TV) and trabecular thickness (Tb.Th) were increased in dasatinib-treated animals, at levels comparable to the ZOL-treated group. These changes were associated with a greater than 50% decrease in osteoclast numbers (Oc.N/BS) and osteoclast surface (Oc.S/BS). While no significant changes in serum calcium levels were observed, hypophosphataemia was induced in the dasatinib-treated animals. Following 8 weeks of treatment, serum levels of the bone resorption marker C-terminal collagen crosslinks (CTX) were decreased in the dasatinib-treated group, relative to the vehicle control, while the levels of the bone formation marker osteocalcin remained unchanged.

Conclusions

This study demonstrates that dasatinib increases trabecular bone volume in a rat model of normal bone remodelling, at least in part, by inhibiting osteoclast activity. While these data suggest that dysregulated bone remodelling may be a possible side-effect of dasatinib therapy, they also suggest that suggest dasatinib may be useful in the treatment of diseases characterised by bone loss.

No conflict of interest to disclose

Wednesday 21 October
HSANZ Free Communications 11

1100-1230
Meeting Room 3

O124

1115

Enhancing the Functional Activity of the OCT-1 Influx Pump in De-novo CML Patients May Greatly Improve Response to Imatinib

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Aim

The primary active influx protein for imatinib (IM) is the human organic cation transporter 1 (OCT-1), and the functional activity of the OCT-1 protein (OA) is predictive of response to IM in CP-CML patients. Patients with a low OA have a poorer response to imatinib compared to those with high OA (MMR by 24 months: 45% vs 85% respectively $p = 0.004$). Increasing dose may overcome the effect of a low OA but is not tolerated by most patients. As an alternative here we investigate strategies to increase the active uptake of IM.

Method

Potential OA enhancers (OA-E) were chosen using the connectivity map (CMAP) which allows the observation of functional interactions that are present between various drugs and genes of interest. In this study, the OA was measured in a cohort of 56 de-novo CML patients with and without the presence of diclofenac; fasudil; and LM1685.

Results

Overall, a significant increase in OA was observed when enhancers were introduced ($p = 0.001$). The mean OA without enhancers was 3.7ng/200000 cells, which was increased to 4.9 with fasudil and 5.3 with diclofenac ($p = 0.026$ and 0.003 respectively). An increase was observed in 64% of all samples tested with fasudil and 80% of samples tested with diclofenac. LM1685 also increased OA in 66% of patients; however, there was no significant change in the mean OA. In patients with an initial OA of $>4\text{ng}/200000$ cells OA-E were less effective, suggesting enhancing OA in patients with a higher OA is of little benefit.

Conclusion

In patients having a low OA and hence increased risk of suboptimal response to imatinib, enhancing the OA may be of great benefit to these patients. The potential to improve response to imatinib by enhancing drug uptake warrants further study and clinical investigation.

No conflict of interest to disclose

A222

Wednesday 21 October
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Meeting Room 3

O125

1130

Short-Term Intense Bcr-Abl Kinase Inhibition is Adequate to Trigger Cell Death in CML-CD34+ Cells But Only if They are Simultaneously Cytokine Deprived

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Aim

Preclinical studies of imatinib set the paradigm of continuous Bcr-Abl kinase inhibition for optimal response in CML. However, clinical studies with dasatinib suggest that intermittent kinase inhibition also leads to good clinical responses. We assessed the impact of the intensity and duration of Bcr-Abl kinase inhibition on proliferation and apoptosis of CML cells.

Method

Cell death triggered by short-term intense (cells were cultured with dasatinib or imatinib for 30min, and following thorough drug washout, cells were recultured in drug free media for remainder of 72hr) and long-term partial (cells were cultured with dasatinib or imatinib for 72hr) Bcr-Abl kinase inhibition was studied in *BCR-ABL*⁺ cell lines and CML-CD34⁺ colony forming cells (CFC). Effect of combined Janus tyrosine kinase (Jak) and Bcr-Abl kinase inhibition on p-STAT5 expression and CD34⁺-CFC was studied in the presence or absence of cytokines.

Results

Despite reactivation of Bcr-Abl kinase activity within 4hr of drug washout, short-term intense kinase inhibition with 100 nM dasatinib induced apoptosis in 70-80% of cells from *BCR-ABL*⁺ cell lines. By contrast, in the presence of cytokines, short-term intense Bcr-Abl kinase inhibition did not trigger apoptosis in BaF3 BCR-ABL cells or in CD34⁺ primary CML cells. However, without cytokines, short-term 100 nM dasatinib reduced CFCs by 75-80%. Cytokines rescued CML-CD34⁺ cells and BaF-3 BCR-ABL cells by activating Jak-STAT5 pathway. In the presence of cytokines, a combination of Jak inhibitor and short-term dasatinib inhibited STAT5 and triggered cell death in BaF3 Bcr-Abl and CD34⁺ CFC.

Conclusion

Cytokines added during or immediately after short-term exposure to dasatinib prevented apoptosis of CML-CD34⁺ cells suggesting that oncogene dependence of these cells can be overcome by exposure to cytokines. Therapeutic strategies combining short-term intense Bcr-Abl kinase inhibition and blockade of cytokine pathways warrant further assessment as a novel strategy for eradication of CML progenitors.

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Meeting Room 3

O126

1145

OCT-1 Activity in CML CD34+ Cells is Not Predictive of Molecular Response to Imatinib Treatment in CP-CML Patients

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Aim

The functional activity of the OCT-1 protein (OCT-1 Activity, OA) in mononuclear cells (MNC) is variable between patients and is predictive of response to imatinib¹. The OA in CML CD34+ cells is significantly lower than that found in mature CML cells. The aim of this study was (1). To assess whether a relationship exists between the OA in MNC and the OA in CD34+ cells. And (2). To identify if CD34+ OA predicts for the achievement of major molecular response (MMR).

Methods

MNC and CD34+ were isolated from imatinib naïve, newly diagnosed chronic phase CML patients. OA was determined using [14C]-labelled imatinib and the OCT-1 inhibitor prazosin [1].

Results

No correlation was found between the OA in MNC and the OA in CD34+ cells in 35 CML patients ($R=0.306$, $p=0.0739$). When patients were divided as having high ($n=23$) or low ($n=12$) OA in their MNC¹, no difference was seen between the OA in their corresponding CD34+ cells (mean low OA: 3.18, high OA: 4.32 ng/200,000 cells, $p=0.236$). Lastly, in 21 patients where 12 month response data was available patients were grouped according to the achievement or not of MMR by 12 months. MNC OA was found to be significantly associated with the achievement of MMR (mean OA, MMR: 13.75, no MMR: 5.31 ng/200,000 cells, $p=0.042$). However, assessment of CD34+ cells failed to demonstrate a relationship between OA and achievement of MMR (mean OA, MMR: 4.11, no MMR: 4.07 ng/200,000 cells, $p=0.409$).

Conclusion

We were unable to demonstrate a relationship between OA measured in a patient's MNC and that in their CD34+ cells. Furthermore, unlike our observations in MNC, CD34+ OA is not predictive of patient's response to imatinib treatment. This suggests, the predictive value of the MNC OA primarily reflects the effective targeting and subsequent eradication of mature CML cells.

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

A224

Wednesday 21 October
HSANZ Free Communications 11

1100-1230
Meeting Room 3

O127

1200

A Population-Based Study of Responses to Imatinib in the Treatment of Newly Diagnosed Patients with Chronic Myeloid Leukaemia in New Zealand

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Aim

New Zealand was one of the first countries to approve and fund imatinib as first-line treatment for chronic phase chronic myeloid leukaemia (CML). To date, most published data has arisen from clinical trials. This study aimed to characterise responses to imatinib in patients with newly diagnosed CML in the New Zealand population.

Method

Laboratory and clinical records of sixty consecutive patients with chronic phase CML in the wider Auckland region receiving imatinib as first-line therapy between March 2003 and February 2008 were analysed for haematological, cytogenetic and molecular responses, event-free, progression-free and overall survival. Toxicities and resistance were also recorded. In addition, clinical practice patterns were evaluated against published guidelines. Statistical analyses were performed by Kaplan Meier and log-rank methods.

Result

Patients in the study had a median follow-up of 30.5 months (range: 3-63 months). Best responses were: complete haematological response 97%, complete cytogenetic response (CCyR) 78% and major molecular response, 62%. Event-free survival (EFS) at 30 months was 77%. Freedom from progression to accelerated phase or blast crisis was 95%, overall survival, 100%. Patients achieving a CCyR by one year had significantly better EFS than those who did not (83% vs 57%, P=0.024). Seventeen percent of patients discontinued imatinib for resistance and 7% with intolerance. The rate of primary resistance was 2%, secondary resistance 15% and incidence of kinase domain mutations in resistant patients, 70%. Imatinib toxicities were similar to other studies.

Conclusion

These outcomes mirror the key IRIS study, and are similar to the recently published Hammersmith population-based study. Treatment approaches met European LeukaemiaNet Guidelines. This study provides a pilot for national evaluations of CML treatment outcomes and clinical management in New Zealand.

No conflict of interest to disclose

Wednesday 21 October
HSANZ Free Communications 11

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Meeting Room 3

O128

1215

OCT-1 SNPs Known to Reduce Function Do Not Account for the Observed Interpatient Variability in OCT-1 Activity or Response to Imatinib in the Australian TIDEL Trial

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Aim

Intrinsic sensitivity of CP-CML patients to imatinib-induced kinase inhibition is related to the functional activity of OCT-1, but not directly related to the level of OCT-1 mRNA. Single nucleotide polymorphisms (SNPs) can influence protein function or substrate recognition. OCT-1 is known to be highly polymorphic, with several reduced-function (R61C, C88R, P341L) or loss-of-function (G401S, G465R) SNPs reported. We hypothesised that the presence of non-synonymous polymorphisms in OCT-1 may explain interpatient variation in OCT-1 Activity (OA) and therefore predict patient response to imatinib.

Methods

Blood was collected from 91 newly diagnosed CP-CML patients, enrolled to the TIDEL trial. Three fragments of the OCT-1 coding region, corresponding to the SNPs above, were amplified by PCR and sequenced. Sequences were aligned in Mutation Surveyor against the GenBank OCT-1 reference sequence. The presence or absence of polymorphisms was assessed against the achievement of a major molecular response (MMR : $\leq 0.1\%$ BCR-ABL, IS) by 24 months, and OA.

Results

Table 1: Achievement of MMR by 24 months based on presence or absence of a reduced-function or loss-of-function SNP in OCT-1. (* denotes homozygosity)

Achievement of MMR by 24 months	R61C (n=12)	P341L (n=1)	G401S (n=2)	G465R (n=2)	No reduced-function or loss-of-function SNP (n=74)
MMR (n=58)	7	1	1	1	48
No MMR (n=32)	5	0	1	1*	26

Of the 17 patients found to have a SNP in OCT-1, only one was homozygous (G465R). R61C was the most common SNP (12/91 patients), while the C88R was not detected. The presence of a reduced- or loss-of-function SNP did not correlate with failure to achieve MMR, as shown in Table 1. Of the 8 patients with a SNP and OA data available, 4 (50%) were in the low OA group and 4 (50%) had a high OA.

Conclusion

The impact of SNPs has been classically associated with [³H]1-methyl-4-phenylpyridinium (MPP+) uptake. However it is known that some SNPs effect substrate recognition, hence a SNP that affects one drug may not necessarily affect another. In this study we cannot demonstrate that genetic variation in OCT-1 is the underlying cause of either the wide interpatient variability in OA or the response to imatinib observed in de novo chronic phase CML patients.

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

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1100-1230
Meeting Room 10

O129

1100

A Role for Klf5 as a Tumour Suppressor in AML

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Kruppel-like factor 5 (Klf5) is a zinc finger transcription factor with roles in proliferation, self-renewal, differentiation, and apoptosis. Klf5 displays oncogenic or tumour suppressor properties in a context-dependent manner, and has been implicated in numerous malignancies. We aim to investigate the role of Klf5 as a novel tumour suppressor in the myeloid system and to determine how aberrant regulation of Klf5 may contribute to the pathogenesis of Acute Myeloid Leukaemia (AML). We show that expression of Klf5 mRNA is up-regulated during granulocytic differentiation of murine myeloid cell lines, and this is confirmed in human and mouse primary haemopoietic systems from bioinformatic analysis of published microarray data. Accordingly, retroviral expression of Klf5 in the murine myeloid FDB1 cell line induces growth arrest and apoptosis, and concurrently drives differentiation. Enforced expression of Klf5 in mouse bone marrow progenitors similarly inhibits cell growth and enhances differentiation, with reduced colony forming ability observed in all cytokine combinations tested. Using a panel of 14 human AML samples, 7 leukaemia cell lines and 4 normal bone marrow controls we show that Klf5 mRNA expression is significantly lower in AML samples compared to normal controls. This finding agrees with our analysis of published microarray data sets evaluating larger AML patient cohorts (n=285). We have employed Sequenom MassARRAY quantitative technology to identify methylation of Klf5 Intron 1 as a potential mechanism for its down-regulation in AML. Treatment of leukaemia cell lines with the methyltransferase inhibitor 5-Azacytidine re-activates Klf5 expression indicating a functional role for the observed methylation. Additionally, retroviral expression of Klf5 in AML cell lines results in changes associated with myeloid differentiation. Collectively, these data are consistent with Klf5 acting as a myeloid tumour suppressor and re-activation of Klf5 may be important in the development of therapeutic strategies for AML.

No conflict of interest to disclose

Wednesday 21 October
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Meeting Room 10

O130

1115

New Targets for the *Protein Kinase* Activity of PI3K: Implications for Deregulated Cell Survival in Acute Myeloid Leukaemia

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Cytokines are potent regulators of cell survival through their ability to activate intracellular signalling cascades via protein phosphorylation. We have previously shown that GM-CSF stimulation of haematopoietic cells results in serine 585 phosphorylation of the GM-CSF receptor, the binding of 14-3-3 family of scaffold proteins and regulation of cell survival¹⁻². We have also shown that Ser585 phosphorylation is deregulated in acute myeloid leukaemia¹. We have therefore sought to identify the kinase that phosphorylates Ser585 using a biochemical purification approach. We have isolated a Ser-585 kinase activity and determined its pharmacological profile against a panel of inhibitors. Strikingly the Ser585 kinase activity was only inhibited by PI3K inhibitors. We have confirmed that PI3K p110 alpha can directly phosphorylate Ser585 in vitro and that p110 alpha selective-inhibitors block Ser585 phosphorylation and abrogate haematopoietic cell survival not only of primary bone marrow progenitors but also in leukaemic stem/progenitor cells³. These results show that Ser585 of the GM-CSF receptor can be phosphorylated by the *protein kinase* activity of PI3K and that these events are deregulated in leukaemia leading to constitutive Ser585 phosphorylation and autonomous cell survival. Blocking PI3K *protein kinase* activity by alpha selective PI3K inhibitors may be a potential strategy in eradicating residual AML leukaemic blasts.

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No conflicts of interest to declare

Wednesday 21 October
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1100-1230
Meeting Room 10

O131

1130

Repression of *Gadd45a* by Activated FLT3 Receptors and DNA Methylation in AML

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The tumour suppressor gene Growth Arrest and DNA Damage Inducible (*Gadd45a*) is involved in induction of cell cycle arrest and DNA repair in normal cells in response to DNA damage. Down-regulation of *Gadd45a* is a hallmark of many solid tumours, such as breast and prostate. In AML, we have shown *Gadd45a* expression to be down-regulated across a broad range of karyotypes and cytogenetic abnormalities (Perugini *et al*, Leukemia, 2009). Our functional analysis is consistent with an important role for *Gadd45a* down-regulation in the continued growth and survival of AML cells. *Gadd45a* is most significantly down-regulated in AML patients harbouring MLL translocations or FLT3 receptor mutations, which together constitute >40% of AML cases. In FLT3-ITD+ cells we have shown regulation of *Gadd45a* downstream of sustained ERK1/2 signalling. Consistent with functional down-regulation of *Gadd45a* by FLT3-ITD we show that over-expression of *Gadd45a* in FLT3-ITD+ myeloid cell lines induces G1/S cell cycle arrest and increases apoptosis. *Gadd45a* expression in AML may also be regulated at least in part by DNA methylation. Using Sequenom Mass Array methodology we identified methylation of the *Gadd45a* promoter on CpG residues previously reported to be methylated in breast and prostate cancer. We found significant promoter methylation in 8/15 AML samples when compared to normal controls. In summary we propose that *Gadd45a* is an important regulator of myeloid cell growth and there are multiple mechanisms of *Gadd45a* down-regulation in AML, including activated receptor signalling and DNA methylation. We are now investigating whether the DNA methylation status and expression of *Gadd45a* is an important prognostic indicator in AML.

No conflict of interest to disclose

Wednesday 21 October
HSANZ Free Communications 12

1100-1230
Meeting Room 10

O132

1145

Membrane Bound Phosphatase Genes Are Epigenetically Regulated in Acute Lymphoblastic Leukemia

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Aim

Gene promoter methylation is an important epigenetic abnormality associated with silencing of tumour-suppressor genes and cancer development. The methylation status of members of a phosphatase gene family was examined in Acute Lymphoblastic Leukemia (ALL).

Method

Methylation specific digestion of DNA derived from ALL patients was performed prior to hybridisation to an Agilent array. Promoter methylation was examined in cell lines and primary leukemia cells with pyrosequencing after bisulfite treatment of DNA. Gene expression was measured in cell lines with real-time PCR.

Result

Genome wide analysis of CpG island methylation in ALL identified abnormal promoter methylation in a large number of genes including members of a membrane bound phosphatase gene family. The methylation status of the promoter of seven phosphatase genes was examined in leukemia cell lines using pyrosequencing and promoter methylation was validated in four members of this gene family. The methylation status of these four validated genes was then examined in primary leukemia samples. The promoters of these four phosphatase genes were significantly methylated in 75% of primary ALL samples analysed (n=53) but promoter methylation was not identified in CLL (n=14), AML/MDS (n=28) or normal samples (n=11). Expression of all 4 phosphatase genes increased in Raji and ALL1 cell lines after *in vitro* exposure to decitabine suggesting that these genes may be epigenetically modified with demethylating therapy.

Conclusion

Four members of a phosphatase gene family were identified to be specifically methylated in ALL. These membrane bound phosphatases may represent tumour-suppressor genes and provide potential targets for demethylating therapy with drugs like azacytidine or decitabine.

No conflict of interest to declare

A230

Wednesday 21 October
HSANZ Free Communications 12

1100-1230
Meeting Room 10

O133

1200

A Distinct Set of MicroRNAs Differentiates Acute Promyelocytic Leukaemia According to FLT3 Mutation Status

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Aim

The PML-RARA fusion gene is central to the pathogenesis of acute promyelocytic leukaemia (APL), however is insufficient alone to induce leukaemia and co-operating mutations have been postulated as “second hit” leukaemogenic events. Foremost amongst these are internal tandem duplications (ITD) and point mutations (PM) in the FLT3 tyrosine kinase which occur frequently in APL (40-50%). Presumably, alternate mechanisms of gene dysregulation are operating in APL patients with only wild type FLT3. MicroRNAs are important regulators of gene expression and their deregulation is linked to the pathogenesis of haematological malignancy. To address what role microRNAs may be playing in the pathogenesis of APL we have examined microRNA expression patterns in FLT3 mutated and wild type APL specimens.

Methods

Total RNA from 24 diagnostic APL bone marrow aspirates (12 wild type FLT3; 9 FLT3 ITD; 3 FLT3 PM) were labelled and hybridized to Agilent microarrays encompassing probes for 723 human microRNAs sourced from miRbase version 10.1. Linear modelling analysis with a false discovery rate cut-off <0.05 was applied to determine differentially expressed microRNAs between FLT3 wild type and ITD samples. Differential expression was validated using RT-PCR (Applied Biosystems) in 17/24 microarrayed samples and a second set of 16 diagnostic APL bone marrow aspirates (8 FLT3 ITD mutation; 8 FLT3 wild type).

Results

Seven differentially expressed microRNAs were identified that were down-regulated (miR-155; miR-378 and miR378*) or up-regulated (miR-10a; miR-99a; miR-100; miR125b) in FLT3 wild type compared to ITD samples. Fold change differences ranged from 1.9 to 6.7. RT-PCR for these seven microRNAs validated these findings.

Conclusions

These microRNAs include several already known to play a role in normal and/or malignant haemopoiesis (miR-155; 10a; 125b) Bioinformatic studies are currently underway to provide insight into leukaemogenic mechanisms in APL by determining potential mRNA targets and biological pathways in which these microRNAs act.

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O134

1215

Up-regulated Expression of a Large MicroRNA Gene Cluster in Acute Promyelocytic Leukaemia

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MicroRNAs are small non-coding RNAs that regulate gene expression and have a central role in cellular differentiation. They act by binding to target messenger RNAs and inhibiting protein translation from that gene. We have been exploring the role of microRNAs in the leukaemogenesis of acute promyelocytic leukaemia (APL) and have used microarrays to determine microRNA expression patterns in 24 diagnostic APL bone marrow specimens. Unsupervised two-dimensional hierarchical clustering analysis of these data identifies a group of 47 microRNAs with 10-100-fold up-regulation in 17/24 APL bone marrow specimens. All of these microRNAs are encoded within a known gene cluster that forms part of a larger imprinted region (DLK1-DIO3) on chromosome 14q32. In mice, microRNA genes in this cluster are expressed predominantly in embryonic tissue, and adult brain and placenta. The two groups with high or low expression did not segregate on the basis of FLT3 mutation status, PML-RARA breakpoint region, white cell count at diagnosis, patient age or sex, or cytogenetic abnormalities additional to t(15;17). To further investigate this we selected four microRNAs encoded in the 14q32 cluster (miR-127; miR-337-3p; miR-299; miR-495) and used quantitative RT-PCR to determine their expression in bone marrow specimens from an independent set of newly diagnosed APL (n=12), other acute myeloid leukaemia (AML) patients (n=10), and from APL patients in molecular remission (n=5). Expression of the 14q32 cluster microRNAs was unique to the APL patients at diagnosis, with 10/12 samples showing up-regulation. Minimal expression was noted in the AML and APL remission samples. Deregulation of 14q32 cluster microRNA genes has been reported in other cancers and our findings suggest this region is also important for leukaemogenesis in APL. Mechanisms responsible for up-regulation of this cluster in the majority of APL patients are under investigation.

This work was supported by the Cancer Institute of NSW Clinical Fellowship Programme

No conflict of interest to disclose

A232

Wednesday 21 October

1100-1230

ANZSBT ASTH Combined Symposium: Anti-thrombotics and Anti-platelets: Problems

They Cause

Hall B
1100

Reversal of Anticoagulants: Old and New. The Emerging Paradigm

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Thromboembolism (TE) is a major contributor to morbidity and mortality. More importantly, it is a preventable disease. Hence, with a growing number of indications, extended antithrombotic therapy use is increasing.

The vitamin K antagonist warfarin is the current conventional choice, but concerns surrounding the safety and practicalities of its use remain an important issue. Heparins, in particular low molecular weight, as alternatives are not without problems. This has not only limited the feasibility of thromboprophylaxis, but has spurred the development of novel parenteral and oral agents – which may offer a more reliable and convenient approach. Many of these emerging options, which predominantly target factor Xa and thrombin, are under phase III investigation and some have already been approved by regulatory bodies. However there are potential obstacles.

The most important complication of anticoagulant use is bleeding. If a patient presents with clinically relevant bleeding, or in situations of recognised risk, such as surgery or trauma - rapid and reliable reversal is required and ideally with a means to monitor the residual antithrombotic activity.

Warfarin is reversed by vitamin K and plasma-based products, but the optimal approach, despite widespread guidelines, remains varied. Apart from idrabiparinux, none of the newer agents have a specific antidote. “Non-specific” prohaemostatic agents, such as recombinant FVIIa, and modalities to assist clearance of these small molecules (e.g. haemodialysis) may have a role. However, there is no robust clinical data demonstrating utility, particularly in bleeding patients. Moreover, there is lack of validated assays for monitoring the antithrombotic effect of these new agents.

As efficacy and safety benchmarks are met, notwithstanding costs, the newer agents offer the potential to refine TE management. Lack of effective antidotes and means of monitoring remains a challenge. Local guidelines and strategies for management need to be considered, as use of these novel agents emerges.

No conflict of interest to disclose

Wednesday 21 October

1100-1230

ANZSBT ASTH Combined Symposium: Anti-thrombotics and Anti-platelets: Problems

They Cause

Hall B

1130

Heparin-induced Thrombocytopenia in the Critical Setting

Lena Napolitano

Abstract not received at time of going to print

Wednesday 21 October

1100-1230

ANZSBT ASTH Combined Symposium: Anti-thrombotics and Anti-platelets: Problems

They Cause

Hall B

1200

Novel Tests for Monitoring Haemostasis and Anticoagulants

Benny Sorensen

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Standard coagulation assays such as the activated clotting time (ACT), prothrombin time (PT/INR), or activated partial thromboplastin time (APTT) gives information only of the very initiation of clot formation. However, formation of a sufficient haemostatic plug is a continuous process with characteristic rate-specific properties. Routine coagulation assays, such as the PT and APTT are often performed using platelet poor plasma. The revised understanding of the haemostatic system has emphasized that all blood cells, platelets in particular, are important for the overall regulation of the haemostatic process.

A problem of special concern includes measurement of levels of fibrinogen a.m. Clauss (standard procedure in most coagulation laboratories). Hemodilution with colloids and dextran interfere with the measurement and reveal "false" high levels.

An ideal laboratory test would have the capacity to determine the clinical implication of a biochemical coagulation diagnosis and provide specific guidance in choice of effective haemostatic intervention.

Currently, there is no single haemostasis laboratory test that has the capacity to accurately illustrate the clinical effects of all types of pro- or anticoagulant interventions. Although the time course of thrombin generation in plasma and the endogenous thrombin potential (ETP) may be useful coagulation parameters, clotting involves components other than thrombin (e.g. platelets, fibrinogen). In particular it should be emphasized that normalization of thrombin generation has limited effect if the predominant characteristic of a coagulopathy is due to a dysfunctional fibrinogen polymerization (e.g. dilutional coagulopathy).

The continuous coagulation profiles of thrombelastometry may provide a more accurate reflection of *in vivo* biology, covering initiation, development and final clot firmness during whole blood clot formation. This method has helped to clarify the mechanism of action of whole blood clot formation, demonstrating the differences from clotting in plasma, and the importance of platelets and fibrinogen. Thrombelastometry has been used extensively in the clinic for monitoring the haemostatic system during cardiac and liver –surgery. It has also been used to investigate hypocoagulation (in haemophilia A, rare coagulation disorders, anticoagulant therapy and dilutional coagulopathy), and the effect of haemostatic interventions by e.g. fibrinogen, activated prothrombin complex concentrate, factor VIIa, factor VIII, factor XIII, and antifibrinolytics.

Evaluating levels of functional fibrinogen using thrombelastometry and commercial assays (e.g. FIBTEM®) do not overestimate levels of fibrinogen in cases of dilutional coagulopathy.

Overall, tailoring laboratory assays to illustrate and correlate with clinical phenotypes is essential for effective coagulation monitoring. Applying an algorithm of pre-, peri- and postoperative tests, including thrombelastometry and evaluation of whole blood platelet aggregation, may enable physicians to achieve this.

