0930 to 1100 La Trobe Theatre
ANZSBT Plenary Session 1 - Hospital Transfusion Safety 1
Chair: Erica Wood

14
The Changing Landscape of Transfusion Medicine: 2004- 2015
Walter H Dzik

Born at the dawn of the 20th Century, Transfusion Medicine has seen tremendous progress and technical achievement during the last 100 years. As the pace of change increases, the prospects for the future are even brighter. This lecture will review the recent past developments and provide conjecture on future advances in each of the following areas: blood donor services, infectious hazards, non-infectious hazards, process risk, diagnostics and therapeutics. Expectations of what may occur by 2010 and 2015 will be presented. Emerging technologies such as gene array and radio-frequency identification are likely to produce advances in the safety of blood transfusion therapy. More into the future, nanotechnology may re-invent the indications for blood therapies. The field of new therapeutics should be the fastest growing area and the lecture will highlight recent advances in drug development and molecular therapeutics as well as highlight coming technology such as robotic surgery. The therapeutic promise offered by nuclear transfer technology and its impact on cellular therapies will be addressed. With these new opportunities come new challenges and the lecture concludes with an impassioned reminder of the real challenges that await us.

15
What Has SHOT Told Us and What Are We Doing About It?
Lorna M Williamson

The UK haemovigilance scheme Serious Hazards of Transfusion (SHOT) has just issued its 7th report. Particpiation remains high, and is now actively encouraged by the Dept of Health. The main areas of concern over this period have been hospital errors, bacterial contamination, and more recently transfusion-related acute lung injury (TRALI). Post-transfusion purpura and transfusion-associated graft-versus-host disease, which were already uncommon, have virtually disappeared since universal leucocyte depletion in 1999. To minimise hospital errors, new guidelines for blood administration have been issued, and hospitals are trialling bar code equipment from patient wrists bands and blood fridges, which presents implementation challenges. SHOT is working with the new National Patient Safety Agency on further measures to reduce errors. Bacterial reduction is being approached in a stepwise manner, with new 2-stage donor arm cleansing being rolled out, and blood bag divert pouches now universal. Bacterial screening and pathogen reduction remain under consideration. Review of donors and components associated with TRALI cases showed a strong association between HLA- or HNA-positive female donors and 'high plasma' components (FFP and platelets). Therefore we are moving towards 'male only' FFP, and approach which requires neither donor testing or deferral. We have achieved 90% success so far, and some centres also use male plasma for suspension of buffy coat pools. Additional options for platelets including additive solution are being considered. Future haemovigilance data will assess the impact of these initiatives.

1130 to 1245 La Trobe Theatre
ANZSBT Plenary Session 2 - Hospital Transfusion Safety 2
Chair: Chris Hogan

16
Tackling the Problem of Bacterial Contamination
James P AuBuchon

Focus on the risks of viral transmission via transfusion has pushed recognition of other transfusion risks to the background over the last several decades. With reduction in the risks of HCV and HIV to very low levels (well less than 1/million units), productive attention can now be directed toward other risks. Bacterial contamination of platelets represents the largest morbidity and mortality risk faced by a platelet recipient, and the risk of dieing due to a contaminated unit is at least 10- if not 100-times greater than the chance of HIV transmission. Approximately 1 in every 1-4,000 units of platelets can be shown to contain bacteria, and the frequency of fatality due to port-transfusion sepsis is 14/million units transfused. Bacterial contamination is often not recognized clinically, however, because of the situation of the patient. A variety of techniques to limit and detect bacterial contamination are available for implementation. Culturing is used most widely. Checking pH, glucose concentration or preservation of swirling are simple and inexpensive techniques but suffer from lack of sensitivity. The development of immunologic techniques that can be accomplished rapidly may shift the testing to immediately before issuance for transfusion, a time at which any contamination would be easier to detect because of growth during the storage period. Not only will detection techniques improve transfusion recipient safety, but they can lead to cost-savings through extension of the platelet storage period as has already been implemented in several European countries.

Panel Discussion
Walter H Dzik , Lorna M Williamson , James P AuBuchon

1415 to 1530 La Trobe Theatre
ANZSBT Plenary Session 3 - Apheresis
Chair: James Isbister

17

Granulocyte Transfusion: Best Practice Collection & Transfusion

Ronald Strauss

Serious and repeated infections with bacteria, yeast, and fungus continue to be a consequence of severe neutropenia. Previous attempts to prevent and/or treat these infections in severely neutropenic patients through use of neutrophil (PMN) transfusions achieved only modest success - largely due to the collection and transfusion of relatively small numbers of PMNs. Granulocyte colony-stimulating factor (G-CSF) has revolutionized the collection of PMNs for transfusion because stimulation of donors with G-CSF plus corticosteroids before leukapheresis permits collection of 6-8 x 10to the10 PMNs per each transfusion. Although questions and concerns have been raised about the biology and potential toxicity of giving G-CSF + steroids to normal allogeneic donors, these issues have questionable clinical significance. Properly controlled, clinical trials have not been performed to evaluate the efficacy and toxicity of PMN transfusions collected from donors stimulated with G-CSF + steroids. Therefore, despite the somewhat compelling 'logic' that transfusing very high numbers of PMNs should be helpful in treating serious infections in neutropenic patients, this practice can not be justified at this time. Indeed, published reports of individual patients and small series of patients have given mixed results (i.e., successes and failures). Hence, the need for properly-designed clinical trails.

18

Ethics and Apheresis: What Choice is There?

Ian Kerridge

Westmead Hospital, NSW

By comparison with many other procedures in medicine, apheresis for collection of haematopoietic progenitor cells (HPC) is a relatively innocuous process. The procedure itself is straightforward, serious adverse effects are rare and many donors experience real benefits. But the apparent simplicity of HPC harvest is misleading as apheresis raises substantial issues of consent, risk assessment and conflict of interest. G-CSF primed apheresis is associated with a number of predictable short-term risks and unpredictable long-term risks, particularly in those who submit to a second or third donation. Donors are often coerced by the very nature of their relationship to the recipient and by expectations of altruism and are infrequently offered a true choice between bone marrow harvest and peripheral HPC harvest. And, as is the case in solid organ donation, HPC donors may consent to HPC collection for a number of different reasons, some of which may negatively impact upon their experience of apheresis and may give rise to concerns regarding late withdrawal from donation. While these issues are complex enough where competent adults are the donors, they are more difficult again where the 'donor' in not competent, is mentally ill or is a child. In this paper I will review the ethics of apheresis, critique the concept of informed consent and the disproportionate concern with unrelated donors, and consider whether current practices are ethically justifiable.

1600 to 1730 La Trobe Theatre

ANZSBT & ASTH Plenary Session 4 - Haemostasis Support in Patients with Massive Blood Loss

Chair: Walter H Dzik

Panel

Walter H Dzik, James Daly, David Andrews, Chris Hogan, Julie Miller

0830 to 1030 La Trobe Theatre
ANZSBT Plenary Session 5 - Platelet Transfusion
Chair: Erica Wood

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Platelet Transfusion - Back to Basics
Lorna M Williamson

As in many aspects of transfusion medicine, practices have developed based on low levels of evidence. Let's review some assumptions and think how these might change in future:-

(1) Indications. 80% of platelet transfusions are given prophylactically to patients with bone marrow failure, yet no trials have demonstrated the value of prophylaxis over treatment of bleeding alone.
(2) Trigger. A trigger of 10 x 10^9/l is now accepted. Reducing this further will depend on accurate counting, challenging at this level.
(3) Dose & frequency. The optimum dosing schedule has not been derived by pharmacokinetic studies, but by what can be conveniently manufactured. An adult dose of 3 x 1011 platelets is accepted, but there is wide variation in practice.
(4) Manufacture. Globally, 3 methods (pooled buffy coats, platelet rich plasma and apheresis) all produce an efficacious product. Although apheresis platelets reduce donor exposure, no functional advantage has been demonstrated.
(5) In vitro assessment of function. A battery of tests is recommended, but only pH has been shown to correlate with post-transfusion survival.
(6) ABO matching. ABO compatible platelets give better increments, and transfusing ABO incompatible plasma can be associated with haemolysis, but ABO matching to patient is often compromised by stock management.
(7) Use of platelet additive solution. A 70:30 mix of PAS:plasma would reduce reactions and perhaps TRALI but this is not standard practice. Is the plasma useful?
(8) CMV safety. Overall, leucocyte depletion alone appears to provide a high degree of protection, but this is not universally accepted.
(9) Assessment of response. 1 and 24 hr increments are standard, but is regression analysis the way forward?
(10) Management of refractoriness. Better HLA and HPA matching is now technically possible. Should these lead to better HLA matching for all?
(11) Is one product for all appropriate? 20% of platelets go to surgical patients who are bleeding. Do they need a product with a different specification?

74
Improving Platelet Transfusion: The Impacts of Pathogen Reduction Technology and Increased Storage Periods
James P AuBuchon

Removing, inactivating or detecting pathogens in platelet units are panaceas that some expect to revolutionize transfusion support for patients. Opting for transfusion without concern for the presence of pathogens may not be such a simple decision, however. The effect of pathogen reduction treatment may reduce platelet number and/or function. Clinical trials have documented that treated platelets provide effective hemostatic support for thrombocytopenic patients. However, platelets may need to be transfused in greater quantity, and thus the treatment technique may create a greater demand for the production of platelets through the supply system. Toxicologic concerns about the treatments must be addressed to ensure that there is net benefit for patients. Testing reported to date suggests that any residual compounds are at concentrations far below that associated with detectable toxicity, but the concept of transfusing a component containing even minute amounts of toxic substances may be unsettling to some. Detecting bacteria may remove the largest remaining infectious threat in platelet units and would leave platelets undamaged to function normally. This opens the option for extending the storage period of platelet units, but any extension of storage would be expected to be associated with a decrease in recovery and survival. To avoid continuing decrements with each proposed change in processing or storage, a new standard for efficacy has been proposed that would compare the recovery and survival of the platelet component to fresh platelets from the same donor. This standard appears useful and applicable in ensuring safe and efficacious platelets for transfusion. The question remains: Which direction is best for platelet recipients?

0830 to 1030 You Yangs 5
ANZSBT 'Therapeutic Goods Administration Symposium'
Chair: Glenn Smith

75

Regulatory Oversight of Blood, Tissues and Emerging Biological Therapies in Australia and New Zealand

Richard Pembrey

Therapeutic Goods Administration

The regulation of blood, blood product, tissue and gene technology therapies are viewed as critical to protecting the health of Australians. In response to the growing community concerns regarding the ethics, quality and safety of tissue and biological therapy products, the Australian Health Ministers Council, in 2002, recommended the development and introduction of a regulatory framework for tissues and emerging biological therapies. The proposal for tissues and emerging biological therapies parallels the regulation of blood and blood components introduced by the TGA in 2000 following similar ministerial recommendations, policy development and consultation A comprehensive system for the regulation of cellular and tissue based therapies within a proposed trans Tasman framework will be characterised by the following principles:

• All therapeutic products definable as cellular and tissue therapies, including cell-based gene therapy products, will be overseen by the system

• The level of regulation will be classified according to the risks posed by the therapies to the individual and the community.

• The classification system will align regulation of therapies to:

- standards
- standards and GMP
- standards, GMP and pre-market evaluation according to the level of risk.

• All facilities responsible for the manufacture and supply of cellular and tissue based products will be required to register their organisation with the TGA.

76

Regulation of Blood Components - a Help or Hindrance to Good Clinical Practice?

Peter Flanagan

New Zealand Blood Service

The application of formal regulatory structures to the field of blood component and blood product manufacture is an understandable response to public and governmental concern over the safety of such products. Internationally the trend is to greater central control of blood service provision and regulation is one of several tools used to achieve this. In both Australia and New Zealand, blood components are treated as medicinal products for the purposes of regulation. The imminent creation of a TransTasman Regulatory Authority provides an opportunity to review systems used for the regulation of these products within Australasia and to ensure that an appropriate balance between clinical need and quality conformance is achieved. The control of human derived medicinal products presents a number of specific challenges. This is particularly the case in relation to blood components where biological variation will impact on the 'formulation' of any given product. Essentially each component becomes a unique batch. Efforts at control and regulation should recognise this. Two broad approaches can be used for regulation. In the first control is achieved through the application of standard processes. In the second the necessary outcome is defined but the process not formally defined. Increasingly regulatory control of blood component manufacture favours control of the process. Control through process can however ultimately restrict clinical flexibility and responsiveness. Care must be taken to ensure that this is recognised and addressed in the development of systems utilised to assure the quality and safety of blood components.

1100 to 1200 La Trobe Theatre
ANZSBT Plenary Session 6 - Governance in the Blood System
Chair: Joanne Pink

78
The Role of the National Blood Authority
Peter DeGraaff

National Blood Authority

Since its establishment on 1 July 2003, the National Blood Authority (NBA) has been making steady progress towards fulfilling its vision of 'Saving and improving Australian lives through a world-class blood supply'. While the NBA's early focus has been on developing organisational infrastructure and meeting compliance requirements as a new Commonwealth agency, it has already begun to bring about improvements to the management of the blood sector in line with the recommendations of the 2001 Stephen Review. As the centralised procurement agency for blood and blood products, the NBA is responsible for managing existing supply contracts with the Australian Red Cross Blood Service, CSL Limited and a number of pharmaceutical companies, and is currently negotiating new contracts with two major suppliers. The NBA has also been working with the signatories to the National Blood Agreement, all nine Australian Government Jurisdictions, to coordinate national demand and supply planning. It is introducing an agreed single national pricing schedule and has developed improved forecasting models and reporting mechanisms to ensure that jurisdictional requirements for blood and blood products are more closely matched to supply. With time, the NBA hopes to play a greater facilitation role in encouraging the better clinical use of blood and blood products as this will not only result in better patient outcomes, but will also contribute to ensuring the adequacy of the blood supply. This presentation discusses why the NBA was established, its roles and responsibilities, and how it is working to bring about reforms in the sector in terms of increased value for money, transparency and accountability.

79
Clinical Governance and Transfusion
Jenny Bartlett

Department of Human Services, VIC

Governance is the exercise of power consequent on the assumptions of responsibility. Clinical Governance is the process and framework through which Health Services achieve accountability for continuously improving the quality of their services and safeguard high standards of care by creating an environment in which excellence in clinical care can flourish in a patient centred environment. In terms of clinical governance as it pertains to safe and appropriate use of blood and blood products it is being rolled out in Victoria at two levels. At a hospital level, as part of clinical governance there is a requirement for organisations to have an active transfusion committee to provide oversight on the usage, appropriateness and adverse events related to blood and blood products. This committee should report through to the hospitals clinical risk/quality committee. Transfusion nurses have been funded to amongst other things resource this committee. At a state level Victoria has established a Better Safer Transfusion (BeST) practice program supported by a BeST advisory committee. The work will support hospital transfusion committees and advise on future work in the area.

80
The UK Approach
Lorna M Williamson

1400 to 1530 La Trobe Theatre
ANZSBT Free Communications 1 - Transfusion Practice
Chair: Carole Smith

81

Blood Transfusion Prescribing Patterns Across the Australian Capital Territory (ACT)

Bethany Crowe [1], Philip Crispin [1,2], Sue Quayle [1,3], Anne McDonald [1,2]

[1] ACT Haemovigilance Project, Canberra, ACT
[2] ACT Pathology, Canberra, ACT
[3] Calvary Hospital, Canberra, ACT

Aim: To identify the medical officers responsible for blood transfusion therapy across the ACT.

Method: Medical records of patients having transfusions were audited in one tertiary referral hospital, one general hospital and one associated private hospital as part of a quality improvement project. The clinical status patients, reasons for transfusion and laboratory data were extracted and the appropriateness of transfusion was assessed independently by two of the authors. Differences were resolved by consensus. The prescriber was also identified where possible, defined as the most senior clinician recorded in the medical record involved in the decision to transfuse.

Result: The prescriber could be determined in 78% of transfusion episodes. In the majority of cases the decision to transfuse was attributed to specialist staff, in all hospitals. Registrars at the tertiary hospital prescribed significantly fewer inappropriate transfusions (12%) than specialists (18.1%, $p<0.05$). This was not found at the smaller public hospital. After hours resident and registrar staff prescribed significantly fewer inappropriate transfusions (3%) than other prescribers across the Territory (18.7%, $p<0.005$).

Conclusion: Medical practitioners with varying levels of experience make medical decisions in hospitals. Who is responsible for these decisions has not been well studied. Our findings demonstrate specialist involvement in the majority of transfusion decisions. The results favour a 'top down' approach to quality improvement in transfusion, rather than targeting junior medical officers. The findings also have significant implications in understanding the dynamics of clinical decision making in the hospital setting and for the targeting of other quality improvement strategies.

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Prothrombinex Use in Cardiac Surgery: Results of a 6 Month Audit

Philip Campbell [1], Lisa Stevenson [1], Charlie Corke [2], Anthony Plowman [3], Morteza Mohajeri [4]

[1] Clinical Haematology, Geelong Hospital, Barwon Health
[2] Intensive Care, Geelong Hospital, Barwon Health
[3] Cardiac Anaesthesia, Geelong Hospital, Barwon Health
[4] Cardiothoracic Surgery, Geelong Hospital, Barwon Health

Cardiac surgery continues to be associated with excessive perioperative bleeding due to poorly characterised haemostatic changes associated with cardiopulmonary bypass (CPB) and the trauma of major surgery. Blood component support varies between institutions and the recent introduction of recombinant VIIa promises to improve perioperative haemostasis, albeit at significant expense. Prothrombin complex concentrates (ProthrombinexTM-HT(PTX), CSL Australia) are utilised increasingly to correct excessive oral anticoagulation and have a number of advantages over FFP including greater coagulation factor concentration (particularly IX), reduced product volume and viral inactivation. There is no data on the use of PCCs in cardiac surgery. During a 6 month period (February - August 2003), 203 patients underwent cardiac surgery at Barwon Health of whom 60 received PTX during the intra-operative or post-operative period (45 M, 15 F; Mean age 68.7 years). 28 (47%) patients underwent simple procedures (either valvular or graft surgery alone) while the remaining 32 (53%) patients had complex surgery (emergency cases, graft/valves, double valves, Bentall's or redos). PTX was employed following the use of standard blood component support when there was evidence of persistent bleeding and abnormal laboratory coagulation studies. 20 patients intra-operatively and 34 patients post-op required no PTX. The mean dose of PTX administered intra-operatively was 595 U (range 500-2000 U) and 240 U post-operatively (range 500-1000). PTX use was associated with documented improvements in bleeding and coagulation studies; mean INRs and APTTs pre- & post-PTX 1.7>1.3 and 65s>52s respectively. 33 (55%) patients were evaluated at 3 months through their physicians for evidence of prothrombotic sequelae. 8 patients were noted to have had potential prothrombotic complications (4 myocardial ischaemia, 1 pulmonary embolus, 1 CVA and 2 thrombophlebitis).

Conclusion: On the basis of this small study, PTX is a useful adjunct to standard blood component support in cardiac surgery and may defer the use of rVIIa in this setting.

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Investigation of Intragam® P Haemolysis Adverse Events

James Isbister [1], Chris Hogan [2], Erica Wood [3], Fiona Wilson [4], Darryl Maher [4]

[1] Royal North Shore Hospital
[2] Royal Melbourne Hospital
[3] ARCBS, Victoria
[4] CSL Bioplasma

Aim: To understand the underlying cause of a cluster of haemolysis-type adverse event (AE) reports received by CSL/ARCBS in patients receiving Intragam® P.

Method: The cluster of AE reports were analysed with respect to patient factors (clinical indication, dose, blood group, concomitant medications and blood products, bone marrow reserve), product factors (batch results, production records) and changes in clinical practice (administration protocols, infusion kits). All Intragam P haemolysis related AEs from clinical trials and post-marketing exposure were reviewed, as well as international literature on this subject.

Result: Sixteen haemolysis type cases were reported to ARCBS/CSL over a 3 month period, ranging from abnormal laboratory test results (positive Coombs test) to clinical symptoms requiring transfusion. Ten of these reports were received in 4 days from 2 Victorian hospitals following the detection of 2 cases, which precipitated a retrospective review that identified additional similar cases. All patients received high dose (doses exceeding 400-600 mg/kg given every 3-4 weeks either in frequency or quantity) and all but one (where evidence suggested an underlying disorder) were of blood group A or AB. Eight patients were recipients of bone marrow transplants. No product or batch related factors or processes likely to have contributed to the haemolysis type AEs were identified. Anti-A and Anti-B titres of all Intragam P batches were investigated and confirmed to be within regulatory approved release specifications and pharmacopoeial limits.

Conclusion: Haemolysis type adverse events are a recognised consequence of IVIG administration and are widely discussed in the literature. This investigation has confirmed the association of high dose IVIG in recipients of blood group A or AB and increased risk of haemolysis type adverse events. It also highlighted the possibility of accentuating any consequent anaemia in patients with diminished bone marrow reserve (eg post bone marrow transplant patients). Pre-infusion ABO blood groups should be determined in patients receiving high dose IVIG and their haemoglobin levels monitored in the days following therapy.

84

Acquired Factor XIII Deficiency Secondary to an Inhibitor: Effect of Factor XIII Replacement and Rituximab Therapy

Alhossain Abdallah [1,2], Elizabeth Duncan [2], Chirely Casey [2], Ken Davis [1,2], Chi-Hung Hui [1,2]

[1] The Royal Adelaide Hospital, Adelaide, South Australia
[2] Institute of Medicine and Veterinary Science (IMVS), Adelaide, South Australia

Factor XIII (FXIII) is the last enzyme in the clotting cascade. Its main function is to convert the loose fibrin polymer into a firm, cross-linked structure. FXIII inhibitor is a rare condition, in which three different types of inhibitors have been described usually due to IgG antibodies. We describe two male patients with acquired FXIII deficiency (age; 73 and 69 years), who had a history of significant bleeding. Functional assay using the acetic acid/urea clot solubility test showed residual FXIII levels between 1-2%. Molecular studies by PCR and DNA sequencing excluded an inherited FXIII deficiency. In addition, we demonstrated an inhibitor pattern for FXIII by in vitro mixing studies with both purified FXIII (Fibrogammin, Aventis Behring) and cryoprecipitates as sources of FXIII. Transglutaminase assay of FXIII (Dade Behring) was normal for both patients, consistent with a type III FXIII inhibitor, which believed to be directed against the activated FXIII-fibrin complex. Both patients showed transient correction of clot solubility to normal after infusion of high doses of Fibrogammin. In view of previous reports describing FXIII inhibitors of the IgG class, we attempted a novel treatment with rituximab (chimeric monoclonal anti-CD20 antibody, Roche) to interfere with production of the inhibitor. However, neither patient showed correction of FXIII activity as measured by clot solubility tests during and up to 2 weeks after therapy. Preliminary results suggest that high dose Fibrogammin is required to correct FXIII deficiency due to an acquired type III inhibitor. The role of rituximab or other immunosuppressive therapy requires further investigation.

85

The Western Australian Experience with a Register of Atypical Antibodies.

Susan Lariat, Brian Fisher

ARCBS-Enterprise

Since 1980 a register of all blood donors and patients in Western Australia (WA) with clinically significant red cell and platelet antibodies has been maintained by the Blood Group Reference Laboratory, Australian Red Cross Blood Service (ARCBS)-Enterprise. The register is updated by ARCBS daily, and prior to 1999 a microfiche version was sent each month to WA transfusion laboratories. In 1999 ARCBS-Enterprise and the Health Department of WA (HDWA) explored the option of transfusion laboratories in public hospitals obtaining immediate electronic access to the register. A secure telnet radio-link was established between a dedicated stand-alone ARCBS server and the HDWA computer network which allowed password protected enquiry-only access to the database by hospital end-users. All end-users are required to sign an agreement to guarantee proper use of the register. The use of this comprehensive database has been successfully incorporated into the pre-transfusion testing protocols of all transfusion laboratories in WA. The benefits of this system include:

- Reduced delay in obtaining phenotyped units for patients with known antibodies.
- Reduced chance of delayed transfusion reactions in patients whose antibody level has fallen below detectable levels.
- A mechanism for ante-natal monitoring and predicting severity of haemolytic disease of the newborn when patients move between care providers during the pregnancy.

ARCBS-Enterprise and end-users are passionate about the continuation of this extremely important resource in the future.

1400 to 1530 You Yangs 5

ANZSBT Free Communications 2 - Keeping it Fresh, Keeping it Cool

Chair: David Jones

session sponsored by Novo Nordisk

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Refrigeration Failures - Bringing the Problem in from the Cold

Neil Waters, Erica Wood, Malcolm Forsyth

Australian Red Cross Blood Service

Aim: Review of four failures of refrigeration facilities in Victorian institutional blood banks reported to ARCBS in the first 4 months of 2004. These incidents at both public and private institutions, in metropolitan and regional settings, resulted in loss of 148 units of red cells, 7 vials of RhD Ig and 2 bottles of Albumex. Incidents were investigated and root causes identified by initial phone interview and visits to blood bank storage facilities.

Result: Case 1: Temperature fell below 0°C at remote site, alarm was activated at monitoring centre (main lab) but without response. Case 2: Power failure resulted in rise in temperature. Alarm did not activate until power was restored. Alarms were set outside required storage temperatures. Case 3: Circuit failure resulted in loss of power. Alarm was activated, but then switched off and no further action taken. Case 4: Compressor failure resulted in alarm activation. Alarm and monitoring devices were deactivated while repairs carried out. Product was not moved to other storage facilities. These cases all highlight the importance of procedures, training, equipment and maintenance.

Conclusion: Losses of precious blood components and products through refrigeration failures are preventable. Vital improvements should include commissioning refrigeration equipment that meets AS 3864, protocol development and staff training in use of procedures to be followed when out of temperature events occur, and performance of regular preventative maintenance, calibration and testing of storage and monitoring equipment. ARCBS can work with institutional blood banks to improve storage and management of blood.

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Trial of Baxter 'Blood in Motion' Transport System

Ruth Power

St Vincent's Hospital, Melbourne

Aim: To evaluate the Baxter 'Blood in Motion' temperature controlled system for transportation of Red Blood Cells (RBCs) by the blood bank at St Vincent's Hospital Melbourne. The trial incorporates transport of RBCs between the blood bank and theatre within St Vincent's, and between St Vincent's blood bank and blood banks within peripheral laboratories. Interest in this system arose in response to the Australian Red Cross Blood Service (ARCBS) proposed Memorandum of Understanding (MOU) and ARCBS amendments to packing of RBCs for transport effective 1st January 2004. According to the MOU, RBCs must be transported in a system which maintains a temperature range of 2-10 degrees Celcius.

Method: RBCs were transported between the blood bank and theatre in the 'Blood in Motion' transport elements on 11 occasions [N(t)=11]. RBCs were transported between St Vincent's blood bank and Werribee Mercy Hospital blood bank in the 'Blood in Motion' transport elements contained within a 'Blood in Motion' silver bag on 6 occasions [N(w)=6]. An acceptable outcome resulted if the temperature displayed on the LCD thermometer inside the system was within range of 2-10 degrees Celcius on arrival at destination.

Result: An acceptable outcome was achieved in 100% of transport episodes to date. Further data will be collected and evaluated.

Conclusion: The Baxter 'Blood in Motion' transport system is a viable alternative to the ARCBS packing system for transport of RBCs (and facilitates compliance with the proposed ARCBS MOU). This system would ensure maintenance of RBC quality and safety during transport by St Vincent's blood bank and minimise wastage of RBCs.

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New Transport System for Maintaining Temperature of Blood Products in Emergency Situations

Michael Haeusler [1], Chris Hogan [1], Surender Juneja [1], Jane Bartlett [2], A Suter [2]

[1] Department of Diagnostic Haematology, Royal Melbourne Hospital
[2] Baxter Healthcare, Pty, Ltd, Sydney, NSW

The RMH is a large, tertiary teaching hospital that is now a designated level 1 trauma centre. Facilities include a helipad and upgraded Emergency Department and Operating Theatre areas.

In many cases it is not possible to provide group specific and crossmatched Red Cell Concentrates (RCC) for individuals requiring urgent and lifesaving treatment. In this situation, scarce O Negative RCC must be used. Previously, the Blood Bank at the RMH issued blood and blood products to the Emergency Department in plastic bags or foam eskies. Once issued these RCC could not be returned to the hospital Blood Bank inventory, as the product transportation and storing conditions to and in the Emergency Department were uncontrolled.

Baxter Healthcare ½ recently has made available an innovative and validated system of transport for blood and blood products. This 'Blood-In-Motion' (BiM) system consists of moulded plastic elements that are filled with a patented Phase Change Material (PCM). Following our adoption of the BiM we found it an easy to use, validated system for the safe temperature controlled transport of RCC in an emergency situation. We have further utilised the BiM system as a 'Massive Exsanguination Pack' (MEP) used in response to a time-critical call from Emergency. The MEP transports 4 units O Neg RCC, 4 units thawed AB FFP & 1 pool Rh Neg platelets. This BiM/MEP system allows the storage of RCC and plasma products under controlled temperature for up to 6 hours. This solution has enabled the RMH to conserve a scarce and valuable resource, and to supply emergency blood products in a timely and controlled manner.

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A Model for Isolated Area Emergency Donor Panels

Steve Flecknoe-Brown [1], Mark Dean [2], Jan Pearce [1]

[1] Broken Hill Hospital, NSW
[2] Gosford Hospital, NSW

Aim: To review the operation of an Emergency Donor Panel in an isolated Australian community.

Result: The Panel has been in operation for 4 years. Two donors have been rejected, one due to development of cancer and one due to biological false positive hepatitis C serology. Thirty five donors remain active on the panel. No donors have sero-converted for transmissable disease during the operation of the panel. The panel has been activated on 3 occasions, all for surgical bleeding, resulting in blood transfused within 40 minutes of activation in all cases. Two cases were for bleeding associated with ruptured AAA and one for bleeding associated with gangrenous bowel.

Conclusion: A properly designed and monitored Emergency Donor Panel can safely supply blood for transfusion in the event of depletion of local stores. The EDP should be activated after 10 units of stored blood have been transfused into a patient undergoing massive bleeding from a correctible source, or in association with the hospital's Disaster Plan.

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Design and Delivery of a 'Donor-centric' Blood Collection Facility

Ross Savvas [1], Matt Voller [1], Peter Moek [2], Simon McGinn [1], Lisa McDonald [1], Kathleen Doherty [1]

[1] Australian Red Cross Blood Service
[2] Brown Falconer Group

Aim: ARCBS collection sites are located in capital city centres, regional and metropolitan static sites and mobile units. The project aimed to establish a 'donor-centric' collection facility that met evolving donor needs.

Methods & Result: Due to Adelaide's high level of urbanisation combined with; (a) the eastern based skew of the existing Pirie Street operation and (b) the demographic profiles of donor and non-donor cohorts, potential sites for further investigation were identified. This process was further enhanced by surveying donors (stakeholder buy in and input) who indicated a preference for a mid-CBD site. This selection was supported by retailing and traffic analysis that reinforced the Currie St. location - a major street frontage with a transport hub to the western suburbs . 'Guiding Principles' for the design and operation of the centre were developed and stated that the site must; - have a WOW factor - make donation easy for donors - have a modern and professional outlook - have its own personality - be creative and bold - not compete with the existing city site - cater for a different market segment to existing sites. The Guiding Principles and recommendations from donor focus groups, were used by the architects to develop concept drawings. These were reviewed by the Donor Advisory Committee, and specifications finalised. Furniture, fittings, refreshments and marketing strategies also focussed on the target donor groups and Guiding Principles.

Conclusion: A donor focus in the planning, design and development of this centre has produced a facility that currently exceeds collection targets and has been nominated for building industry awards.

1400 to 1530 You Yangs 4
ANZSBT Free Communications 3 - Components and Safety
Chair: Rosemary Sparrow

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A Proteomic Approach for Identifying Proteins that Accumulate During Storage of Red Cell Products

Kristen M Glenister, Angela M Anniss, Jessica J Killian, Rosemary L Sparrow,

Australian Red Cross Blood Service

The aim of this project was to identify potential protein mediators of adverse transfusion reactions in stored red cell concentrates. Previously, research into protein mediators of adverse transfusion reactions has focussed on specific proteins, such as cytokines. In this study, a global approach was taken which involved two-dimensional electrophoresis based Proteomics, which has the potential to uncover previously unrecognised mediators or novel proteins. Red cell products with and without pre-storage leukoreduction (each n=6) were prepared and stored according to standard blood bank procedures. Supernatant samples were taken at several time points until product expiry. Proteins were separated by two-dimensional electrophoresis and those that accumulated in the supernatant were selected for identification by mass spectrometry. The protein profile of supernatant from leukoreduced red cell products was less complex compared to non-leukoreduced products (from 4.4 fold fewer proteins at day 1 to 1.6 fold fewer at day 43). Several proteins were observed to be predominantly present in leukoreduced products which may potentially be beneficial to red cell survival. These proteins were identified as being involved in the maintenance of a stable extracellular environment. Conversely, a number of proteins, which may have detrimental effects, were predominantly expressed in non-leukoreduced products. These proteins included a neutrophil chemoattractant (activator) and a potential acute-phase reactant. A number of proteins identified by mass spectrometry were matched to theoretical proteins of unknown function. The findings confirm the usefulness of Proteomics to investigate storage effects on blood products. The clinical relevance of these proteins is the focus of our future investigations.

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Response of Allogeneic Mononuclear Cells to Stored Red Cell Concentrates

Rosemary Sparrow, Katherine Patton, Geraldine Healey

Research Unit, Australian Red Cross Blood Service, Melbourne, Australia

Red blood cell (RBC) transfusion has been implicated in certain adverse patient outcomes. Immunomodulation may play a central role. The aim of this project was to determine the ability of supernatants and cellular fractions of RBC concentrates to modulate the immune response of allogeneic mononuclear cells (MNCs), particularly monocytes. Non-leucocyte-reduced, buffy-coat-depleted and leucocyte-filtered RBC concentrates were prepared and stored according to standard blood bank procedures. RBC samples were collected on day 1 and fortnightly until product expiry, and centrifuged to obtain supernatant and cellular fractions. On the day of RBC sample collection, whole blood (WB) and MNCs were prepared from ABO-compatible allogeneic donors. Induction of monocyte CD11b and CD54 was determined by flow cytometry by incubating allogeneic WB with RBC supernatants or cellular fractions, followed by staining with fluorescently-labelled anti-CD14, anti-CD11b and anti-CD54. Cytokine release was determined by incubating MNCs with RBC supernatants and culture supernatants were assessed by ELISA for IL-8, TNFalpha ; and IL-10. Supernatant and cellular fraction from non-leucocyte-reduced RBC concentrates induced expression of CD11b and CD54 on allogeneic monocytes. Buffy-coat-depleted and leucocyte-filtered RBC concentrates had minimal effect on monocyte CD11b or CD54 expression. Cytokine release from MNCs incubated with RBC supernatant suggested a tenuous balance between proinflammatory (IL-8 and TNF alpha;) and immunosuppressive (IL-10) responses. All RBC product types induced cytokine release from MNCs. The results from this study indicate that stored RBC concentrates can modulate allogeneic MNCs. Both proinflammatory and immunosuppressive responses were evident. Leucocytes in RBC concentrates appear to favour a proinflammatory response by MNCs.

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Significant Differences in the Borna Virus p24 Region Between Nucleotide Sequences from Humans in Australia and Reference European Strains

Sandra Kamhieh [1,2,3,4,5], Liv Bode [2], Wayne Bolton [4], Jennifer Hodgson [5], Robert Flower [1,2]

[1] PaLMS and Royal North Shore Hospital, Northern Blood Research Centre, Sydney, Australia
[2] Robert Koch Institute, Berlin, Germany
[3] Free University, Berlin, Germany
[4] Australian Red Cross Blood Service, Sydney, Australia
[5] University of Sydney, Faculty of Veterinary Science, Sydney, Australia

Aim: Borna Virus (BV) is a negative strand RNA virus associated with neurological diseases in animals. An association with human neuro-psychiatric disease has been suggested. The hypothesis investigated was that potentially infectious levels of blood borne BV may be present in humans. Levels of antibody to BV in human and cat sera were studied by ELISA with antigen from infected horse brain, immunofluorescence with infected cells, Western blot and BV epitope peptide-ELISA. Monoclonal antibodies were used in capture ELISAs to measure BV antigen levels (BVAg) in plasma and RT-PCR and sequencing used to investigate relatedness of BV sequences from humans in Australia with European sequences.

Result: In 1% of blood donors, free BV antigen was detected in plasma and reactions with BV p24 or p40 antigens confirmed by Western Blot. Circulating immune complexes, antibody to brain-derived antigen and reactions with two of the eight peptides tested were also detected. Strong positive reactions in tests for circulating immune complexes, free plasma antigen and free antibody, were observed in a small number of blood donors. Individuals with high levels of plasma antigen were investigated by RT-PCR and sequencing. Sequence from the p24 region of the Australian human BV genome was 5% different from European reference strains.

Conclusion: These data support the hypothesis that a form of BV is endemic in humans in Australia, including potentially infectious levels in blood donors. The extent of difference, in regions other than p24, between BV in Australia and strains infecting animals overseas remains to be clarified.

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Microbial Contamination in Cord Blood (CB): A Retrospective Analysis of Three Test Protocols

Leigh Mison, April Goodear, Marcus Vowels

Sydney Cord Blood Bank, Sydney Children's Hospital

Aim: Since 1995, the SCBB has used three protocols to screen CB for contaminating microbes. The protocols differed in the number of culture bottles inoculated and sample volume used. The aim of this study was to compare microbial detection rates for each method.

Method: CB units (n=9,618) were tested for microbial contamination using BacT/ALERT blood culture bottles (BioMerieux). Of these, 6,836 had 20ml plasma waste fraction inoculated into adult anaerobic and aerobic bottles (Group A), 365 had 1ml final stem cell product into an aerobic paediatric bottle (Group B), and 2,417 had 1ml final product into a paediatric bottle and 10ml plasma waste fraction into an adult anaerobic bottle (Group C). All bottles were incubated for a minimum of 5 days. Detection rates for microbe contamination were calculated for each group.

Result: Microbe detection rates were: Group A -345/6836 (5.0%), Group B - 3/365 (0.8%) and Group C - 66/2417 (2.7%) ($p<0.0001$). Organisms detected by A but not by B (and to a lesser extent C) included Streptococcus, Staphylococcus, Bacteroides and Proprionibacterium.

Conclusion: Contamination detection rates differ significantly, depending upon the sample volume and type of culture bottle used. A protocol that uses the recommended sample volume and both anaerobic and anaerobic test bottles is likely to detect the highest number of contaminated units. Testing of a small sample from the final product using only a single paediatric aerobic bottle is likely to miss detection of the majority of anaerobic and some aerobic organisms. Prospective studies to define a standardised method for microbial detection for CB are required.

0800 to 1015 John Batman Theatre
ANZSBT Plenary Session 7 - Rh D Immunoprophylaxis
Chair: Amanda Thomson

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RhD Prophylaxis in Australia: 1966 and Beyond
Amanda Thomson

Royal North Shore Hospital, Sydney/ ARCBS Endeavour

In the late 1960s clinical trials showed that the incidence of Rh D immunisation of Rh negative women could be dramatically reduced by the administration of Rh D immunoglobulin (Ig) after delivery. In 1968 Australia became the first country to be self sufficient in Rh D Ig with the introduction of the Rh programme. Despite appropriate use of Rh D Ig 1-2 % of Rh negative women still became immunised. Studies subsequently showed that immunisation rates could be further reduced by prophylactic administration of Rh D Ig during pregnancy. Routine antenatal prophylaxis has been practiced in Canada and USA for more than 20 years. The first NHMRC Clinical Practice Guidelines on the use of Rh D Ig were issued in 1999. These acknowledged that routine antenatal prophylaxis was best practice, however could not be recommended due to supply constraints. Subsequent initiatives included the licensing of the 250IU dose of Rh D Ig, expansion of plasma collection and importation of product. Staged introduction of routine antenatal prophylaxis began in late 2002 with introduction of prophylaxis for primigravidae. Stage 2, which provides antenatal prophylaxis for all Rh D negative women, is to be implemented by 1 January 2005. Stage 3 will see cessation of the need for use of imported product. A Joint Consultative Committee of involved specialties is helping to ensure consultation and communication for the implementation process. The possibility of developing a laboratory Rh D Ig which could be produced in unlimited quantities, in a standardised formulation with no reliance on human derived sources has been entertained for many years. Results of trials of possible options have been intermittently published. However, it is likely that the 'liquid gold' anti D plasma donated by our amazing volunteers will be needed for many years to come.

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Rh(D) Immunoglobulin - Where Does it Come From?
Tanya Davison, Brenton Wylie

Australian Red Cross Blood Service

Australian Rh(D) Immunoglobulin is produced by CSL Bioplasma using plasma containing anti-D collected by the Australian Red Cross Blood Service (ARCBS) from Australian voluntary blood donors. The ARCBS Rh Project was established, initially in New South Wales in 1967, with an aim of providing an adequate supply of Australian derived Rh(D) Immunoglobulin. In 1999, the NHMRC 'Guidelines on the prophylactic use of Rh(D) Immunoglobulin (Anti-D) in obstetrics' stated that universal prophylaxis with Rh(D) Immunoglobulin to RhD negative women with no preformed anti-D antibodies at 28 and 34 weeks gestation is generally regarded as best practice. With the availablity of Rh(D) Immunoglobulin at that time, the NHMRC recommended a phased implementation commencing with all Rh(D) negative primigravidae with no preformed anti-D antibodies (Phase 1). Phase 1 commenced in late 2002. The NHMRC recommemnded that full antenatal prophylaxis (phase 2) would be implemented when domestic supplies of Rh(D) Immunoglobulin increased sufficiently to cover the increased demand. The ARCBS plays an integral part in the implementation of universal antenatal prophylaxis by being the supplier of plasma containing anti-D for production and as the distributor of the final Rh(D) Immunoglobulin product. In 1999, the ARCBS supplied an average of 15x106 IU of anti-D per month to CSL Bioplasma. Implementation of phase 1 occurred following the introduction of two new products; the Australian derived Rh(D) Immunoglobulin 250IU dose for first trimester indications and the temporary importation of WinRho SDFTM for postnatal use. Implementation of phase 2 and 3 requires the ARCBS to increase the amount of anti-D collected to an average of 30x106 IU and 45x106 IU per month, respectively. Phase 3 of the program is full antenatal prophylaxis supported entirely by domestic product. In 2002, additional funding was allocated to the ARCBS Rh Project to implement strategies to ensure that the input targets for phase 2 and 3 are achieved. In mid 2004, following months of primary immunisation and boosting of both new and existing donors, input levels began to consistently meet or exceed 30x106 IU, and planning for phase 2, implementation began. This presentation will give an overview of the strategies used by the ARCBS to increase the volume of anti-D collected from these donors.

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Implementation of Antenatal Prophylaxis: A Midwife's Perspective
Vickie Kyriakopoulos

Family Birth Centre, Royal Women's Hospital, Carlton

In July 2003, The Royal Women's Hospital, Melbourne implemented the use of Prophylactic Rh D Immunoglobulin in obstetrics to help reduce the incidence of Haemolytic Disease of the Newborn (HDN), as recommended by the Chief Medical Officer in December 2002. Successful implementation required the coordinated efforts of a large working party including haematologists, laboratory staff, blood bank, product technicians, CSL, clinical managers, midwives, obstetricians, clerical staff and educators. New practice guidelines, system processes, procedures and staff education needed to be developed and coordinated to support the successful implementation across the Royal Women's Hospital complex maternity care programme sites. This talk firstly outlines how this implementation was achieved across a complex organisation as is The Royal Women's Hospital, and the tools that were invaluable in contributing to its consistent success. Examples of materials developed to support staff, from discussion guidelines to materials developed to aid pregnant women to make an informed choice will be shown. The second part of this talk offers an insight into the concerns that pregnant women raise regarding the offering of prophylactic Anti - D, from a midwifery perspective. What does the consumer think and how can we best serve their needs? What are the practical considerations that help Rh D negative women choose what is best for themselves and their babies? It is hoped that this talk contributes practical information to anyone involved in the delivery of prophylactic Anti-D to pregnant women for the prevention of HDN.

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RhD Antenatal Prophylaxis - The Pathology Laboratory Perspective
Ken Davis

Institute of Medical, Adelaide, SA

Stage 1 of routine antenatal RhD immunoglobulin prophylaxis was recommended in late 2002, for RhD negative women having their first child. This was as a result of a 1999 National Health Medical Research Council [NHMRC] recommendation as best practice to further reduce the incidence of HDN due to anti-D. Revised guidelines were released in 2003 by the National Blood Authority with NHMRC endorsement. It is likely that stage 2 [for all pregnancies in RhD negative women] will be in place by early 2005. The introduction of antenatal prophylaxis has raised a number of issues for pathology laboratories:

a) frequency of antenatal testing within this new program
b) laboratory protocols where the presence of RhD immunoglobulin is confirmed or suspected
c) reporting formats in such circumstances
d) inventory and supply issues including product traceability
e) testing of maternal and cord samples at delivery in women who have received antenatal prophylaxis The Australian & New Zealand Society of Blood Transfusion released revised antenatal testing guidelines in early 2004 to assist laboratories in dealing with some of these issues. Collaboration with ARCBS, CSL and RANZCOG has also resulted in useful information being made available covering other aspects of this new initiative. The presentation will explore some of the above issues leading to more general discussion during the symposium.

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Rhesus Alloimmunisation: Forgotten but not Gone
Shelley Rowlands

Department Maternal Fetal Medicine, Royal Women's Hospital, VIC

The prophylactic use of rhesus immunoglobulin has led to a significant reduction in the incidence of haemolytic disease of the newborn secondary to rhesus alloimmunisation, which now only occurs in 1 in 1000 live births. Despite prophylaxis, antibodies to the D antigen are still the commonest cause of rhesus isoimmunisation. Although now a rare pregnancy disorder rhesus isoimmunisation continues to be a challenging obstetric complication and while the face of this disease has undergone many changes over recent years, the principles of management remain unchanged. Pregnancies at risk of the disease are identified by past obstetric history and the antenatal detection of red cell antibodies with serial measurement of antibody levels. In cases of heterozygous paternity the real risk of fetal disease can be determined using new DNA techniques to determine the fetal blood cell type with tissue obtained from amniocentesis, and now by molecular analysis of circulating fetal DNA in the maternal plasma and serum. Sensitised pregnancies are monitored for the development of fetal anaemia. Since its introduction into clinical practise by Liley over 40 years ago, spectral analysis of amniotic fluid bilirubin as an indirect measure of fetal haemolysis has been the primary method of screening for fetal anaemia. Noninvasive evaluation is now possible with the use of doppler ultrasound to detect changes to fetal blood flow secondary to fetal anaemia, most commonly using the middle cerebral artery, and ultrasound evaluation of fetal haemopoetic organs and for the diagnosis of hydrops. Ultrasound guided intrauterine transfusion of red blood cells remains the treatment for the severely affected fetus. Perinatal survival rates in the non hydropic fetus are reported at over 90 % and long term studies have revealed normal neurological outcomes for more than 90% cases.

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Bravo! The Rh Project Donor
Robyn Barlow

Australian Red Cross Blood Service

In 1966, Sydney hosted the conference of the International Society of Blood Transfusion. At an Rh Symposium, experts revealed that several countries had conducted trials proving the safety and efficiency of anti-D in preventing Rh Haemolytic Disease of the Newborn. There have been no other major advances in the prevention of this tragic disease since that time. The source of anti-D in Australia is donated by a small group of Rh Project donors, and we depend entirely on them for the precious antibody which prevents Rh Disease. They have given magnificent service and thousands of babies have been saved. The Rh Project donors' contribution over 37 years is remarkable, inspiring and a triumph over disease.

1045 to 1200 John Batman Theatre
ANZSBT Plenary Session 8 - Transfusion Medicine: The Human Resource
Chair: Graeme Woodfield

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Life Blood - Nurses at the Front Line of Transfusion Practice
Sanchia Aranda

Peter MacCallum Cancer Institute, Vic

In 2002 the Victoria Government, through a Blood Matters Collaborative, supported the introduction of Transfusion Nurse roles in participating Victorian Hospitals. The TN role, based on haemovigilance roles internationally, was key to the introduction of change initiatives undertaken as part of the collaborative project. TNs required a broad knowledge of transfusion practice along with training in organisational change and implementation of change concepts. Training for the TNs was provided through a Graduate Certificate in Transfusion Practice developed specifically for the project. This program has now been developed for online delivery. This paper will outline the role development and role vision developed for TNs throughout the Blood Matters Collaborative and considers the issues that have arisen since completion of the project in terms of role sustainability. Important issues include the place of TNs within organisational structures, development of sustainable systems of role integration and the ongoing integrity and sustainability of programs developed for role preparation.

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Transfusion for Doctors: Why, What & Who
Gordon Whyte

Monash University, Bendigo

Training doctors in transfusion medicine needs a new perspective because the existing knowledge based structure has neither attracted enough doctors nor made the system safer. Safety: Intra-hospital transfusion systems, where most transfusions are delivered by overworked, under skilled PGY1s or PGY2s or nurses, are usually inherently unstable and unsafe. The system itself has to be designed to protect the patient in real hospital life situations. That means competency based training of all staff connected with transfusion and fail safe system design with continuous quality review. Knowledge: The range of knowledge required in a blood centre underscores the difficulty of pigeon holing transfusion medicine in a particular College. More fundamentally, the issue is about what practical benefit the knowledge brings to its owner in terms of jobs or recognition. It includes 'Health assessment of well donors 'Relevant viruses, bacteria and prions, 'Immunology at molecular, cellular, process and tissue levels and the associated clinical syndromes and their management 'Haematology at molecular, cellular, process and tissue levels of inflammation and coagulation and the congenital anaemias 'Cytotoxic, immunological or inflammatory effects of chemotherapy and transplantation in malignant haematology 'Resuscitation and immunological or inflammatory conditions in medicine & surgery, with implications for blood products 'Public health particularly for management of public safety, regulatory systems and health economics Transfusion knowledge is broad and often task specific. Developing appropriate task specific experts requires training and research publications to develop credibility and professional networks.

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Training/Human Resource Issues in Transfusion - Laboratory Scientists, 25 minutes
Ralph Green

1200 to 1300 John Batman Theatre
Ruth Sanger Oration
Chair: Ken Davis

Ruth Sanger Oration 'Life, Death and Oxygen: A Story of Science, Dogma, and Serendipity'
James Isbister

As a cosmic element Oxygen is the third most abundant element in the universe. Its presence in the atmosphere and solubility in water makes life on Earth possible. As a human element Oxygen makes up approximately 60% of our body weight and access to too little or too much is fatal. Medically, Oxygen is an essential therapeutic element and its technical delivery has led to the development of blood transfusion, ventilators and intensive care units. The discovery and evolution of our understanding of Oxygen as 'The Molecule that made the World' is a story of genius, serendipity and politics. To the haematologist and transfusionist Oxygen's relationship to the red cell and the haemoglobin molecule is a constant source of amazement and fascination. The red cell, the only cell in the body that is not dependent on Oxygen for energy production, has, in conjunction with the cardiovascular and respiratory systems, the awesome responsibility of delivery adequate amounts of Oxygen to every cell in the body 24 hours a day 7 days a week. Haemoglobin, probably the most studied molecular in biology, has been pivotal in the broader understanding of molecular structure and function and continues to reveal it's secrets. Oxygen, as an economic element is essential to industry and as an environmental element is closely linked to conservation of planet Earth. Many sports have a close and frequently dubious interest in enhancing delivery of oxygen to the muscles. On the basis of doubtful people who feel the need are increasingly being enticed to 'Flood their bodies with Oxygen as therapy against our polluted World'. It is the presenter's aim that this lecture will illustrate the paramount role of Oxygen to our existence and it's central importance in the history of medicine, and blood transfusion in particular.

1400 to 1530 John Batman Theatre
ANZSBT Free Communications 4 - Immunohaematology
Chair: Jennifer Condon

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Varying Specificities of Several anti-Mi(a) Antibodies Purified by Affinity Chromatography on Peptides Representing the Mi(a) Antigen are not the same as an anti-Mi(a) Monoclonal Antibody

Robert Flower [1,2], Marie Lin [3]

[1] PaLMS, Royal North Shore Hospital
[2] University of Sydney, Sydney, NSW
[3] Mackay Memorial Hospital, Taipei, Taiwan

Aim: Anti-Mi(a) has been used as a generic term to describe a number of antibodies to variant MNS (vMNS) antigens. The aim of this study was to compare the specificty reported for an anti-Mia) monoclonal antibody with monospecific polyclonal antibodies recovered from human sera by affinity chromatography. The hypothesised pattern was equal reactions with QNTDK28HKRDT and QNTDM28HKRD peptides. Sepharose columns one with each form of the peptide (K28 or M28) were prepared. A 10 ml pool of sera that reacted with the M28-peptide AND strongly agglutinated Vw cells, was subjected to affinity chromatography on the M28-peptide column.

Result: A concentrated eluate reacted only with the M28-peptide by ELISA and agglutinated all Vw, HUT, MiIII and Mi VI cells tested. This was an anti-Mi(a) but with different pattern of ELISA reactivity to that reported for the monoclonal antibody. In a sequential second affinity chromatography, the fraction that did NOT bind to the M28-peptide was applied to the K28-peptide column: The concentrate reacted with K-containing peptide and agglutinated all Vw, HUT, MiIII and Mi IV cells - an anti-Mi(a) with a different (lysine-specific) pattern of reactivity. In one case an antibody that also reacted with cells with an Sta phenotype was isolated from the K28-peptide column.

Conclusion: In human antisera to Mi(a) there are several patterns of reaction for antisera that agglutinate Vw and Mi(a) cells. None of these resembled the MAb (hypothesis refuted). Complex reactivities predicted by early serolgists in the field can now be studied using specific antisera.

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Molecular Characterization of FUT1(H) Gene in the Bombay Phenotypes Detected in India

Vasantha K, Ajit Gorakshakar, Roshan Colah, Swati Kulkarni, Dipika Mohanty

Institute of Immunohaematology (ICMR)

Bombay phenotype, one of the rare blood group was discovered in India in 1952 by Bhende et al. The frequency of this in Western India is about 1 in 7600.

Aim : Our Institute gets referral samples from various hospitals and blood banks with problems in grouping and cross matching for identification of blood group and atypical antibodies. We have identified many Bombay phenotype cases out of these by serological procedures. We undertook molecular characterization of FUTI(H) gene on this large series of Bombay phenotypes to see whether the earlier reported mutations on Indian Bombay phenotypes from South Africa and Reunion Island are present in our population or not.

Method : Serological confirmations of Bombay phenotypes was done as per standard procedures. Thirty of these serologically confirmed samples were undertaken for molecular characterization of FUTI(H) gene. DNA extraction was done from these samples and FUTI(H) gene was amplified by performing nested PCR followed by digestion with restriction enzyme Nae 1 to screen for T 725 G mutation. The digested product was run on agarose gel electrophoresis and analysed.

Result : Out of these 30 samples 27 samples were homozygous for T 725 G mutation and one sample was heterozygous for T 725 G mutation which serologically had shown absence of H antigen on red cells and two samples did not show the presence of this mutation.

Conclusion : The T 725 G mutation of FUTI(H) gene appears to be the common mutation amongst the Bombay phenotypes detected in India.

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The Quantitation of D Antigenic Sites in Partial D and Weak D Variants by Flowcytometry

Swati Kulkarni [1], Dipika Mohanty [1], Snehalata Gupte [2], Vasantha K [1], Sanmukh Joshi [3]

[1] Institute of Immunohaematology
[2] Surat Raktadan Kendra, Surat
[3] Indian Red Cross Blood Bank, Mumbai

Aim: The aim of the study is to quantitate D antigenic sites on RBC in weak D and partial D variants in Indian population by flowcytometric method.

Method: D antigenic sites were estimated in 48 cases of partial D, eight cases of weak D (identified by serological methods) and normal Rh phenotypes subjects by flowcytometric technique. The indirect immunoflourescence method was employed using thirty epitope specific monoclonal anti-D as primary antibody. The D antigenic sites were calculated using standard RBC with known D antigenic sites.

Result: The mean (± I S.D) D antigenic sites per RBC in weak D and partial D were 4503 ±2067 and 6750 ± 3426 respectively. In R1R1 phenotype the range of D antigenic sites was 16500 ±2500 and in R1r it was 10600 ± 2520. In four cases of weak D sites were in range of 1700 to 3500 and in other four it was 5000 to 7200 D sites/RBC. Among partial D variants DVI had minimum, and DVa had maximum number of D sites.

Conclusion: Flowcytometry is a very good tool for demonstrating minor differences in D antigen sites in partial D and weak D when serological methods are not conclusive.

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Purification of RBC Agglutinating Antibodies by Affinity Chromatography on Peptides Representing Specific Variant MNS Antigens

Robert Flower [1,2], Marie Lin [3]

[1] PaLMS, Royal North Shore Hospital
[2] University of Sydney, Sydney, NSW
[3] Mackay Memorial Hospital, Taipei, Taiwan

Aim: Variant MNS (vMNS) phenotypes are characterised by the presence of multiple antigens that provoke complex antibody responses. The hypothesis tested was that red cell-agglutinating antibodies of a single specificity could be purified by affinity chromatography on peptides representing vMNS antigens. The Vw and Hut/Mut antigens vary only at glycophorin residue 28, Vw methionine-28, Hut and Mut lysine-28. The Mur peptide TYPAHTANEV was aa 33 to 42 of GpMur. For each experiment a 5 ml pool of 4 and 10 sera that agglutinated Mi-positive cells of known MNS-peptide-ELISA activity, was subjected to affinity chromatography on a single peptide.

Result: For a serum pool that was strong peptide-ELISA positive with the lysine-28 peptide but was methionine-28 peptide negative, the antibody eluate was lysine-28 pos by ELISA and agglutinated all MUT and HUT pos cells tested but not Vw cells or Mi(a) negative cells. For the MUT antigen the hypothesis that a rbc-agglutinating antibody of a defined specificity could be purified by affinity chromatography on a linear peptide representing the antigen was confirmed. In a similar fashion a specific anti-Mur was prepared. However, for one serum pool the antibody eluted from the Mur-column reacted with Hut (MiII) as well as Mur-positive cells, suggesting that Mut.Mur was recognised as a single antigen.

Conclusion: Purified monospecific polyclonal antibodies that define vMNS phenotypes may be useful in resolving difficult serological investigations. The complexity of responses to vMNS glycophorins is confirmed by the recovery of an antibody reacting with sequentially adjacent antigens as a single entity.

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Molecular Characterization of Partial D Variants in India

Swati Kulkarni [1], Roshan Colah [1], Ajit Gorakshakar [1], Snehalata Gupte [2], Dipika Mohanty [1]

[1] Institute of Immunohaematology(ICMR)
[2] Surat Raktadan Kendra, Surat

Aim: To find incidence of partial D variants in India and classify them by molecular techniques.

Method: Partial D variants were identified by screening blood samples of subjects from Western India by using partial D kit (Scottish National Blood Transfusion Service). Molecular characterization of partial D variants was performed using multiplex PCR(M-PCR).

Result: The incidence of partial D was 0.15% in the population studied. Forty eight partial D variant samples identified by serological studies were classified by M-PCR. 29% of partial D variants identified were of DFR and 14.6% were of DVI category. 77% of partial D variants identified in Indian population were characterized by M-PCR. Fifteen families of partial D variants were studied and ten more individuals of same variant were identified. All partial D variants were of R1r (DCe/dce) phenotype. The family studies showed partial D hybrid (RHD-CE-D) was linked with Ce gene.

Conclusion: DFR was most frequently found partial D in Indian population. Majority of partial D variants characterized in Indian population arise due to substitution of part of RHD exon with RHCE equivalent.

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Fetal Loss Due to Anti-Hro in a Lady Having Rare Genotype of Rh (D- - / D- -)

Vasantha K, Seema Jadhav, Swati Kulkarni, Dipika Mohanty

Institute of Immunohaematology(ICMR), Parel, India

Aim: A lady of 30 years was referred to our Institute for investigations who had an obstetric history of one living child, followed by one still birth and then a termination of pregnancy due to suspected fetal ascitis.

Method: Blood grouping and Rh genotyping was done by us by standard serological procedures and patient's serum was investigated for atypical antibodies by panel of cells. Fourteen family members were investigated for ABO grouping and Rh genotyping.

Result: Patient's blood group was identified as O Rh Positive showing a rare Rh genotype D- - with presence of only D antigen and absence of C, c, E and e antigens. Confirmation of this was done by absorption of patient's red cells with anti-C, anti - c, anti -E and anti -e antisera and testing the eluate reactivity for presence of the respective antigens. Patient's serum reacted with all panel red cells of the common Rh genotypes and the antibody is anti - Hro which is the most potent antibody a D- - individual produces. Amongst the family members of the propositus, seven probable heterozygote D- - individuals were identified by testing with incomplete anti -D by saline technique.

Conclusion: The patient had a rare Rh genotype D- - / D- - and had produced anti - Hro antibody due to fetomaternal leak during pregnancies and this must have been responsible for the fetal loss. No other family member exhibited this rare Rh genotype in homozygous form.

1400 to 1530 La Trobe Theatre
ANZSBT Free Communications 5 - Quality Improvement in Hospital Transfusion Practice
Chair: Merrole Cole-Sinclair

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Strict Specimen Labelling Criteria - A Safety Initiative Experience from a Hospital

Marija Borosak, Slav Curcic, Hans Schneider, Alison Street, Merrole Cole-Sinclair

Pathology Service, The Alfred, Prahran, VIC

Aim: Correct patient and specimen identification and labelling are critical to ensuring that the correct results are issued for the right patient, and that medical decisions and treatments given on the basis of these test results are appropriate. This is particularly critical in pre-transfusion testing with the risk of ABO mismatch transfusion and potential fatality a real possibility.

Background: Over 11months, data relating to mislabelled specimens demonstrated a basal rate of 2 'wrong-blood-in-tube' (WBIT) incidents per month and 8 of 23 were for pre-transfusion testing. These incidents were detected only where inconsistencies either in clinical information or previous test results were apparent. Intervention Our pathology service developed a set of strict minimum identification criteria for ALL pathology specimens and a policy of rejection of specimens that do not comply. These criteria align with ANZSBT guidelines for pre-transfusion samples. An 8week education and notification phase followed by strict application of the criteria in February 2004 and a subsequent follow-up phase was conducted.

Result: Smooth implementation of a major network-wide initiative has occurred over the last 5 months, as evidenced by the low specimen incident rate of 1%. Early indicators of success include an increased awareness of WBIT episodes with three self-reported episodes of WBIT since implementation. Unlabelled specimens have reduced by at least 50% compared to the same period prior to intervention.

Conclusion: Our experience may be of interest to other institutions considering similar initiatives. A reduction in WBIT events is the most preferred outcome, however it may be many months before this can be appreciated due to low detection levels.

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Audit of Nurses- Adherence to Pre-transfusion Checking

Janine Furmedge [1,2], Hans Surya [1], Jane Darlington [3], Helen Savoia [1], Paul Monagle [1,4]

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[2] Murdoch Children's Research Institute, Melbourne, Australia.
[3] Paediatric Intensive Care Unit, Royal Children's Hospital, Melbourne, Australia.
[4] Department of Paediatrics, University of Melbourne, Australia.

Background: Errors in blood product administration occur not infrequently and cause morbidity and mortality. Pre-transfusion checking at the bedside is the final opportunity to detect errors and is thus a vital step in preventing wrong transfusions. Unfortunately, pre-transfusion checking is the area where most common errors are documented. We developed a number of strategies to improve nurses' adherence to pre-transfusion checking guidelines.

Method: Pre-transfusion checking practices were audited by direct observation. Nurses in Haematology/Oncology unit and PICU were targeted because both units are major blood users in our hospital. Pre defined key measures of adherence to best practice recommendations were noted, including checking of the blood product, the recipient's wristband and the compatibility report. An audit of practice was carried out before and after the introduction of a multifaceted educational intervention, comprised of information transfer (posters, in-services, and hospital web-based clinical practice guidelines), audit feedback, and the provision of pre-transfusion checking reminders on the reverse side of the issued compatibility report.

Result: There was significant improvement in the results for two aspects of pre-transfusion checking following intervention. Checking of the blood unit for normal appearance (Haem/Onc Pre intervention 43%, Post 81% and PICU Pre 11%, Post 58%) and checking the patien's identification wristband prior to administering a blood product. (Haem/Onc Pre 19%, Post 81% and PICU Pre 68%, Post 79%).

Conclusion: Baseline compliance with pre transfusion checking was poor in a tertiary hospital. The introduction of a multifaceted educational intervention appears to be effective in improving nurses' adherence to pre-transfusion checking.

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Improving Hospital Transfusion Practice: Evaluation of Progress at Two Metropolitan Hospitals

Karen Botting [1], Neil Boyce [1], Sanchia Aranda [2], Felicity Topp [3]

[1] Blood Matters Consortium Project, Australian Red Cross Blood Service, Victoria
[2] Peter MacCallum Cancer Centre
[3] The Royal Melbourne Hospital

Aim: Blood Matters was a project funded by the Victorian Department of Human Services to introduce sustainable improvements in hospital transfusion practice. An evaluation was conducted at two metropolitan hospitals to review progress against stated aims and identify key elements leading to changes in transfusion practice and highlight any unintended consequences.

Method: During the project, each hospital chose to focus improvement efforts in key areas. Measurable, specific goals were developed. Using a case study approach, the evaluation was conducted by review of the aim-specific data collected during the project and by semi-structured interviews from which key themes (eg assumptions underpinning decisions) were identified. Interviews were conducted with at least 10 representative individuals from each organisation whose roles were considered necessary for change in their institution's transfusion practice.

Result: Measurable improvements have been made (eg an 87 percent improvement in reduction of patient identification error) and the Transfusion Nurse position made operational at both sites. Examples of key success factors identified are: a committed transfusion team to direct improvements in the organisation; integration of transfusion data reporting into existing organisational quality/risk management structures to provide ongoing visibility for transfusion issues and; mentorship and support for the Transfusion Nurse role.

Conclusion: This project has, in the short term, successfully generated improvements in hospital transfusion practice and influenced hospital systems to enable changes to be sustainable in the longer term.

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Recidivism in Transfusion Quality Improvement: Support for the Role of Transfusion Nurses

Sue Quayle [1], Philip Crispin [1,2], Bethany Crowe [1,3], Anne McDonald [1,2]

[1] ACT Haemovigilance Project, Canberra ACT
[2] ACT Pathology, Canberra ACT
[3] Calvary Hospital, Canberra ACT

Aim: To evaluate longer term outcomes of a quality improvement strategy in transfusion.

Method: A Clinical Health Improvement Program (CHIP) quality improvement project was conducted in a 290 bed acute general hospital, to decrease the rate of transfusions with high pre-transfusion haemoglobins. Strategies for improvement included: formation of a Transfusion Committee; development of transfusion pathway; implementation of a Maximum Blood Order Schedule; educational strategies targeting clinical staff at all levels; promulgation of the NHRMC Clinical Practice Guidelines on the Use of Blood Components; and feedback to clinicians. The CHIP project was superseded by the ACT Haemovigilance Project, and an ACT wide concurrent transfusion audit was undertaken. There was no formal feedback on clinician performance during, or for 6 months prior to, the Haemovigilance audit. Red cell transfusion was assessed for compliance with the NHMRC Guidelines and compared with CHIP results.

Result: The CHIP project demonstrated successful implementation of the selected strategies, leading to a reduction in transfusions with pre-transfusion haemoglobins greater than 90g/L. Follow up auditing commencing six months later demonstrated a progressive, and statistically significant, increase in inappropriate transfusions over the following six months.

Conclusion: Although improvement in transfusion practice was demonstrated with a multi-factorial approach to quality improvement, the improvement was not sustained in the medium term. Our results indicate the need for organisational commitment to long term quality improvement strategies in transfusion, and support the development of Transfusion Officers or Nurses to improve bedside clinical practice.

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The Transfusion Medicine Team - A Hospital Perspective

Marija Borosak, Slav Curcic, Geoff Magrin, Alison Street, Merrole Cole-Sinclair

Pathology Service, The Alfred, Prahran, VIC

Background and aim: Three serious transfusion-related incidents over a 9month period highlighted the clinical risk in transfusion during 2001-2002. This lead to the development of a strategy to comprehensively review and implement transfusion safety initiatives across the Bayside health network of which the Alfred is a major hospital. Intervention The initiative commenced in January 2003 with the assembly of a multi-disciplinary team that consisted of medical, nursing, scientific as well as hospital executive representation. This was possible with the appointment of a Transfusion Nurse as part of the Victorian Department of Human Services Blood Matters Collaborative and a Transfusion Medicine Registrar, co-appointed by the hospital and Australian Red Cross Blood Service. The activities consisted initially of process mapping blood transfusion practice. Key areas were then selected for targeting process improvement, namely; - Patient and product identification, - Sample labelling, - Blood component administration.

Result: Initiatives implemented were; - Widespread medical and nursing education on transfusion issues. - Increase transfusion information availability. - Network-wide implementation of strict criteria for specimen labelling (all specimens) in line with ANZSBT pre-transfusion testing guidelines. - Development of a pre-transfusion testing and blood request form. - Development of a blood administration form to reconcile all transfusion related documentation in the medical record. - Policy and guideline development.

Conclusion: Sustained improvement requires ongoing dedicated personnel to maintain gains, continue to audit practice, analyse incident data both actual adverse incidents as well as near-miss events and to respond to these challenges within an ongoing and operational quality improvement framework

0900 to 1030 La Trobe Theatre
ANZSBT Plenary Session 9 - Transfusion Initiatives: Project to Practice
Chair: Carole Smith

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Blood Matters - Translating Ideas Into Action
Neil Boyce

Australian Red Cross Blood Service

On behalf of the 'Blood Matters' Planning Group. The Blood Matters Collaborative was structured around a series of specific projects designed to improve distinct aspects of the sequence of care processes that ultimately determine the safety of each and every fresh blood product (red cell, platelet, fresh frozen plasma and cryoprecipitate) transfusion. These commence with the appropriateness of the clinical decision to transfuse blood products, then move to ensure that procedures for patient, sample and blood product identification and blood administration are robust and that any procedural errors and adverse events associated with transfusion are captured, analysed and acted upon. Finally and very importantly the collaborative sought to improve the patient and carers understanding of the risks and benefits of transfusion. It was an ambitious program of work but there seemed little alternative other than addressing all of the interdependent processes that determine the quality and safety of transfusion. All elements in this chain contribute to the actual levels of transfusion safety. With support from the Clinical Innovation Agency and planning group members, local multi-disciplinary hospital teams set out to deliver changes to improve transfusion safety. The collaborative methodology, initially developed by the Institute of Healthcare Improvement in the United States of America, has been adopted and used by a large number of health systems internationally and within Australia. It is based on answering three key questions [Q1. What are we trying to accomplish? Q2. How will we know that a change is an improvement? Q3. What changes can we make that will result in improvement?] and testing ideas through the plan, do, study, act cycles of change. Individual projects monitored progress using 'run charts' to record their measured performance. These indicated progress towards achieving locally set targets and collaborative improvement goals. Our objectives were translated into tangible benefits for patients requiring blood product transfusion. Fundamental to the collaborative approach to improving transfusion practice is the belief that changes can be tested rapidly on a small scale without risk. For example, an idea can quickly be tried with one patient, one clinic or ward, a single consultant or hospital. Only if it proved successful was it suggested for wider adoption as an improvement in care across an entire Health Service or system of care. Our successes reinforced the value of using a systematic method for translating ideas into practice such as that used by the collaborative. The governance structures and teams established during our participation in the collaborative have helped us to identify key local issues and implement strategies to enhance the quality of our transfusion services to our patients. Our aim is to ensure that the continued review and improvement of transfusion practices becomes an integral part of the way that clinical teams and hospital and health system clinical governance structures work. The role of state or national bodies is to support this to happen. We hope to build a series of improvement partnerships that all focus on optimising the quality of transfusion services and the outcomes of patients receiving transfusion during their clinical care.

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NSW Blood Transfusion Improvement Collaborative: Where to From Here

Amanda Thomson

Royal North Shore Hospital, Sydney

The NSW Blood Transfusion Improvement Collaborative completed its project in mid 2003 and presented the final report in November 2003. The focus of the collaborative was on the appropriateness of red cell transfusion using the 'breakthrough' methodology of the Institute for Healthcare Improvement. Results showed that the goal of 50% reduction in inappropriate transfusion of red cells had been met. The largest improvements were seen at sites that used vetting of transfusion requests based upon a restrictive threshold as per the guideline parameters. This was most effective when combined with endorsement, distribution and education re the guidelines. There has been no further funding for BTIC. In NSW there are currently no systems in place for monitoring the safety and appropriateness of transfusion practice. It is essential that this is addressed particularly in view of the findings of the Collaborative and the NSW Coroner indicating that there are inappropriate transfusion practices and serious preventable adverse events associated with transfusion occurring. Necessary measures include improvement in the governance of transfusion practice in the clinical setting, establishment of measurement systems to provide data on the management and use of blood and improvement in education for the staff who prescribe and administer transfusions. Valuable transfusion practice improvement work is progressing in a number of other States and overseas. However, securing commitment for resources to allow these to proceed is an ongoing problem. With the restructuring of the state and national systems of management of blood and transfusion services, there is the opportunity to establish a coordinated approach to ensuring safe and appropriate transfusion practice.

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BloodSafe - From Project to Practice in South Australia

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[3] Department Of Health, Adelaide, South Australia

BloodSafe is a joint initiative between the South Australian (SA) Department of Health, Australian Red Cross Blood Service, hospitals and transfusion service providers. It has successfully established quality assurance programs to improve the safety and quality of blood management practice within the SA health system. BloodSafe began as a project in September of 2002 initially with 12 months funding from the Safety and Quality Council. Four Transfusion Nurse Consultants were appointed across five major metropolitan teaching hospitals. Audit of practice within these hospitals demonstrated three major problem areas- transfusion specimen collection, the decision to transfuse and administration of blood. A further 12 months funding was granted including extension of the project to include a Nurse Educator for the state to introduce initiatives into country and private hospitals. Reaudit of practice after interventions and education demonstrated that the Transfusion Nurse Consultants were effective change agents. In particular red cell use outside the NHMRC/ASBT guidelines fell from 18% to 4% ($p < 0.01$) in stable adult orthopaedic patients audited. A number of other important aspects of transfusion practice also showed significant improvement including documentation, consent and administration. Recurrent funding for BloodSafe Transfusion Nurse Consultants was granted by the SA Department of Health from July 2004. Factors contributing to the success of BloodSafe include a multidisciplinary team, Transfusion Nurses of clinical nurse consultant level, demonstration of a clear need for the initiatives, evidence of successful practice improvement, statewide collaboration and interventions, benchmarking across hospitals, and impartiality of the BloodSafe name with acceptance by stakeholders.

1100 to 1300 John Batman Theatre
HSANZ/ANZSBT/ASTH Presidential Symposium
Chair: Mark Hertzberg, Ken Davis, Hatem Salem
session sponsored by Pall Medical

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Mutation Screening in Imatinib-Treated Chronic Myeloid Leukaemia (CML) Patients is Indicated in Primary Refractory Patients and Those with Greater than Two Fold Rises in the BCR-ABL Level

Susan Branford [1], Rebecca Lawrence [1], Chani Field [1], Zbigniew Rudzki [1], Andrew Grigg [2], Kerry Taylor [2], John F Seymor [2], Simon Durrant [2], Peter Browett [2], Anthony P Schwarer [2], Chris Arthur [2], John Catalano [2], Michael F Leahy [2], Robin Filshie [2], Kenneth Bradstock [2], Richard Hermann [2], David Joske [2], Kevin Lynch [3], Tim Hughes [1]

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[3] Novartis

Imatinib provides significant therapeutic benefit for patients with CML. However, resistance can occur in any disease phase. Somatic mutation within the BCR-ABL kinase domain is the main mechanism of acquired resistance and monitoring patients for mutations provides an important guide for clinical management. It is unclear how frequently patients should be monitored. We evaluated 214 patients receiving stable doses of imatinib for up to 39 months (median 15 months) to determine if molecular monitoring of BCR-ABL transcript levels could guide the frequency and timing of mutation analysis. We hypothesised that the emergence of a mutation would lead to a rise in the BCR-ABL level. Of the 214 patients, 5 were in blast crisis at the start of imatinib therapy, 27 in accelerated phase, 48 in late-chronic phase and 134 in early-chronic phase (defined as less than 12 months since diagnosis). BCR-ABL transcript levels were measured using real-time quantitative PCR at 1 to 6 month intervals (total 1,533 samples, median 5 per patient). The patients were screened for mutations at least every 6 months by PCR isolation of the BCR-ABL allele and direct sequencing (804 samples). Fifty-seven patients had a greater than 2-fold rise in BCR-ABL in consecutive samples (median rise 3.0-fold). Mutations were detected in 35 (61%) of these patients. In 37%, the mutations were retrospectively detectable prior to the rise (median 4 months). Only 1 of 157 patients (0.6%) with stable or decreasing BCR-ABL had a mutation detected ($P<0.0001$). Irrespective of the disease phase, failure to achieve a 1-log reduction in BCR-ABL by 6 months of imatinib therapy, which occurred in 17% of all patients, was associated with a significantly higher probability of having a mutation detected by 24 months ($P<0.0001$). We conclude that the highest incidence of mutations occurs in patients with a rise in BCR-ABL of more than 2-fold and in those who fail to achieve a 1-log reduction by 6 months of imatinib therapy. Mutation screening can be reliably and cost-effectively restricted to these patients.

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An Essential Role for Trp570 and Phe568 in the Cytoplasmic Domain of Glycoprotein Iba for filamin A Binding

Susan L Cranmer [1], Inna Pikovski [1], Pierre Mangin [1], Philip E Thompson [2], Teresa Domagala [1], Mark Frazzetto [1], Hatem H Salem [1], Shaun P Jackson [1]

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Binding of the platelet glycoprotein (GP) Ib/V/IX receptor to von Willebrand factor (vWf) is critical for platelet adhesion and aggregation under conditions of rapid blood flow. The adhesive function of GPIb a is regulated by its anchorage to the membrane skeleton through a specific interaction with filamin A. In this study, we examined the amino acid residues within the cytoplasmic tail of GPIb a that are critical for association with filamin A, using a series of 25-mer synthetic peptides that mimic the cytoplasmic tail sequences of wild-type and mutant forms of GPIb a . Peptide binding studies to purified human filamin A have demonstrated a major role for the conserved hydrophobic stretch L567FLWV571 in mediating this interaction. Progressive alanine substitutions of triple, double, and single amino acid residues within the Pro561-Arg572 region suggested an important role for Trp570 and Phe568 in promoting GPIb a binding to filamin A. The importance of these two residues in promoting filamin A binding to GPIb a in vivo was confirmed from the study of Chinese Hamster Ovary (CHO) cells expressing GPIb a Trp570Ala and Phe568Ala substitutions. Phenotypic analysis of these cell lines in flow-based adhesion studies revealed a critical role for these residues in maintaining receptor anchorage to the membrane skeleton and in maintaining cell adhesion to a vWf matrix under high shear conditions. These studies demonstrate a novel filamin A binding motif in the cytoplasmic tail of GPIb a that is critically dependent on both Trp570 and Phe568.

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Adhesion of Stored Red Blood Cells to Vascular Endothelium Increases with Duration of Product Storage and Leucocyte Burden

Angela Anniss, Kath Patton, Rosemary Sparrow

Australian Red Cross Blood Service

Adherence of red blood cells (RBCs) to vascular endothelium impairs blood flow, decreases oxygen delivery and leads to vaso-occulusion. RBCs are relatively non-adherent however little is known of how changes to RBCs for transfusion during storage may affect their adherence properties.

The aim of this study was to monitor adherence of stored RBCs to vascular endothelium under conditions of continuous flow in vitro. Specifically the influence of RBC storage time and leucocyte burden of stored red cell preparations was investigated. Human umbilical vein endothelial cells (ECs) were grown to confluence on fibronectin-coated coverslips. Non-leucocyte-reduced, buffy-coat-reduced and leucocyte-filtered RBC products were prepared according to standard blood bank procedures. RBC samples were collected at multiple time points until product expiry and perfused across an EC monolayer using a parallel flow chamber mounted to an inverted microscope. Perfusion of RBCs was controlled for shear stress and temperature. RBC-EC interactions were recorded using a digital camera attached to the microscope. The number of RBCs adhering to the EC layer progressively increased with product storage time. RBCs from products stored for 28 and 42 days were significantly more adherent than fresher cells. RBCs from products containing leucocytes were also significantly more adherent to the EC layer on days 28 and 42 of storage than RBCs from leucocyte-reduced products.

Our findings indicate that product storage time and leucocyte burden increase the adhesion of RBCs to an EC layer. These results may lead to greater understanding of the interaction of transfused RBCs with recipient endothelium and the biological consequences of this adherence.

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Development of a Platform in Zebrafish for Studies of Immunity

Maria Flores, Scott Mead, Chris Hall, Enid Lam, Kathryn Crosier, Philip Crosier

The University of Auckland, Auckland, New Zealand

The zebrafish immune system has considerable structural equivalence with that of mammals. The utility of this model system for genetic and embryological studies led us to develop a platform in zebrafish for studies of immunity.

This has involved identification of zebrafish orthologues of genes that might regulate B and T lymphocyte development, analysis of their expression patterns and identification of promoter regions for linkage to EGFP to mark lymphocyte compartments. Initial work has involved the Src family kinase Blk and the Runx family member Runx3.

Zebrafish *blk* was identified, mapped and shown to have conserved syntenic relationships when compared with mammalian genomes. It is first expressed in individual cells throughout the developing pancreas and later in aggregations of cells close to the gut, suggestive of developing gut-associated lymphoid tissue (GALT). This expression parallels that of *Igμ* and *rag1*, supporting the identification of *blk* as a B cell-specific marker in zebrafish. To further characterise zebrafish GALT, we are generating transgenic fish that express EGFP under control of the zebrafish *blk* promoter.

Zebrafish runx3 is expressed in the thymic rudiment during early development. This expression is present in the haematopoietic mutant *cloche* but absent in the endodermal mutant *casanova*, suggesting that Runx 3 is initially derived from thymic epithelial cells and not thymocytes. Runx3 is a regulator of mammalian gastric epithelial cells and a tumour suppressor gene in this environment. Our results raise the possibility of a similar role in development of the thymic epithelium.

Together, these studies have provided additional tools for studying the immune system.

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Suppressor of Cytokine Signalling-3 (SOCS3) negatively regulates G-CSF-driven Emergency Granulopoiesis

Ben Croker, Donald Metcalf, Nicos Nicola, Warren Alexander, Andrew Roberts

The Walter and Eliza Hall Institute of Medical Research

Aim: G-CSF drives emergency granulopoiesis during the body's response to infection. When used clinically to accelerate neutrophil production or to mobilise stem cells, some patients develop unexpected inflammation or marked splenomegaly. We aimed to determine how G-CSF signalling is switched off to prevent development of toxicity. We hypothesised that the intracellular protein, SOCS3, was responsible for inhibiting G-CSF signalling.

Method: Socs3 knockout mice die early in utero precluding analysis. We therefore generated mice with a conditional deletion of SOCS3 specifically in haemopoietic cells. Bone marrow (BM) cells were assayed in vitro for G-CSF responsiveness. Neutrophil activation and function were also assayed. Young mice were injected with G-CSF 5μg/day, or placebo, for 4 days, then analysed.

Result: When stimulated with G-CSF in vitro, SOCS3-deficient BM cells exhibited enhanced and prolonged STAT3 activation, and increased proliferation and survival. Progenitor cell proliferation was also specifically increased in response to G-CSF. Mice developed neutrophilia and a spectrum of acute inflammatory pathologies with age. Prior to the development of inflammation, young mice, and age-matched controls were injected with G-CSF. The experiment was terminated after 4 days because of hindleg paresis in the SOCS3-deficient animals injected with G-CSF. No similar toxicity has ever been observed in wild-type mice injected with G-CSF. Mutant mice displayed enhanced neutrophilia, progenitor cell mobilisation, and splenomegaly, and histology revealed inflammatory neutrophil infiltration into multiple tissues, including the spinal cord.

Conclusion: SOCS3 plays a critical role during G-CSF-driven emergency granulopoiesis to switch off the response and limit tissue damage by activated neutrophils.

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Oestrogen Regulation of the Anti-coagulant Protein S

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Aim: The anticoagulant Protein S (PS) is coded for by the PROS1 gene and serves as a co-factor to APC inactivation of FVa and FVIIIa. Previous Studies have shown a reduction in circulating PS levels with increasing oestrogen (E2) levels resulting in an increased thrombotic risk.

Method: We have identified a potential oestrogen response element (ERE) spanning -350° -367 within the 5` UTR of PROS1. Using an EGFP expression vector, clones containing this ERE, the entire 5' UTR (948bp) and the Sp1 binding site (-66° -75) have been transfected into HepG2 cells and expression measured by flow cytometry.

Result: The ERE sequence alone was able to independently drive transcription. Surprisingly, the PROS1 5` UTR vector increased expression with increasing E2 levels.

Conclusion: These results suggest that the ERE is responsible for part, but not all, of the regulation of PS levels by E2. Further regulation is likely to be exerted by E2 regulated binding proteins. The discovery of an ERE in the PROS1 promotor and identification of previously undescribed (or novel) interacting proteins is a significant step forward in explaining the in vivo observation of E2s influence on circulating PS levels.

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Cytokine Expanded Myeloid Precursors Function as Regulatory Antigen Presenting Cells and Induce Tolerance Through IL-10 Producing Regulatory T Cells

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[2] Centre for Immunology and Cancer Research, University of Queensland, Brisbane, Qld
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[4] Department of Stem Cell Transplantation, Royal Brisbane Hospital, Brisbane, QLD

Stem cell mobilisation with the synthetic G-CSF and Flt-3 receptor agonist Progenipoietin-1 (ProGP-1) is superior to G-CSF for the prevention of graft-versus-host disease (GVHD). In this study we evaluated the contribution of ProGP-1 and G-CSF expanded donor antigen presenting cells (APC) to the amelioration of GVHD after allogeneic stem cell transplantation (SCT) in a well described murine SCT model (B6 into B6D2F1). The role of indirect antigen presentation in allogeneic responses was examined by adding populations of cytokine-expanded donor APC to haematopoietic grafts that would otherwise induce lethal GVHD. ProGP-1 and G-CSF expanded myeloid DC, plasmacytoid DC and a novel granulocyte-monocyte precursor population (GM)that differentiate into class IIpos, CD80/CD86pos, CD40neg APC during GVHD. Whereas addition of plasmacytoid and myeloid donor DC augmented GVHD, GM cells induced transplant tolerance. When purified GM cells from ProGP-1 treated B6 (H2b), DBA/1 (H2q) or B6 class II-/- (H2b) mice were added to control allogeneic grafts (H2b), only the B6 (H2b) GM cells provided long-term protection, confirming the protection was MHC class II restricted. GM cells added to T-cell depleted control grafts supplemented with IL-10-/- T cells failed to rescue animals from GVHD, demonstrating a requirement for donor T cell IL-10 production for GM mediated protection. Thus, G-CSF and ProGP-1 expand granulocyte-monocyte precursors that function as regulatory APC which induce transplant tolerance via the class II restricted generation of IL-10 secreting antigen specific regulatory donor T cells. The data suggest that G-CSF derivatives may have an application outside the context of GVHD, including disorders characterised by a loss of self-tolerance.

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Testing for Homologous Blood Transfusion in Elite Athletes - RPAH AT ATHENS 2004.

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Following the 2000 Sydney Olympics and the introduction of testing for erythropoietin in elite athletes, it became apparent that many athletes had reverted to blood doping using homologous and autologous transfusions. The Science and Industry Against Blood doping research consortium approached Dr Nelson at RPAH to determine if a test could be developed to detect recent blood transfusions. A grant was provided by the US Anti-Doping Agency to develop a flow cytometric assay which detects the expression of a panel of minor blood group antigens. Most blood bank reagents are IgM and are not satisfactory as they cause aggregation. A series of IgG polyclonal antibodies were sourced and the optimal titre of each antibody to detect minor negative and positive red cell populations in in vitro mixtures was determined. The test was validated by four Sydney flow cytometry laboratories who tested unknown samples in a proficiency exercise. In April 2004, IOC, WADA and ATHOC agreed to introduce the test into the Athens laboratory in time for the 2004 Olympics with assistance from RPAH. From May till August, RPAH sourced suitable antibodies, performed antibody titrations, provided a standard operating procedure, consulted daily with both the Athens lab and the Anti-doping lab in Lausanne and conducted a series of 3 Proficiency Testing exercises for both labs. No false positives were found in 254 antibody/sample test combinations. The Athens lab used a panel which included antibodies to C, c, E, Jka, Jkb, Fyb , Fyb , M, N and S. Reagents for anti s, K and e were also provided by RPAH. Two weeks before the Games, the lab personnel moved into the IOC Athens Testing lab. A total of 350 endurance athletes were tested. This included placegetters plus randomly selected competitors.

HAA 2004 ABSTRACTS WEDNESDAY 20