

**ANZSBT ORAL ABSTRACTS PRESENTED AT THE ANNUAL SCIENTIFIC MEETING OF THE HAEMATOLOGY SOCIETY OF AUSTRALIA AND NEW ZEALAND, THE AUSTRALIAN AND NEW ZEALAND SOCIETY OF BLOOD TRANSFUSION AND THE AUSTRALASIAN SOCIETY OF THROMBOSIS AND HAEMOSTASIS (HAA) HELD IN AUCKLAND, NEW ZEALAND, 17-20 OCTOBER 2010**

**Improving Transfusion Practice with Technology**

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Errors in transfusion and the resulting morbidity and mortality are well documented. Recent national initiatives in England to reduce transfusion errors, including by the National Blood Transfusion Committee, the British Committee for Standards in Haematology and National Patient Safety Agency (NPSA), have focussed on implementing recommended manual procedures for good practice, but have at best been partially effective.

Our approach was to 're-engineer' bedside and laboratory transfusion procedures using barcode patient identification, bedside handheld computers and electronically controlled blood fridges. This was demonstrated to simplify transfusion procedures and improve practice:-

Pre and post audits showed improvement from 11.8% to 100% of staff following the process for correct patient identification at the bedside.

Rejected blood samples due to inaccurate, incomplete or illegible labelling were reduced to 0.1%.

Reduced wastage of blood.

Reduced blood usage.

Reduced nursing and laboratory workload.

The median time to deliver urgently required red cell units to patients from the time of a telephone request was 18 minutes (range 5-47 minutes). After implementation of electronic remote blood issue from electronically controlled blood fridges, red cell units were obtained in a median time of 45 seconds (range 30 seconds to 2 minutes).

The electronic system provides a simple mechanism for compliance with UK regulatory requirements for the traceability of blood, and the documentation of transfusion and training. Feedback from patients was positive; none objected to a barcode on their identification wristband.

The project was taken through pilot stages through to its full implementation across the acute hospitals in Oxfordshire. Our group wrote a national specification for the electronic transfusion process, which is now being implemented both in the UK and internationally. There is the potential to use the same approach for other clinical procedures such as drug administration.

## Update on Current Issues in the Immune Response to Blood Type Antigens

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### Background

The immune system is educated to detect and react with foreign antigens and to tolerate self-antigen. Pregnancies and transfusion of blood challenge the immune system by introducing foreign antigens. These foreign antigens may or may not cause immune responses. Immunization with the RhD antigen is a classic and strong immune response related to blood transfusions and pregnancies. Processing of this large antigen results in many peptides which can be presented on different HLA class II molecules. Immunisation can therefore be induced in many individuals with different HLA class II molecules. Anti-RhD antibodies have variable ability to cause hemolysis of erythrocytes.

### Aim

To understand the mechanism of immunization with human platelet antigen (HPA) 1a antigen and identify the individuals at risk for being immunized.

### Methods

Identification and characterization of antigens, antigen specific B cells, T cells and the MHC restriction of antigen presenting cells (APC) and the collaboration between the different cell types via their specific receptors. Studies of effector functions of T cells and B cells (cytokines and antibodies).

### Results

HPA 1a is an antigen with one amino acid difference from the other allotype, HPA 1b. Both B cells and T cells from women immunized with HPA 1a antigens during pregnancy and the immune synapsis have been studied. The effect of antibodies and T cell stimulation are studied in order to predict the effect of the immune response both related to platelets and other cells carrying the  $\beta 3$  integrin and the HPA 1 antigen.

### Summary/conclusions

Despite emerging knowledge about the immune responses to some of the blood group antigens, better understanding of the immune responses are needed in order to identify individuals at risk for immunization.

## Blood Provision for Difficult Patients

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Provision of blood for any patient with an alloantibody should take the clinical significance of the antibody into consideration. Clinical significance can usually be assessed reliably from serological reactions eg thermal range, mode of reactivity, reaction strength and from published data for an identified specificity. Less readily available are *in vitro* functional cellular assays and *in vivo* <sup>51</sup>Cr survival studies. Guidelines are available which recommend when antigen-negative and crossmatch compatible blood should be selected for transfusion. In the context of my talk 'difficult' refers to patients with a rare blood group ie lacking a high incidence antigen and transfusion dependent patients.

It can be problematic and time consuming finding compatible blood for patients with a rare phenotype and corresponding antibody to a high incidence antigen. If the antibody is identified and compatible blood is judged to be a requirement but is not available locally there are other options: Frozen blood banks, National and International panels of donors of rare blood type or, on rare occasions, autologous donation or a family member, usually a sibling.

Transfusion dependent patients eg sickle cell disease, thalassaemia syndromes, severe aplastic anaemia, myelodysplastic syndromes and other congenital or acquired chronic anaemias can also cause problems with blood provision. These patients can be exposed to many allogeneic red cell antigens and alloimmunisation rates are high. Multiple antibody production can make antibody identification difficult. Extended red cell phenotyping is recommended for these patients and molecular genotyping can be used when transfused cells are present. Provision of blood for sickle cell patients can be especially difficult for several reasons: high incidence of alloimmunisation, high incidence of transfusion reactions and hyperhaemolysis even after transfusion of compatible blood, high incidence of autoantibody and mixtures of antibodies that change over a period of time. Closely antigen-matched donor units, often also ethnically matched, are recommended for these patients.

# Apoptosis in Platelets Following Pathogen Reduction Technology

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## Background

Pathogen reduction technology (PRT) systems such as the Mirasol<sup>®</sup> system (CaridianBCT) have the potential to reduce the residual risks of transfusion transmitted infection. In addition, PRT may enable extension of the platelet shelf life. However, *in vitro* data have demonstrated Mirasol treated platelets are more apoptotic, indicated by increased phosphatidylserine exposure and mitochondrial depolarisation. Further, clinical trials have demonstrated reduced corrected count increments and survival following transfusion of Mirasol treated platelets.

## Aim

To investigate changes in apoptosis signalling in buffy coat-derived platelet concentrates following Mirasol PRT treatment over 7 days of storage.

## Methods

Two ABO matched buffy coat platelet units in plasma/SSP+ (MacoPharma) were pooled and split for each replicate. The test unit was treated in the Mirasol PRT system, while the control remained untreated. Both units were stored as per standard blood banking conditions for 7 days and samples were taken on days 1, 5 and 7 for analysis. Apoptosis was determined through measurement of cell viability, phosphatidylserine exposure and mitochondrial depolarisation by flow cytometry (n=10). Further, expression and activity of Bcl-2 family proteins and caspase-3 and -9 were determined by western blotting and ELISA (n=3).

## Results

Platelet mitochondrial integrity was maintained during the storage period. However, there was a small, but significant decrease in platelet viability following Mirasol treatment and throughout 7 day storage ( $p < 0.05$ ). In addition, the results show a significant increase in phosphatidylserine exposure from day 2 of storage ( $p < 0.05$ ). Western blotting demonstrated an increase in pro-apoptotic Bcl-2 family proteins Bak and Bax from day 5 in Mirasol treated platelets during storage. In addition, caspase-9 and caspase-3 activation was increased from day 5 in Mirasol treated units compared to controls. Further, there was increased cleavage of the caspase-3 substrate, gelsolin, in the Mirasol treated units, evident from day 5. These results indicate increased apoptosis signalling is occurring in the Mirasol treated units compared to controls.

## Conclusion

Mirasol PRT treatment activates several proteins regulating apoptosis, leading to increased phosphatidylserine exposure and reduced platelet viability during storage. These changes may influence the survival of Mirasol treated platelets.

# Characterisation of a Cryopreserved Platelet Product Suitable for Transfusion

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## The Australian Red Cross Blood Service

### Background

Due to the short shelf life and constant clinical need for platelets, it can be difficult to maintain appropriate inventory levels. Platelet shortages occur reasonably frequently whereas at other times there may be a platelet surplus, resulting in unnecessary discards. If platelets could be effectively cryopreserved, then wastage in times of surplus could be minimised. Further, there is an unmet need in the Australian Defence Force for suitable blood products, usually in cases of trauma in remote areas where maintaining fresh products is not feasible given their short shelf-life and the distance from Australia.

### Aim

The aim of this project was to develop a robust protocol for cryopreservation and thawing of platelets within The Australian Red Cross Blood Service and to fully characterise the cryopreserved platelet product using relevant *in vitro* testing.

### Method

Buffy coat-derived platelet concentrates were transferred to a cryopreservation bag (Miltenyi Biotech) with 6 % DMSO. The platelets were centrifuged and the supernatant was expressed, leaving approximately 25 mL, in which the platelets were resuspended and frozen at -80 °C. The cryopreserved platelet units (n=9) were rapidly thawed in a 37 °C water bath, reconstituted in 50 % plasma/SSP+ (MacoPharma) and stored at 22 °C with agitation for 1 hour. Platelet recovery and quality were examined 1 hour and 24 hours post-thaw and compared to the pre-freeze sample.

### Results

Upon thawing, the *in vitro* recovery of frozen platelets was between 70-85 %. The major differences between frozen and liquid stored platelets were a significant reduction in aggregation in response to ADP and collagen; increased CD62P expression; decreased viability; increased apoptosis and some loss of mitochondrial membrane integrity. Similar results were found immediately upon thawing, and at 24 hours post-thaw, indicating that these platelets could have a shelf-life of up to 24 hours. The observed differences are consistent with cell damage, which is to be expected during the freeze-thaw process.

### Conclusion

We have developed a simple, robust method for freezing and thawing platelets. Platelet recovery and function was similar, if not superior, to those reported by other researchers, suggesting that they could be used in transfusion to prevent bleeding.

## **Optimising the Management of Pre-operative Iron Deficiency Anaemia (IDA) and Peri-operative Transfusion Decision Making in Colorectal Cancer Surgery Patients**

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Pre-operative IDA is common in patients having colorectal cancer surgery with 2 RCTs demonstrating that 2 weeks of oral iron can reduce transfusion rates. Baseline audit conducted at TQEH, a public metropolitan teaching hospital, found that 43% (26/60) of colorectal cancer patients were anaemic prior to surgery (8/2007-3/2008). The peri-operative transfusion rate was 41% with 30% of patients (20/60) anaemic at 3 months.

The aim of this clinical practice improvement project was to optimise pre-operative anaemia management and peri-operative transfusion decision making to reduce red cell transfusion and its associated adverse outcomes including increased length of stay (LOS).

Identified barriers included the need to improve awareness of the relationship between anaemia, transfusion and outcomes, limited use of oral iron, short time to theatre and lack of assessment of iron status. Interventions included raising awareness and education of clinical staff and in suitable patients, the use of a "Blood Boost" bag containing iron tablets and patient information. A nurse initiated oral iron prescribing protocol was developed and approved by the hospital for use by the colorectal clinical practice nurse.

Pre-operative Hb was a strong determinant of transfusion rate, with a steep increase when Hb was <110g/L. Overall transfusion rate has declined from 41% to 28% (30/107) in 2008-09, 25% (26/106) in 2009-10, with a transfusion rate of 17% (8/46) for the last half of this period. Overall LOS has decreased by 1 day. Over the 2 year period an estimated 31 patients avoided a 2 unit transfusion equating to around AUS\$1400 per patient in blood product/administration costs and AUS\$700 in reduced LOS, an overall estimated saving of AUS\$65,000.

# Development of High Resolution Melting Analysis of Kidd Blood Group Antigen; a Novel Approach to Determining JK Status

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## Background:

Kidd incompatibility is a serious problem, often impeding safe blood transfusion. Anti-JK alloantibodies have been found, alone or in combination with other specificities, in 46% of delayed haemolytic transfusion reactions (SHOT annual report 2009). Multiply transfused patients may carry circulating antigens from different donors, making it problematic to determine a phenotype by serology alone. This creates difficulty in identifying alloantibodies and, as a result, may hinder transfusion. DNA based techniques, such as high resolution melting analysis (HRMA), may overcome the difficulties associated with haemagglutination and urea lysis. At the time of submission there have been no publications concerning the application of HRM to *JK* genotyping.

## Aim:

To develop an HRM-based assay, a form of real-time quantitative PCR, to genotype patients for their *JK* status and identify patients with the JK<sub>null</sub> phenotype caused by an intron 5 splice acceptor site mutation of *JK*. The ultimate goal is to genotype all relevant JK<sub>null</sub> variants.

## Approach:

Primers were designed to flank the G838A transition, the single nucleotide polymorphism (SNP) responsible for determining the JK a or b status of a patient. The fluorescence melting profile of the resulting 133 nucleotide amplicon, labelled with Roche HRM Dye, was measured using a Roche Lightcycler 480 II instrument to determine whether the fluorescence pattern matched a JKa+b-, JKa-b+ or JKa+b+ profile. Likewise, primers were designed to flank the splice acceptor site of intron 5 of *JK*, a mutation of which is responsible for the JK<sub>null</sub> phenotype that shows increased prevalence in individuals of Polynesian heritage. The fluorescence profiles of the 85 nucleotide amplicons were similarly analysed and compared, in this case, to JKa-b- and wild-type samples.

## Conclusion:

Although the work is still in progress, results so far show that JKa+b-, JKa-b+ and JKa+b+ are able to be differentiated using this technique, as were JK<sub>null</sub> individuals.

# **Red Blood Cell (RBC) Membrane Loss Contributes to Altered Properties of Stored RBCs: Opportunities for Improvement with Alternative Additive Solutions**

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**Australian Red Cross Blood Service**

## **Background**

During refrigerated storage RBCs undergo numerous physicochemical changes, known as the storage lesion, which affects their function and survival. This includes loss of RBC membrane by microparticle (MP) vesiculation, which results in reduced surface area, altered morphology and changes to cell surface molecules that may influence RBC rheology. New RBC storage solutions may offer improved RBC quality by limiting membrane changes.

## **Aim**

To compare the effect of new RBC additive solutions on the loss of RBC membrane, shape change, susceptibility to lysis and adhesion to endothelial cells (ECs) under flow conditions.

## **Methods**

Three ABO-matched leukocyte-reduced whole blood units were pooled, split into triplicate, processed, and one each of the RBC concentrates was suspended in SAG-M (control), PAGGS-M and experimental Erythrosol-4 additive solutions (n=4 sets). Samples were collected at time-points up to 49 days of refrigerated storage. RBC shape and number of glycophorin-A and annexin V-binding MPs were determined by flow cytometry. Adhesion of RBCs to ECs under shear stress conditions and RBC osmotic fragility by hypotonic lysis were measured, along with routine RBC quality parameters.

## **Results**

Compared to SAG-M, RBCs stored in PAGGS-M or Erythrosol-4 had lower numbers of MPs, increased osmotic resilience and lower haemolysis suggesting improved retention of RBC membrane during storage. RBCs stored in Erythrosol-4 were significantly smaller in size and had increased extracellular potassium suggesting increased RBC dehydration. Compared to RBCs in PAGGS-M or SAG-M, RBCs in Erythrosol-4 had increased adhesion to ECs at days 42 and 49. No differences were seen in other routine quality parameters.

## **Conclusion**

Storage of RBCs in PAGGS-M or Erythrosol-4 improved the retention of RBC membrane and osmotic resilience compared to the standard SAG-M additive solution. Further development of new additive solutions may offer improved RBC product quality.



## The Translation of Dendritic Cell Biology into Diagnostics and Therapeutics.

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Dendritic cells (DC) represent unique populations of antigen presenting cells, which initiate and direct immune responses as well as reinforcing immunoregulatory mechanisms to maintain tolerance. The definition of DC in human blood, bone marrow, tonsil, lymph nodes, spleen and other tissues such as the skin, respiratory tract, heart, kidney, gut and liver has increased interest in how DC progenitors adapt to these unique locations and how they react to different signals to generate both innate and cognate immune responses, which maintain host homeostasis but may in certain circumstances contribute to pathogenic outcomes.

Previous observations defined several potential human blood DC populations, whilst more recent work has confirmed that the human CD141 DC population has unique characteristics, which distinguish it from the larger CD1c population (Jongbloed et al. *J Exp Med* 2010;207:1247). Ongoing proteomic analysis has identified unique cell surface markers associated with the CD1c DC subset and these will increase the pool of monoclonal antibodies (mAb) available to study the myeloid and plasmacytoid DC populations.

The CMRF-44 mAb detects the presence of activated DC and the presence of activated CD11c DC predicts for acute graft versus host disease (GVHD) after allogeneic bone marrow transplantation, raising the question as to whether these reflect systemic activation of blood DC or the migration of DC activated elsewhere. Recent data associating the expression of CCR5 on circulating CD11c DC with clinical AGVHD, supports the latter concept and is the subject of ongoing functional studies. In parallel with these diagnostic evaluations of DC surface markers, the prospect of clinical intervention with therapeutic anti CD83 mAb (Wilson et al. *J Exp Med* 2009;206:387) continues to progress with the recent production of a fully humanized mAb (Jones et al. *J Immunol Methods* 2010;354:85), that inhibits allogeneic responses *in vitro* and *in vivo*. The investigation of DC subpopulations in malignancies such as multiple myeloma and prostate cancer is yielding new information and strategies for using DC surface molecules to purify DC or target them in therapeutic vaccination strategies akin to that recently approved by the FDA for prostate cancer. Studies in inflammatory disease focusing initially on psoriasis have identified mechanisms, whereby defective plasmacytoid DC signalling may fail to generate appropriate Treg responses.

These new insights into DC subpopulations and their cell surface molecules are now the subject of a wider translational DC Biology and Therapeutics Program that involves clinical colleagues in evaluating mAbs to DC surface molecules as potential diagnostic and therapeutic agents.

## **VCJD and the UK Blood Supply**

**MacLennan S**<sup>1</sup>

<sup>1</sup> *Clinical Director – Products, NHSBT; Professional Director of UK Joint Professional Advisory Committee (JPAC)*

Variant CJD has been the key driver in blood safety in the UK for almost 15 years since it was first recognised in 1996. To date there have been 173 cases in the UK, 4 of whom are still alive.

Estimating the potential size of the vCJD epidemic has proved very difficult. All cases so far have shown methionine homozygosity at codon 129 of the prion protein gene, but it is unclear whether individuals of a different genetic makeup will develop the disease or be able to transmit.

Transfusion transmission of vCJD has been documented in animal studies and in humans. After cattle feed security was assured, transfusion transmission became the major risk for prolongation of the outbreak. As a result, UK blood services have performed extensive risk assessment exercises, and introduced a variety of measures to reduce the risk.

The first measure employed was ceasing the use of UK plasma for fractionation in 1998/9. Plasma was sourced from the USA, which continues until present. This was shortly followed by universal leucodepletion in 1999 (subsequently shown to be ineffective for eliminating risk of vCJD, although conveying other benefits).

A decision to import FFP for children born after 1996 was made in 2002, following demonstration of transfusion transmission in sheep, because this age group would not have been exposed through diet (later extended to all children and cryoprecipitate manufacture). FFP was pathogen inactivated with MB because of the perceived increased viral risk.

Donors who had been transfused were excluded from donation in 2004, later extended to donors whose blood had been transfused to recipients who had later developed vCJD. Any recipient who has received blood from an implicated donor has been informed so that precautionary measures can be taken with regard to surgical instruments and blood / tissue donation.

Risk assessment of options for further risk reduction led to increasing the proportion of platelets sourced from apheresis (currently to 80%). Further possible measures to reduce risk currently being considered by the DOH are prion filtration, use of double dose red cells, and import of FFP for all. There are currently no screening tests available.

## **Five Strategies to Reduce the Risk of Allogeneic Blood Transfusion Related Mortality**

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Following a review of the relative frequency of the causes of allogeneic blood transfusion-related mortality in the US today, five possible strategies for further reducing such transfusion-related mortality are presented. The five strategies (in hierarchical order) are: 1) Avoidance of unnecessary transfusions through the use of evidence-based transfusion guidelines, to reduce all potentially fatal (infectious as well as non-infectious) transfusion complications; 2) Reduction in the risk of transfusion-related acute lung injury (TRALI) in recipients of platelet transfusions through the use of single-donor platelets collected from male donors, or female donors without a history of pregnancy or who have been shown not to have white-blood-cell (WBC) antibodies; 3) Prevention of hemolytic transfusion reactions through the augmentation of patient identification procedures by the addition of information technologies, as well as through the prevention of additional red-blood-cell (RBC) alloantibody formation in patients who are likely to need multiple transfusions in the future; 4) Avoidance of pooled blood products (such as pooled whole-blood-derived platelets) to reduce the risk of transmission of transfusion-transmitted infections (TTIs) and the residual risk from known TTIs (especially transfusion-associated sepsis [TAS]); and 5) WBC reduction of cellular blood components administered in cardiac surgery to prevent the poorly-understood increased mortality seen in cardiac-surgery patients in association with the receipt of non-WBC-reduced (compared with WBC-reduced) transfusion.

## Bacterial Safety of Platelet Components

### MacLennan S <sup>1</sup>

<sup>1</sup> Clinical Director – Products, NHSBT; Professional Director of UK Joint Professional Advisory Committee (JPAC)

Since the inception of the SHOT Haemovigilance Scheme in 1996, 33 incidents of transfusion transmission of bacteria in platelets have been confirmed, with 9 deaths. Contamination occurs most commonly from the donor skin at the time of venepuncture. Organisms implicated are typically skin commensals or environmental organisms such as *Staph epidermidis* and *Bacillus cereus*.

There are several strategies to reduce the risk. Donor selection will ensure that donors with bacterial infections do not donate. Robust arm cleansing of the donor arm pre-donation removes most bacteria, although spore-forming bacteria may be more difficult to remove. A formulation containing 70% isopropyl alcohol with 2% chlorhexidine gluconate has been demonstrated to be the most effective. Diversion of the first 20-30mL of blood away from the main collection pack is also effective. The combination of improved arm cleansing and diversion has been shown to reduce the risk of contamination by up to 80%.

Bacterial screening has been adopted by the majority of Blood Services in Europe. The aim is to detect contamination to prevent the component being transfused; however the 'false negative' rate may be up to 50% meaning that contaminated platelets can go undetected. This is because platelets are sampled early in their shelf life when only a very few bacteria may be present and missed by the sampling; these can then go on to proliferate within the pack. A longer pre-sampling hold, or repeat sampling later in the shelf life, can help to reduce the false negative rate.

Pathogen inactivation (PI) technology can inactivate bacteria and other pathogens with chemicals and / or energy within the platelets prior to storage. Two systems are currently licensed and a third is likely to be licensed in the near future. PI has the advantage of inactivating viruses, protozoa and lymphocytes in addition to bacteria, and may be useful in reducing the risk from emerging pathogens. Disadvantages include loss of platelets in the process, and efficacy of the treated component, and cost. The UK Advisory Committee on Safety of Blood, Tissues and Organs has recently considered options for reducing risk of bacteria in platelets and decided not to recommend PI at the present time due to questions on patient safety, increased donor exposure, and efficacy.

## **Adverse Effects Associated with Stored Blood – Fact or Fiction?**

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A meta-analysis examined whether the available data support an adequate suspicion that transfusion of old red blood cells (RBCs) is associated with increased mortality, organ failure, infection, prolonged mechanical ventilation, and prolonged stay in the hospital or the intensive-care unit. Such suspicion is required for intentionally exposing patients enrolled in randomized controlled trials (RCTs) to the known or probable—but rare—risks of old RBCs, to document (and prevent) purported common adverse effects of old RBCs. Observational studies presenting adjusted results were eligible for analysis if the adequacy of the adjustment for confounding factors could be assessed.

Among 22 observational studies, there had been only 2 matched analyses of trauma patients which—after adjusting for transfusion dose—reported a significant ( $p < 0.05$ ) adverse effect of length of RBC storage on multiple-organ failure or mortality. Both of these studies were small ( $n = 63$  and  $n = 202$ , respectively), and they did not necessarily adjust for all the other confounding factors. Integration of adjusted findings on the same outcome, from observational studies conducted in the same setting, produced summary results that were either negative (in 6 analyses) or impossible to evaluate owing to uncontrolled confounding by the number of transfused RBCs (in 2 analyses).

Across 2 small RCTs, transfusion of old RBCs was associated with a significant reduction in mortality in the as-treated (summary odds ratio, 0.38; 95% confidence interval, 0.14-0.99;  $p < 0.05$ ; i.e., a result opposite from what would have been expected), but not the intention-to-treat analysis. Although this finding should be discounted as the product of: chance owing to the small number of patients; inappropriateness of as-treated (as opposed to intention-to-treat) analyses; and integration of clinically-heterogeneous studies; this unexpected finding should nonetheless make Safety Monitoring Boards more vigilant in ensuring the safety of the patients enrolled in the on-going RCTs of the purported adverse effects of old RBCs.

# Bacterial Pre-Release Testing of Platelets: The Australian Red Cross Blood Service Experience

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## Aims

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

## Methods

Seven Blood Service testing laboratories (5 new facilities) use the BacT/ALERT® 3D automated microbial detection system. Closed system sampling (15-20mL) from each platelet component occurs at 24h; samples are inoculated for aerobic and anaerobic culture. Platelets are released 'negative to date' while culture continues over component shelf-life. Initial machine positive (IMP) and all follow up results are notified to transfusing laboratories. Communication and education was undertaken to inform clinicians and laboratory personnel of bacterial screening prior to introduction. In the period following implementation (late April 2008 – end June 2010) Blood Service transfusion medicine staff provided clinical follow up of cases where transfusion had occurred prior to IMP notification.

## Results

Feedback from clinicians showed understanding of the clinical rationale to undertake bacterial screening and follow up. Initial concerns related to management of different clinical scenarios and potential workload for laboratories and clinical staff. Of 260123 platelet components screened, there were 2807 (1.08%) IMP notifications of which 780 (28%) had associated transfused components (977 platelets or their associated components [red cells, plasma]. Of all screened platelets, 480 were confirmed positive/indeterminate (0.18%). In 229 (27%) of these the organisms were deemed clinically significant (not *Propionibacterium* species). Transfusion was prevented in 76% (173/229) of these cases due to early notification. Sole anaerobic yield of significant organisms was 2.1% (60/2807) including two confirmed positive cases of *Clostridium perfringens* where transfusion was prevented. Transfusion occurred in 76% (267/352) of *Propionibacterium* species (confirmed/indeterminate), with a mean time to detection of 4.2 days. One high probability septic transfusion reaction was reported in this period related to red cells where no platelet was manufactured.

## Conclusion

Successful implementation of bacterial contamination screening of platelets has occurred in Australia, contributing to a small but important further improvement in transfusion safety.

# Extended Storage of Apheresis Platelets Collected in Additive Solution using the Trima Accel

## Version 6.0

Johnson L, Winter K, Reid S, Hartkopf-Theis T, Kwok M, Marks D

### The Australian Red Cross Blood Service

#### Background and Aim

Collecting apheresis platelets into additive solution allows more plasma to be collected for fractionation, clinical use and also has the potential to reduce transfusion reactions, particularly TRALI. Until now, collection of apheresis platelets with the Trima system required manual addition of additive solution. Trima Version 6.0 software allows immediate, automated, metered addition of platelet additive solution during the collection process, and has not previously been examined. The aim of this study was to evaluate the platelet quality, during extended storage, following collection with the new Version 6.0 Trima system.

#### Method

Apheresis platelets were collected using the Trima Accel apheresis system (CaridianBCT). The test platelet units (n=15) were collected into 48 % plasma/platelet additive solution (SSP+; MacoPharma) using Version 6.0 software, whilst the control units (n=6) were collected into 100 % plasma. All units were stored for nine days, and *in vitro* cell quality parameters were evaluated on days 0, 2, 5, 7 and 9 post-collection.

#### Results

Platelets collected in SSP+ maintained a very stable pH, which was significantly higher than control units on day 5, 7 and 9 of storage. The rate of glucose consumption was significantly lower in additive solution than control platelets in plasma. Consequently, lactate production in control units was significantly higher than those in additive after day 5. Expression of CD62P was significantly higher in control platelets by day 5 of storage. Further, the HSR and mitochondrial membrane potential were maintained better in platelets collected in SSP+. Control platelets displayed higher levels of ADP-induced aggregation, whilst there was no difference in response to collagen. Importantly, little change in platelet viability was observed in either control or SSP+ units as reflected by calcein-AM and FM4-64, and low levels of apoptosis determined by annexin V staining. Cytokine secretion also increased during the storage period to a similar degree in all platelet units examined.

#### Conclusion

These data indicate that apheresis platelet concentrates collected and stored in additive solution, using Trima Version 6.0 software, maintained platelet metabolic and cellular characteristics, which were equivalent, if not superior, to platelets collected in 100 % plasma at day 5 of storage. Furthermore, this data also suggests that apheresis platelets in additive solution could be stored for up to 7 days.

## **WBIT via MCV**

**Osborn W, Davis A, Akers C, Magrin G**

### **Alfred Health**

WBIT via MCV

#### **Aim**

Wrong blood in tube (WBIT) has long been recognized as a serious source of error that can lead to ABO mistransfusion. We retrospectively reviewed the incidence of WBIT due to MCV delta check failure.

#### **Background**

Alfred Health incorporates a major tertiary referral teaching hospital with a trauma service, ICU, cardiothoracic service, bone marrow transplant unit and is a state service for burns and heart and lung transplantation. Alfred Health performs over 36,000 group and antibody screens and dispenses approximately 20,000 red cell units annually. It has been vital to improve blood transfusion practices by the monitoring, facilitating and reporting of data to initiate strategies to enhance the quality and safety of transfusion practices.

#### **Methods**

We have retrospectively monitored the number of WBITs detected by a change in the Mean Cell Volume (MCV) of the red cells greater than 5 fL. The MCV delta check is a quality control initiative written into middleware rules that flags this >5 fL change for all patients within a 28 day period. The MCV remains relatively constant in this time frame unless transfusion or therapy has been initiated. These MCV delta flagged patients are checked for clerical errors, rerun and the transfusion history sourced. If there has been no record of recent transfusion the specimens are ABO and Rhesus D (RhD) grouped and checked against a historical blood group if available. ABO Rh(D) group and in some cases red cell phenotyping mismatch have helped identify WBIT. From January 2007 until June 2010, 118 WBITs were detected of which 26 were identified via MCV delta check method.

This gives a mean result of WBIT due to MCV delta check flag of 22%.

#### **Conclusion**

These findings demonstrate that the MCV delta check rule will increase the number of WBITs identified and we recommend that this is incorporated into routine laboratory practice in order to improve transfusion safety.



## **Unexpected development of anti-C and anti-E following apheresis platelet transfusion: are microparticles to blame?**

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Apheresis platelets (APs) have gained favour over those made from whole blood on the presumption that APs are less likely to provoke alloimmunization to red blood cell (RBC) antigens. This may be true, but RBC antigens vary in their potential to provoke alloimmunization, intrinsically and according to a given patient's predisposition. And, platelets do not express Rh antigens on their surface. Rh(D) is among the most antigenic of RBC antigens; as little as 50  $\mu$ L of Rh (D) RBCs have provoked alloimmunization in Rh(D)-negative patients. Although Rh(C) and Rh(E) are thought to be less antigenic than Rh(D), here we describe cases of Rh(C) and Rh(E) alloimmunization from single AP transfusions. Case 1: A 71 y.o. female with a history of delayed haemolytic transfusion reaction developed anti-C after Rh(C)-mismatched AP transfusion for aortic aneurism repair. Case2: A 57 y.o. female developed anti-E after Rh(E) mismatched AP transfusion for chemotherapy-related thrombocytopenia, Case3: A 73 y.o. male developed anti-E and anti-c production following Rh(Ec)-mismatched AP transfusion for myelodysplastic syndrome. By flow cytometry, APs contain an average of less than 1  $\mu$ L of RBCs. As microparticles have been implicated in antigen trafficking, we postulate that microparticles contained in the apheresis platelet products may facilitate alloimmunization to Rh antigens. RBC-derived microparticles (RDMP) averaged  $210.7 \times 10^6$  (95%C.I. [166.2 – 254.2  $\times 10^6$ ]) on one manufacturer's device and  $232.3 \times 10^6$  (95%C.I. [194.3 – 272.9  $\times 10^6$ ]) on another's. Thus, while platelet products for transfusion have improved with respect to RBC contamination, but our investigation quantified RDMPs as well as RBCs. RDMP may be more immunogenic than RBCs themselves, because they can easily be phagocytosed by recipient antigen-presenting cells. So, extended red cell phenotyping of platelet donors may be warranted for some patients.

## **A<sub>2</sub> Platelets: A Solution to Apheresis Platelet Inventory Imbalance?**

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**Background:** In WA the Blood Service supplies predominantly apheresis platelets, 92 % of 8900 units in 2009-10. RPH receives 31% of State supply. Inventory imbalance of A and O platelets may result in delays in transfusion or transfusion of non ABO identical platelets that may have reduced survival.

**Aim:** To assess the efficacy of transfusion of blood group A<sub>2</sub> platelets in group O patients.

**Method:** Patients were stringently selected for transfusion of A<sub>2</sub> platelets by RPH criteria:

- ◆ Group O with anti-A detectable in serum
- ◆ Haematology in-patient
- ◆ Afebrile
- ◆ Non-refractory and non-alloimmunised to platelet transfusions
- ◆ Non group-incompatible transplant.

Efficacy of platelet transfusion was assessed by pre, 1 hour, 24 hour post transfusion raw platelet counts. The Blood Service supplied platelets for this study.

RPH tested platelet A antigen expression by flow cytometry, compatibility against patient serum and patient anti-A titre pre-transfusion.

**Results:** Preliminary results (n=12) confirm A antigen expression of A<sub>2</sub> platelets (mean<6%) is similar to expression of group O and markedly decreased compared to A<sub>1</sub> platelets.

Comparison of platelet increments in group O patients receiving O and A<sub>2</sub> platelets x10<sup>9</sup>/L:

A<sub>2</sub> platelets: mean 1 hour=28, 24 hour=12, range: 1hr= 14 to 40, 24hr= -1 to 29

O platelets: mean 24 hour=18, range: 2 to 46

All patients receiving A<sub>2</sub> platelets demonstrated increments  $\geq 14$  at one hour. No adverse events reported. This study is ongoing.

**Conclusion:** Transfusion of A<sub>2</sub> platelets to group O patients is effective in raising platelet count. Identification of group A<sub>2</sub> apheresis platelets would increase the inventory available for group O including those HLA/HPA alloimmunised patients, assist with stock inventory management particularly in remote areas and decrease wastage

*This study was approved by the RPH Ethics Committee*

## **Flow Cytometric Analysis of Stored Platelets: What Happens After Filtration?**

**Nollet K, Saito S, Ohto H**

**Fukushima Medical University**

**Aim.** Prior work has addressed the effects of pre-storage filtration of whole blood on subsequent vesicle formation (HAA 2009; *Transfusion Medicine* 20(5):215-216; *Archives of Pathology and Laboratory Medicine* 134(5):771-775). The present work is a first report of post-filtration vesicle formation in apheresis platelets.

**Methods.** After institutional ethics committee approval, health adult males with no prior transfusion history were recruited as apheresis platelet donors. Pairs of group O, group A, and group B apheresis products were pooled and equally divided into four parts. One part was retained without filtration, and the others were passed through leukoreduction filters from three different manufacturers. All products were stored for in an enclosed, temperature-controlled platelet agitator. Samples were periodically withdrawn over a two-week interval to measure leukocyte-derived microparticles (LDMP) and platelet-derived microparticles (PDMP) by flow cytometry.

**Results.** No manufacturer's filter was seen to be superior or inferior with respect to LDMP and PDMP formation during storage. LDMP counts never exceeded 150/uL, except for a single measurement of 551/uL in one filtered sample. PDMP counts ranged from 6/uL to 362/uL over 8 days of storage, and from 378/uL to 4996/uL on day 14.

**Conclusions.** Leukocyte filtration is a relatively mature technology that generally performs as advertised. Nevertheless, prior work suggests that continued vigilance is warranted, especially as the clinical implications of microparticles are better understood. With appropriate protocols – still being developed – flow cytometry may be a useful tool for quality assurance and quality control of blood products for transfusion.

## **Matching for HLA-C Antigens Correlates with Improved Outcomes in Platelet Transfusions Selected Using the HLAMatchmaker Algorithm.**

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<sup>1</sup> Australian Red Cross Blood Service, <sup>2</sup> Department of Haematology, Peter MacCallum Cancer Centre, <sup>3</sup> Department of Epidemiology and Preventive Medicine, Monash University

### Introduction

Prolonged platelet transfusion support is frequently complicated by HLA alloimmunisation and platelet refractoriness. Platelet transfusions from HLA-compatible donors may improve platelet increment. The HLAMatchmaker computer algorithm determines donor-recipient compatibility based on HLA type at amino acid level. It has been used successfully to improve donor selection to achieve adequate corrected count increments (CCI) following platelet transfusion. Previous studies have examined HLAMatchmaker considering only HLA-A and HLA-B antigens. We hypothesised that matching for HLA-C antigens in addition to HLA-A and HLA-B antigens when selecting platelet donors would improve CCIs. This study aimed to evaluate any association between number of HLA triplet mismatches (TMMs), as determined by HLAMatchmaker, with 1h and 24h CCI in platelet refractoriness due to HLA alloimmunisation.

### Methods

Using the HLAMatchmaker algorithm, apheresis platelet donations provided to platelet-refractory patients were selected according to number of HLA-A, -B and -C antigen locus TMMs. Multiple linear regression was used to assess the association between number of HLA-A, -B, and -C TMMs and 1h and 24h CCIs.

### Results

311 HLA-matched platelet transfusions were transfused to 12 patients (2-57 transfusions/patient). 1h and 24h CCIs were available for 256 and 136 transfusions respectively.

Number of HLA-A and HLA-B TMMs correlated with an inferior 1h CCI (reduction in CCI of  $470 \times 10^{11}/L$  per TMM,  $p < 0.001$ ). Number of HLA-C TMMs predicted a further reduction in 1h CCI ( $630 \times 10^{11}/L$  per TMM) over and above the effect of number of HLA-A and HLA-B TMMs ( $p = 0.006$ ). Number of HLA-A and HLA-B TMMs also correlated with inferior 24h CCI ( $p < 0.001$ ). There was no statistically significant correlation between number of HLA-C TMMs and 24h CCI.

### Conclusion

Number of HLA-A and HLA-B TMMs correlated with inferior 1h and 24h CCI. Number of HLA-C TMMs correlated with an inferior 1h CCI and was independent of the number of HLA-A and HLA-B TMMs. Consideration should be given to inclusion of HLA-C antigens when using the HLAMatchmaker algorithm to improve platelet donor selection and clinical outcomes.

## **Intra-uterine Transfusions .**

### **A Collaborative Approach New Zealand Blood Service and Maternal Foetal Medicine National Women's Health Auckland City Hospital .**

**Donegan R**

**New Zealand Blood Service**

#### **Background**

In December 1962 National Women's Hospital made International headlines when the first successful pre-birth transfusion was performed by Sir William Liley.

National Women's Health continues to be at the forefront in care of pre-term infants affected by severe haemolytic disease of the fetus/newborn (HDFN).

In 2007 an 8 week old infant who had received intra-uterine(IUT) and top-up transfusions at National Women's Health died .The Health and Disability report highlighted poor follow-up and co-ordination of care between services. NZBS investigated and implemented a process to improve communication with Maternal Foetal Medicine (MFM) and follow-up care and management of babies with HDFN

#### **Method**

This is a retrospective audit of the management and outcomes of women with significant red cell antibodies that have been referred to MFM.NZBS is responsible for alerting the Lead Maternity Carer (LMC)of the antibody identification results. If a significant antibody capable of causing HDFN is detected then antibody titres are performed. Results of the titres determine whether the patient is referred to MFM. MFM perform regular ultrasound monitoring (to assess for signs of anaemia due to haemolysis) and decide when intervention with an IUT is required, as well as performing the procedure.

#### **Results**

Between January 2007 and May 2010 MFM has performed 57 red cell IUT to 23 women. The indications for IUT was maternal red cell antibodies to Rh,Kidd,Kell and Duffy blood group antigens .Regular communication is held between NZBS and MFM . As the Transfusion Nurse Specialist I attend the IUT and work with MFM. We provide women and their families with information regarding ongoing management of their babies including possible future transfusion and post delivery follow-up.

#### **Conclusion**

Preparation and co-ordination of the IUT has significantly improved. Post delivery exchange and top-up transfusions are less frequent. Women and their families are better prepared for ongoing monitoring and management.

# **Implementation of a Simple Tracking Method for Red Cells held in DHB Remote Blood Fridges**

**King F**

**New Zealand Blood Service**

## **Background**

NZBS Wellington Blood Bank issue red cells to named patients within a clinical area upon request. Several of these clinical areas hold remote storage refrigerators on-site where issued red cells are stored and, if not transfused, returned to Blood Bank. In 2008 a corrective action request was issued by IANZ requiring Blood Bank to prove that returned red cells from remote fridges had not been out of controlled storage for greater than 30 minutes and could safely be returned to stock.

## **Aim**

To develop and implement a simple method of tracking red cells into and out of remote blood fridges held within Capital & Coast District Health Board (C&CDHB)

## **Method**

Following discussion with NZBS staff and CCDHB clinical staff a simple tag was developed that is attached to all red cells units issued to Operating Theatre and Delivery Suite. This tag documents the unit number and department of issue and has space to detail date, time and signature for each time the unit is entered into or removed from the fridge. On return to Blood Bank, if the tag shows there is evidence the unit has been out of controlled storage for more than 30 minutes or if details are incomplete, the unit is discarded and the tag filed. Extensive education was undertaken prior to implementation and an amnesty period of one month was allowed.

## **Results**

During the amnesty month, both departments returned between 45 – 65% units with tags incomplete. Further teaching was undertaken and major reduction in wastage was achieved. Delivery Suite and Operating Theatre now waste approximately 7% and 10% of returned red cells respectively per month.

## **Conclusion**

The tracking tag is a simple, cheap and effective method of tracing red cell units in and out of remote fridges, once issued from Blood Bank.

## **Learning from STIR's most common reported event:the complexity of acute transfusion reactions**

**Birznieks D<sup>1</sup>, Stevenson L<sup>1</sup>, Wilks D<sup>1</sup>, McNicol L<sup>2</sup>, Wood E<sup>3</sup>**

**<sup>1</sup> Blood Matters-Department of Health, Victoria, <sup>2</sup> Austin Health, Melbourne, Victoria, <sup>3</sup>**

### **Australian Red Cross Blood Service, Australia**

The Blood Matters program is an initiative of the Department of Health, Victoria and the Australian Red Cross Blood Service. STIR (Serious Transfusion Incident Reporting) is the voluntary haemovigilance system of the program, where hospitals and laboratories from Victoria, Tasmania, Australian Capital Territory and Northern Territory participate. The system aims to:

- measure and monitor serious transfusion incidents, including near misses, relating to administration and handling of fresh components and pretransfusion samples.
- derive recommendations for better, safer transfusion practice and disseminate these to health services, governments and the Blood Service.

Health services submit initial reports electronically and the STIR office provides detailed case report forms relevant to the event type (e.g. acute transfusion reaction, ATR). Information (de-identified for institution) regarding the case is returned to STIR for data entry and review, including attribution of causality and severity, by an expert clinical group.

Since 2006, STIR has been notified from 63 institutions of 667 transfusion episodes resulting in 676 adverse events (In 2008-09 274 events were reported with a denominator of 511,037 blood components issued). The majority (61%) were associated with red cells. ATRs, which include febrile non-haemolytic, allergic (including anaphylaxis), acute haemolytic, transfusion-associated circulatory overload and transfusion-related acute lung injury, represent the majority of reports (55%). Allergic reactions are a high proportion of ATR reports (39%) with severity ranging from anaphylaxis (20%) to severe allergic reactions requiring adrenaline (26%) to mild allergic (53%).

ATRs are unpredictable, often complex in causality and with a wide variation in severity. It may be difficult to distinguish one type of reaction from another in the acute setting. Recognising and managing these reactions requires education and training of clinical and laboratory staff. With the growing number of ATR reports, STIR is developing a picture of this clinical complexity. Cases and recommendations are summarised in the STIR report for 2008-09, which aims to assist health services in management of these events.

Acknowledgements to all members of the STIR expert group and participating hospitals. Information about the program and the STIR system is available at <http://www.health.vic.gov.au/best/tools/stir.htm>

# **Efficacy and Safety Profile of Prothrombinex-VF at Royal Perth Hospital: A Six Month Prospective Audit**

**Kruger P<sup>1</sup>, Herrmann R<sup>2</sup>, Le Viellez A<sup>3</sup>, Burrows S<sup>4</sup>**

**<sup>1</sup> Royal Perth Hospital Haematology Department, <sup>2</sup> Haematologist, Royal Perth Hospital, <sup>3</sup> Scientist in Charge, Transfusion Medicine, Royal Perth Hospital, <sup>4</sup> Biostatistician, Clinical Trials WA and Royal Perth Hospital.**

## **Background**

Prothrombinex-VF powder for injection (PTX) is licensed for warfarin reversal and previous studies have suggested that relatively low doses are efficacious. New indications for PTX administration are developing and studies of efficacy are warranted.

## **Aims**

To assess usage patterns of PTX at Royal Perth Hospital and analyse efficacy and safety profile.

## **Methods**

Prospective audit of every patient prescribed PTX over a six month period at Royal Perth Hospital from November 2009 to May 2010. Data was collected for diagnosis, comorbidities, dosage of PTX/Fresh Frozen Plasma (FFP)/phytomenadione, investigation results pre/post dose, and adverse reactions.

## **Results**

334 vials of PTX were given to 85 patients over 107 prescriptions. Indications included warfarin reversal for haemorrhage/imminent procedure (n=66), non-warfarin reversal administered for intraoperative bleeding (n=20), and coagulopathy (n=21). Prescriptions differed from the Warfarin reversal Consensus Group Guidelines (WRCG, MJA 2004) in most (61/66) patients. Subtherapeutic doses of PTX (ie <25IU/kg) were efficacious when given with fresh frozen plasma (FFP) or phytomenadione. Haemostasis was achieved in 15/28 bleeding warfarinised patients with medical management alone. PTX was administered in intraoperative bleeding and it was a cofactor in haemostasis, however factor levels were not investigated. Safety analysis revealed no hypersensitivity reactions, no immediate thrombotic complications, and no worsening of disseminated intravascular coagulation.

## **Conclusion**

Subtherapeutic doses of PTX, in combination with either FFP or phytomenadione, are efficacious in rapid warfarin reversal. PTX without FFP is also efficacious in warfarin reversal. Further studies should be considered before future revision of Warfarin Reversal Guidelines. Prospective studies of efficacy of PTX in non-warfarinised patients for cessation of intraoperative bleeding are warranted. There were no adverse effects associated with PTX administration noted in this audit.



# **Core temperature changes in whole blood plasma reduced within the ambient temperatures of Neonatal Intensive Care Units prior to exchange transfusion.**

**Rishworth S**

**New Zealand Blood Service**

## **Background**

Exchange transfusion is undertaken to replace a calculated volume of a neonate's circulation with donated allogeneic blood. The procedure is performed to correct and manage severe anaemia at birth and to treat severe hyperbilirubinaemia, usually as a result of haemolytic disease of the newborn (HDN). With the availability of RhD immunoglobulin, intrauterine transfusion and phototherapy the frequency of exchange transfusion has decreased.

Warming of the allogeneic blood during a neonatal exchange is recommended to prevent hypothermia; however concerns regarding potential over-heating from in-line warmers have been documented. In 2004 the British Committee for Standards in Haematology (BCSH) updated their guidelines for transfusing children and neonates, noting that most clinical units within the United Kingdom allowed the blood to approximate the ambient temperature rather than add in-line warmers.

The national recommended ambient temperature of a Neonatal Intensive Care Unit (NICU) in New Zealand is 23°C - 26°C.

## **Aim**

To measure the changes in the core temperature of whole blood plasma reduced (WBPR) in NICU ambient temperatures. The secondary aim is to identify if the core temperature achieved in the NICU setting prior to a neonatal exchange transfusion could minimize the requirement for in-line warmers.

## **Method**

Ambient temperature was measured for five to seven days within five Level III NICUs in New Zealand to obtain mean, maximum and minimum ambient temperatures.

WBPR was exposed to various ambient temperatures and handling contact in a lab-setting to replicate the fate of a unit from a Blood Bank environment (21-22°C) to a NICU environment (23-26°C), for a period of 90 minutes. Core temperatures were measured at 5 or 15 minute intervals.

## **Preliminary Results**

WBPR handled once, then left static in 24.4°C - 25.5°C reached 12.8°C at 60 minutes and 15.6°C by 90 minutes. However WBPR that were handled in 21-22°C for seven minutes, then handled in temperatures of 24.5 - 26°C took much less time to warm. WBPR exposed to gentle inversion every 15 minutes reached 17.3°C at 60 minutes and 19.6°C at 90 minutes. WBPR exposed to gentle inversion from T = 15, at five minute intervals, reached 18.8°C at 60 minutes and 21.4°C at 90 minutes.

## **Comment**

Early findings are suggestive that gentle inversion of the WBPR in the NICU environment can hasten the rise of the core temperature. Further testing is currently underway.

## Ten Years and Over 3600 Cases of Recombinant Activated Factor VII Use: What does the Haemostasis Registry Data Tell Us?

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**Background:** Recombinant activated factor VII (rFVIIa) is approved for the treatment of spontaneous and surgical bleeding in patients with haemophilia A or B and with antibodies to either factor VIII or factor IX. However rFVIIa continues to be used off-license for critical bleeding episodes despite the absence of strong RCT evidence.

**Aims:** The aim of the Haemostasis Registry was to collect data on the off-license use of rFVIIa in Australia and New Zealand.

**Methods:** Monash University established the Haemostasis Registry with an educational grant from Novo Nordisk Pharmaceuticals. Ethics approval was obtained at 96 hospitals, including all major users of rFVIIa.

**Results:** Approximately 3600 off-license rFVIIa cases have been reported to the Registry (2000-2009). Major areas of use were cardiac surgery (~43%), other surgery (18%) and trauma (13%). The majority (~77%) of patients received a single dose of rFVIIa with a median (IQR) dose of 91 (73-103) mcg/kg. 74% of cases documented a response (decrease or cessation) to bleeding following rFVIIa and the median number of RBC units was reduced following administration of rFVIIa ( $p < 0.001$ ). Clinician perceived control of bleeding following rFVIIa was strongly correlated with outcome at 28 days ( $\chi^2_2$ ,  $p < 0.001$ ). Stepwise logistic regression analysis revealed that pH was the most important factor in determining response to bleeding and patient survival. Importantly, size of dose was not associated with either outcome measure; low doses (40 mcg/kg) appear to be just as effective as larger (100 mcg/kg) doses in cardiac surgery patients. A case control study determined that risk-adjusted adverse events rates were not significantly different in cardiac surgery patients treated with rFVIIa compared with untreated cases.

**Conclusions:** The Haemostasis Registry is the largest dataset of its kind and provides critical observational data on the use of rFVIIa in an area unlikely to be supported by new RCT data.

# Diagnosis and Management of Fetal / Neonatal Alloimmune Thrombocytopenia

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## Background

Thrombocytopenia (platelet count  $<150 \times 10^9/l$ ) is present in almost one percent of newborns. The most common cause of severe thrombocytopenia (platelet count  $<50 \times 10^9/l$ ) is fetal exposure for maternal IgG alloantibodies against HPA 1a antigen on fetal platelets. In 1:12,500-25,000 pregnancies the fetus or newborn suffers from FNAIT induced intracranial hemorrhage which may cause death or survival with lifelong disabilities. The pathophysiology of the disease is very similar to hemolytic disease of the newborn (HDN). Clinically, the condition is under-diagnosed, there is no consensus of treatment in pregnancy and unlike the situation in HDN, no prophylactic treatment is yet offered.

## Aim

The aim of the study was to identify and characterize FNAIT in a prospective screening study and to reduce neonatal morbidity and mortality by a new intervention approach including development of passive immunization with anti-HPA 1a antibodies.

## Material and methods

Screening for HPA 1bb women was performed in 100,448 pregnancies. Blood samples from HPA 1bb women were analyzed for the presence and quantity of anti-HPA 1a antibodies throughout pregnancy and post partum and typed for HLA DRB3\*0101. Platelet counts in the newborns were registered. T cells and B cells were isolated from HPA 1bb women who gave birth to babies with thrombocytopenia. ABO typing was performed in 158 HPA 1bb women who had given birth to children with variable platelet count. Birth weight was registered retrospectively.

## Results

A woman at particular risk of giving birth to a child with severe anti-HPA 1a-induced FNAIT have the following characteristics; she is HPA 1a negative, HLA DRB3\*0101 positive, has anti-HPA 1a antibodies  $>3.0$  IU/ml and blood type A. Without antenatal screening for mothers at risk of having a child with FNAIT, only a small percentage of risk pregnancies are identified. Seventy five percent of the women seemed to be immunized after the pregnancy and 25% during their first pregnancy. The antibody level increased during the first pregnancy, whereas in subsequent pregnancies the antibody level typically decreased. It was possible to isolate HPA 1a reactive T cells and B cells from immunized women. The birth weight in newborns was significantly reduced in mothers with high antibody levels. Passive immunization with anti-HPA 1a in un-immunized HPA 1bb women may show comparable results with anti-D to prevent immunization with RhD antigens.

## Summary/conclusion

In order to prevent immunization towards the HPA 1a antigen and avoid fetal and neonatal clinical complications, the women at risk has to be identified. This may be done in general antenatal screening programs. It is conceivable that prevention may be accomplished in 75% of the risk pregnancies by passive administration of anti-HPA 1a antibodies. Dissection of the B- and T-cell interaction and the antibody patterns during pregnancies can provide important information about break of tolerance to fetal antigens. The possible link between ABO type and severity of FNAIT may open exciting perspectives in immune biology.

## **Intra-Uterine Transfusion for Neonatal Allo-immune Thrombocytopenia**

**Parry E**<sup>1</sup>

<sup>1</sup> *Maternal-Fetal Medicine Service, National Women's Health, Auckland City Hospital, ADHB*

Neonatal Alloimmune Thrombocytopenia (NAIT) is a rare event which can be devastating. The diagnosis is usually made after an affected pregnancy with antenatal or postnatal detection of fetal/neonatal intracerebral haemorrhage. Some cases are an incidental finding on neonatal blood testing.

Once platelet antibodies are identified in the mother, paternal platelet antigen testing is performed. Planning for subsequent pregnancies can then be made based on the past obstetric history and paternal antigens.

In my talk I will cover aspects of management of the pregnancy. I will include invasive testing for fetal platelet antigen typing. The role of ivig and steroids will be discussed including timing. I will also spend some time describing how and when intra-uterine transfusion is performed and the risks.

## **Non-Invasive Prenatal Assessment of Fetal RHD Type: Guarding against Pre- and Post-analytical Errors in Molecular Blood Group Typing Assessments**

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**Introduction** Quality assured non-invasive prenatal diagnostic (NIPD) tests can assess fetal *RHD* status to type the fetus in pregnancies at risk of haemolytic disease of the newborn. The accuracy for these analytical tests is assessed with reference to serology as the gold standard and is dependent on robust patient identification and quality systems for sample processing. From a survey of 400 RhD negative pregnancies, of which 173 gave paired samples, we report a low rate of quality system failures in the pre-analytical phase and low rate of discordant molecular and serology typing in the post-analytical phase. The latter resolved in favour of molecular typing.

**Methods** EDTA whole blood samples were collected from 400 Rh(D) negative pregnant women in two locations. Plasma was harvested by a double spin procedure and DNA extraction performed before testing for *RHD* DNA of fetal origin. Samples were blinded for testing and only unblinded when Rh(D) type of the baby was reported from cord blood serology.

**Results** Fetal *RHD* assessment was possible for 97.5% (390/400) of cases. Ten cases carried *RHD* molecular variants and were inconclusive. Sixteen cases were lost to follow up. Among the remaining 374 cases representing 547 samples, five results showed apparent discrepancies between NIPD and post delivery Rh(D) phenotype. Three of the five were tracked to pre-analytical steps: two were adjacent cases, detected as one was a repeat sample and the NIPD result did not match the first result. Instead results were consistent with a sample swap before plasma preparation.

For two of the five cases the predicted phenotype was concordant when full serological data was obtained: In one case review of clinical notes showed that the initial cord blood RhD serotype reflected intrauterine transfusion of Rh(D) negative units rather than the baby's RhD positive status. For the other case a positive DAT due to an ABO incompatibility contributed to a weak RhD positive reaction. Follow up testing confirmed the serology was RhD negative, concordant with the *RHD* genotype.

**Conclusion** A number of quality control measures are built in to the analytical testing steps. As further precautions we request a second sample where the *RHD* is not detected (37% of cases) as assurance to guard against a false negative report which could have clinical impact. This survey suggests that the pre-analytical phase is the most vulnerable point of error and final reporting of results only after two samples have been processed also guards against pre-analytical quality system failures. Two post analytical errors resolved in favour of molecular typing and show the importance of clinical collaboration to resolve molecular and serology discrepancies when evaluating NIPD assays.

# Transfusion-Related Acute Lung Injury (TRALI) in a Two-Event Ovine Model Following Transfusion with Supernatant from Stored Human Packed Red Blood Cells (PRBC)

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**Background:** Transfusion-related acute lung injury (TRALI) continues to be a frequent cause of transfusion-related morbidity and mortality worldwide. We have previously reported the development of an ovine model of TRALI based on transfusion with supernatant from date-of-expiry human platelet components. We now report the further application of this TRALI model to investigate transfusion with supernatant from another human cellular blood product, packed red blood cells (PRBC).

**Methods:** In a two-event ovine model, lipopolysaccharide (LPS) was infused as a first event, and heat-treated pooled supernatant from date-of-expiry human PRBC (d42-PRBC-S/N) was transfused as a second event. Saline-infusion was used as a control for both events, and transfusion with fresh supernatant (d1-PRBC-S/N) also acted as a control for the second event. A range of respiratory, cardiopulmonary and haematological measures were recorded, and TRALI was defined by the development of both hypoxemia (either during or within 2 hrs of transfusion) and post-mortem histological assessment of pulmonary oedema.

**Results & Discussion:** TRALI was not observed in “sham” groups receiving either saline (n=5) or LPS (n=6) as a first event followed by saline transfusion. TRALI developed in only one of five LPS-treated sheep transfused with d1-PRBC-S/N, however subsequent analyses of baseline respiratory data indicated that underlying pathology may have predisposed this animal towards development of TRALI. While saline-treated sheep transfused with either d1-PRBC-S/N (n=4) or d42-PRBC-S/N (n=3) did not develop TRALI, four of five LPS-treated sheep transfused with d42-PRBC-S/N did develop TRALI. This indicated that both LPS-infusion and d42-PRBC-S/N transfusion were required for the development of TRALI. Also associated with TRALI were decreased pulmonary compliance, decreased end tidal CO<sub>2</sub> and increased arterial partial pressure of CO<sub>2</sub>.

**Conclusions:** This in-vivo ovine model has demonstrated that: (i) LPS-infusion (as a model of clinical infection) made the sheep susceptible to the development of TRALI; (ii) supernatant from date-of-expiry PRBC was capable of causing TRALI; and (iii) TRALI pathogenesis followed a two-event mechanism. Results from this study provide further evidence of contributing roles in TRALI pathogenesis for both biological response modifiers that accumulate in cellular blood components during storage and the patients’ underlying clinical condition.

## **Severe Iron Deficiency In Paediatric Patients: 10 years experience.**

**Kalumba K<sup>1</sup>**

**<sup>1</sup> Peter Mc Callum hospital/ Melbourne Pathology, <sup>2</sup> Royal Children's Hospital**

### **BACKGROUND**

Iron deficiency is common with potential to cause significant morbidity and mortality, particularly in children. Management of severe iron deficiency anaemia in hospital ranges from oral therapy to transfusion.

Our study assessed patterns of therapy and their outcomes in children admitted to the Royal Children's Hospital (RCH), Melbourne over 10 years.

### **METHOD**

Retrospective study of children admitted to RCH with severe iron deficiency anaemia (Hb < 70g/L & ferritin < 15 ug/dL) between January 1998 and December 2008.

Information was obtained from hospital medical records and information systems.

Exclusion criteria included incomplete records and other causes of anaemia.

### **RESULTS**

42 patients identified: 55% male, age 2 months to 18 years (mean: 7.3 years).

Hb range 26 – 69g/L (mean 50.6g/L). Mean ferritin level 2.9 ug/L. 26% below tenth percentile for weight. Half the patients were referred by their general practitioner. None were compromised clinically or on other routine laboratory testing.

Poor diet was prevalent (40%). GIT blood loss and inflammatory bowel disease were present in 19% & 9.5% respectively.

50% received red cell transfusion, 45.2% were treated with oral iron and 4.7% were managed with IV iron infusion. There was no significant difference in Hb increment beyond 14 days of therapy between the 3 groups.

### **CONCLUSION**

Severe iron deficiency anaemia is a chronic condition. In the absence of end organ hypoxia, transfusion as the primary therapy appeared over-utilized in our institution, with exception of patients with concomitant acute blood loss.

In the absence of significant differences in outcome between therapies, oral iron supplementation and iron infusion were underutilized. Investigation of the barriers to use of iron supplementation is warranted to alter practice.

# Baseline Iron Deficiency without Anaemia is associated with Future Deferral from Whole Blood Donation in Premenopausal Women

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## Introduction

Iron deficiency is a potential risk for blood donors, especially premenopausal females, and may result in anaemia. Donors who develop anaemia are deferred. How baseline iron indices affect subsequent donor health is poorly understood. We sought to identify factors associated with, and outcomes from, donor iron depletion.

## Methods

As part of a study investigating hepcidin as a diagnostic test of iron status, non-anaemic, premenopausal, female whole blood donors completed the SF-36 health survey and had samples taken for ferritin prior to donation. At subsequent donation, any change in haemoglobin and deferral for anaemia was noted.

## Results

Of 266 non-anaemic donors at baseline, 22.3% had ferritin < 15 ng/mL. Baseline iron depletion was associated with number of donations over previous 12 months (OR 1.8 [95%CI 1.4, 2.3] per donation,  $P < 0.001$ ). There was no association between iron depletion and impaired health (SF-36). After minimum 6 months follow-up, 151 (58.1%) donors had returned for donation. There were no significant differences in baseline haemoglobin ( $P = 0.15$ ) or ferritin ( $P = 0.17$ ) between donors who did/did not return. Mean haemoglobin was lower at follow-up (131.3 g/L) than baseline (133.7 g/L) ( $P < 0.01$ , t-test). Anaemia at subsequent donation was seen in 8.6% and associated with baseline iron depletion (OR 20.3,  $P < 0.001$ ). 10/151 (6.6%) of returning donors were deferred: 90% of these were iron depleted at baseline, compared with 30/141 (21.4%) in those not deferred (OR 33.0,  $P < 0.001$ ). Baseline haemoglobin was higher in non-deferred (mean 134.5 g/L) than deferred (mean 124.4 g/L) donors ( $P < 0.0005$ , t-test). Both ferritin ( $AUC^{ROC} = 0.88$ ) and haemoglobin ( $AUC^{ROC} = 0.87$ ) were predictors of deferral.

## Conclusions

Iron status in female premenopausal blood donors is related to donation history. Although iron depletion in eligible donors is not associated with concurrent health impairment, they are more likely to develop anaemia subsequently and be deferred from future donations. Screening for iron deficiency may offer opportunities for early intervention. Prevention of donor iron deficiency, through donor education, research into new diagnostics, and investigation of iron replacement strategies, is a high priority for the Blood Service.



## **RhD Usage in New Zealand - A Multi-Centre Audit**

**King F**

### **New Zealand Blood Service**

#### **Aim:**

The aim of the audit was to assess

- the proportion of RhD negative mothers appropriately treated with RhD immunoglobulin.
- other indications for RhD immunoglobulin by tracing RhD immunoglobulin issues.

#### **Method**

A retrospective audit was undertaken by Transfusion Nurse Specialists at eight District Health Boards (DHBs). Fifty births within Public Hospitals and Birthing Centres at each DHB where the mother was known to be RhD negative were assessed. A further eighty issues from Blood Bank or Donor centres at each DHB were assessed, ensuring these did not overlap with the assessed births. Data was obtained from NZBS, DHB and community laboratories, clinical notes and Lead Maternity Carers.

#### **Results**

460 births from RhD negative mothers and 640 RhD Immunoglobulin prescriptions were audited.

95% of RhD negative women who gave birth to RhD positive babies received RhD Immunoglobulin and 98% of those received it within 72 hours of birth. 2% of women received Anti-D after giving birth to an RhD positive baby.

The majority of prescriptions were for obstetric indications in the third trimester. Only 8% of prescriptions were in the first trimester and only 7% of these were for the recommended dose of 250iu, the rest receiving 625iu. Only 5% of prescriptions were for Routine Antenatal Anti-D Prophylaxis.

Four DHBs seldom performed Kleihauer tests (2% of births and antenatal sensitising events), whereas the other four DHBs varied between 81-97%. This marked difference correlated with the absence or knowledge of a policy on Kleihauer testing within the DHB.

Administration was documented in 99% of available records and consent in 93%.

# Unravelling the Mechanism of Adhesion of Stored Red Blood Cells to Vascular Endothelium

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## Background

Red blood cells (RBCs) progressively develop storage lesions during refrigerated storage, including loss of membrane, lipid scrambling, changes to cell surface receptors and altered morphology, which affect their function and survival. Using an *in vitro* model to simulate blood flow, we have previously shown that stored RBCs become progressively adherent to endothelial cells (ECs), which may have implications for *in vivo* vascular rheological behaviour. The biological mechanism(s) of adhesion of stored RBC to ECs is unknown.

## Aim

To investigate the mechanism(s) of adhesion of stored RBCs to vascular ECs under flow conditions.

## Methods

Leucocyte-reduced RBC units were prepared and stored according to standard blood bank procedures and sampled at nominated time points up to day 42 of storage. For some experiments, samples were fractionated into 'young' and 'old' RBCs based on differential centrifugal density. Adhesion of stored RBCs to ECs was determined using a temperature and rate-controlled flow perfusion microchamber system mounted on an inverted microscope and recorded through a CCD camera and imaging software. Strength of RBC adhesion was determined by incrementally increasing the flow rate of the perfusion medium to achieve shear stresses of 0.5-4.0 dyne/cm<sup>2</sup>. Morphology score was used to categorise the shape of adherent RBCs.

## Results

The number of adherent RBCs increased with storage duration. The majority of adherent RBCs had seeming normal discoid shape, which suggests that morphologically-related changes are not the primary mechanism of stored RBC adhesion to ECs. More subtle changes to RBCs during storage and chronological aging may play a greater role in the adhesiveness of stored RBCs to ECs.

## Conclusion

Storage-induced changes to RBCs increase their adhesiveness to ECs under flow conditions. RBC morphological changes did not appear to be the primary mechanism of adhesion to ECs. Other mechanisms related to storage-induced changes and RBC chronological age may be implicated.

## Regulation of Blood in Australia

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In Australia, the safety and quality of blood and blood components is regulated by the Therapeutic Goods Administration (TGA) through the Therapeutic Goods Act 1989. This legislation has required the Australian Red Cross Blood Service (ARCBS) to be licensed via the code of GMP, initially from 1992 for supply of plasma for fractionation, and from 2000 for all fresh blood and blood components, and to comply with standards such as the Council of Europe Guide. The TGA's risk based approach to regulation also includes post-approval monitoring.

An integrated strategy for blood safety is required for the minimisation of transfusion transmitted infections and for provision of safe and adequate blood transfusion services. Over the last 10 years the TGA and ARCBS have developed and refined processes for supporting evidence to meet regulatory requirements for fresh blood products. Review and endorsement of this evidence has become an effective mechanism for shaping the regulatory framework.

An ongoing challenge for the TGA and the ARCBS is the management of emerging and re-emerging infectious disease and the potential impact on the blood supply. Both organisations contribute to the scientific investigation of emerging infectious agents, which includes surveillance, testing methods, standards development and regulatory controls with the aim of implementing an agreed strategy for each identified emerging EREID agent.

Improvements in testing and the development of new technologies need careful consideration and evaluation to determine their relative value in risk management of the Australian blood supply. The TGA and ARCBS risk assessments are independent of funding considerations. This may also entail development of agreed frameworks with other relevant stakeholders regarding implications for donor look-back, as well as retrospective management of manufactured products,

Of prime importance in the regulation of the Australian blood supply is a transparent and shared understanding of the principles of risk and a collaborative approach between the TGA and the ARCBS.

No conflict of interest to disclose.

## The New Zealand Situation

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The pursuit of high quality is a basic principle for any health enterprise.

This presentation provides an overview of blood transfusion services in New Zealand, remembering the early years where nationally consistent standards and approaches were largely absent, through to the establishment and operation of today's national blood service which operates in a highly regulated environment to provide safe, high quality and efficient blood services for the people of New Zealand. We outline how the regulator and the blood service successfully interact to ensure high quality product and manufacturing standards are maintained.

In 1993 blood and blood products became incorporated within the regulatory regime established by the Medicines Act 1981, and the first GMP audits for medicines manufacturing licences were carried out by auditors from the then Department of Health. This was the beginning of a regulatory relationship between Medsafe (the Medicines and Medical Devices Safety Authority) and the then hospital-based blood transfusion services in New Zealand, today the New Zealand Blood Service (NZBS), which has promoted the development of a world-class, quality blood service that enhances and protects the New Zealand blood and blood products supply.

Prior to the formation of NZBS, hospital-based transfusion services operated to a local New Zealand set of *Minimum Standards* based on WHO guidelines. Current standards developed by NZBS are primarily based on Council of Europe Guidelines. Medsafe formally approves and audits against these standards and a consensus approach is taken when changes to standards are required. Some examples will be presented.

## **JPAC – A UK Perspective**

### **MacLennan S<sup>1</sup>**

<sup>1</sup> *Professional Director of UK Joint Professional Advisory Committee (JPAC); Clinical Director – Products, NHSBT*

The Competent Authority for blood in the UK is the Medicines and Healthcare products Regulatory Agency (MHRA), an Executive agency of the Department of Health formed in 2003 by the merger of the Medicines Control Agency and the Medical Devices Agency. The MHRA oversees conformance by Blood Services ('blood establishments') and hospitals ('blood banks') to the Blood Safety and Quality Regulations 2005 (BSQR), which implement the requirements of the European Blood Safety and Quality Directive (2002) and its 3 technical Directives (2004 and 2005). The MHRA inspects blood establishments every 2 years for compliance with the BSQR and GMP, and ensures compliance to the BSQR by blood banks through questionnaires and targeted visits. It also collates haemovigilance data required by the BSQR from hospitals.

The Joint Professional Advisory Committee of the UK Blood Transfusion Services (JPAC or 'Red Book Committee') was set up in 1990 to develop Guidelines for the 4 UK Transfusion Services. Since then, 7 editions of the 'Red Book' have been published (with the 8<sup>th</sup> planned for early 2011), and the role of JPAC has widened to include acting as an advisory committee to the Medical Directors of the UK Blood Services in addition to producing guidelines. A representative of the MHRA sits on JPAC. The Red Book contains guidelines reflecting best practice, sets standards to be met by the products, and describes technical details of the processes involved, as well as including the legally binding requirements detailed in the BSQR. It contains much more detailed guidance than the BSQR, and is more easily amended to reflect changes in the environment and improvements in practice (the only amendment so far made to the EU Directive and BSQR has been a temporary derogation on donor Hb and post-flu deferral which has now expired).

In addition to the Red Book Guidelines for Blood Services, JPAC sets guidelines on selection of donors of blood, tissues, stem cells and cord blood, and produces the Handbook of Transfusion Medicine which outlines clinical use of blood.

All guidelines can be found on [www.transfusionguidelines.org.uk](http://www.transfusionguidelines.org.uk).

## Pandemic Planning and Influenza – What Have We Learned from H1N1?

### Seed C <sup>1</sup>

<sup>1</sup> Australian Red Cross Blood Service

In April 2009 a new H1N1 influenza virus of swine origin emerged resulting in the first influenza pandemic since 1968. As of July 2010 the WHO reports that worldwide more than 214 countries have reported laboratory confirmed cases of pandemic influenza H1N1 2009, including over 18,000 deaths. This is the first influenza pandemic since the inception of dedicated response plans, which provides the opportunity to assess their efficacy.

The 'we' within the question 'what have we learned from H1N1' varies by perspective. Globally, perhaps the most important lesson was that the WHO response plan did not adequately address a pandemic virus with low virulence, like H1N1 2009. Planning had understandably focused on the 'worst case' scenario, a virus similar to the 1918-1919 pandemic strain with a high case fatality rate (>1%). Ultimately the lack of 'flexibility' to address the milder clinical outcome of the H1N1 virus led to criticism of global plan, particularly that the trigger point to level 6 (full pandemic) was premature and ultimately unnecessary for such a virus.

From an Australian population perspective the national pandemic plan was similarly limited in this capacity although there were 'real time' adjustments in an attempt to compensate. This was a valuable lesson that resulted in the addition of a new phase with a focus on treating and caring for patients at higher risk of severe outcomes. Generally, though, the effects of the pandemic were mild and comparable to a moderate seasonal flu period. Workplace and school absenteeism rates were similar to those observed in 2007. The 191 deaths associated with H1N1 infection (0.01% mortality) was markedly lower than the 3,000 estimated annual deaths in Australia attributed to seasonal flu. Other valuable lessons included;

Reliance on costly anti-viral stockpiles was misplaced, given their use had no obvious impact in slowing or altering the overall pandemic

The finding that pregnant women with complications were often IgG-2-deficient suggests a possible avenue for identifying high risk individuals as well as potential therapeutic options

From an Australian Blood Service perspective the impact of the pandemic has thankfully been mild and no greater than that observed during a moderate influenza season. Nevertheless valuable lessons emerged; not the least that the existence of a comprehensive dedicated response plan linked to the public health response was valuable. Other key lessons included;

The 'mild' nature of H1N1 2009 highlighted the need to incorporate viral 'severity' assessment within our plan's response trigger points

Involvement in international blood service 'alliances' was pivotal in effective pre-planning as well as rapid information sharing, which streamlined the response to H1N1

The value of a highly developed 'surveillance' system for emerging pathogens which allowed rapid assessment of the characteristics of the virus and informed the response

Minimising cross infection within facilities using simple infection control measures (e.g. hand hygiene, cough etiquette) and social distancing reduced the overall impact to donors and staff

The logistics of providing 'convalescent' plasma and IVIg (or a hyperimmune Ig) for therapeutic use were explored and may provide a model for future use

Further evaluation of and research into the potential impacts on patients, staff and blood inventory management, are required as priorities, to address severe shortages predicted during a more severe pandemic, including wider awareness and uptake of the National Blood Supply Contingency Plan by health services and clinicians

Undoubtedly the mild nature of the 2009 pandemic will lead some to question the value of developing dedicated response plans. However history has taught us that those 'that fail to plan, plan to fail'!

## How to Improve Evidence Base for Transfusion Medicine

**Murphy M**<sup>1</sup>

<sup>1</sup> *NHS Blood & Transplant, Oxford Radcliffe Hospitals and the University of Oxford*

There are considerable data that current transfusion practice varies widely between different hospitals and clinical teams. Clinicians may not be aware of guidelines of good practice or may be reluctant to follow them because of perceived weaknesses in the evidence-base for their recommendations.

Transfusion medicine is no different to many other clinical specialties in that the evidence base for much practice has not developed to the point at which it can be universally applied with confidence. The lack of a strong evidence base should be seen as a specific challenge to identify transfusion issues that are high priority for further research. Success in meeting this challenge is essential both for the safety of patients and the effective use of an increasingly scarce resource.

Further development of the evidence base for transfusion medicine requires:

New systematic reviews of randomised controlled trials (RCTs) where the topic has not already been systematically reviewed. Broad overviews of RCTs may be a useful tool as a prelude to specific systematic reviews.

Critical assessment of observational evidence on topics where RCTs are absent or are small and inconclusive.

Adequately powered RCTs performed to high standard. These may need to be preceded by surveys, audits and pilot RCTs.

Cost effectiveness studies.

Research further evaluating and developing the effectiveness of methods for changing clinicians' practice.

Given the common themes but limited resources for blood services worldwide, there is a need for more international interaction and collaboration. A number of international research groups and organisations are undertaking or interested in undertaking evidence based medicine projects in transfusion medicine. Increased collaboration between them should be pursued to develop the transfusion medicine evidence base, avoid duplication, increase opportunities to disseminate the work that has already been done, and plan future research.

## **Patient Blood Management Guidelines – An Update**

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<sup>1</sup> *Australian & New Zealand Society of Blood Transfusion representatives Clinical Reference Group, Patient Blood Management Guidelines*

A comprehensive review and update of the 2001 Clinical Practice Guidelines for the use of Blood Components is currently underway steered by the National Health and Medical Research Council (NHMRC), Australia New Zealand Society of Blood Transfusion (ANZSBT) and National Blood Authority (NBA).

The guidelines have a clinical rather than blood product focus. A series of six modules of evidence based Patient Blood Management Guidelines will be progressively developed: critical bleeding/massive transfusion, peri-operative (elective surgery), critical care, medical, obstetric and paediatric/neonatal populations. Patient blood management optimises the use of donor blood and reduces transfusion associated risk.

An Expert Working Group, which included representation from clinical Colleges and Societies, defined the scope of the new Guidelines and constructed six generic questions, to be applied to each module's population. These questions included whether anaemia is an independent risk factor for adverse outcomes, the effect of transfusion of red cells and components, the thresholds at which blood components should be transfused and the use of non transfusion measures to improve haemoglobin. In addition, a number of specific questions for each module's population will also be addressed.

Using the formulated research protocol, systematic reviews of the relevant literature are being undertaken with the results synthesised by a Clinical/Consumer Reference Group (CRG) to produce a series of Evidence Statements and Evidence-based Recommendations to guide clinical practice. In many situations where guidance is necessary, good quality evidence has been found to be lacking. In these situations, Practice Points, based upon consensus CRG opinion, are developed. An NHMRC Guidelines Assessment Register ensures the systematic review and processes comply with NHMRC standards. A comprehensive communication strategy has been developed to ensure that the clinical community is kept informed and involved in the Guideline development and to facilitate dissemination and implementation.

Due to the scope and extent of the work, the development process is necessarily long. The Critical Bleeding/Massive Transfusion module is pending final approval by the NHMRC, the draft Perioperative module is shortly to be released for public consultation and the systematic literature review for the Intensive Care and Medical modules has just begun.

Aspects of the content and development process of the initial two Guideline modules will be discussed.



## **An ABC of Red Cell Genotyping**

### **Flower R <sup>1</sup>**

<sup>1</sup>*Research and Business Development, Australian Red Cross Blood Service*

The mechanism by which red cell antigens have been studied and defined has been agglutination testing. This technique has been applied to define immunogenic polymorphisms in red blood cell surface molecules. Molecular genotyping is possible because the genetic polymorphisms producing these red cell antigens are effectively all known. Most of these are single nucleotide polymorphisms (SNPs), usually from point mutations that produce a single defined antigenic change. When the mutation is inactivating and the protein is not expressed, a null phenotype results. A further level of antigenic complexity arises as a consequence of recombination between chromosomes, sometimes resulting in expression of a normally silent pseudo-exon through splice site re-activation. Design of systems for red cell genotyping should include consideration of the convenience and sensitivity of amplification and analytical techniques. In recent years, the focus has moved to real-time techniques to minimise post-amplification manipulation. Consideration of complications specific to genotype analysis should be a component of the design of testing algorithms. The same serological phenotype can be produced by different genetic changes (Rh(D) neg: deletion vs the “African” insertion). Mutations may suppress antigen expression (the GATA box mutation in the Duffy system). Failure to detect new mutations, in rare circumstances, may result in failure to predict the phenotype. To obtain reliable results process design and interpretation procedures must be optimal. Large scale application of genotyping raises new technical challenges, such as a high throughput DNA extraction. Standardisation of molecular genetic terminology also remains a significant issue, as naming of every allele, including silent and intronic variants, may make reporting complex. Systematic investigation based on genotyping has the potential to improve transfusion practice by concurrent typing of a full profile of red cell SNPs in problem investigations, plus reliable typing of Direct Antiglobulin Test-positive patients and multi-transfused patients. In addition, the identification of donors with rare genotypes, using relatively low cost high throughput multiplex screening, has the potential to renew the dwindling supplies of blood available for patients with rare blood types.

## Clinical Applications of DNA Technology

Poole J<sup>1</sup>

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The molecular mechanisms responsible for blood group polymorphisms are diverse. The majority result from single nucleotide polymorphisms (SNP's) which encode amino acid substitutions either in the extracellular membrane domains of red cell proteins and glycoproteins (eg Rh, Kell, MNS) or in transferase enzymes that catalyse the synthesis of carbohydrate antigens (eg ABO). Other mechanisms include gene deletion, nucleotide deletion, sequence duplication, nonsense mutation, intergenic recombination between closely linked genes giving rise to hybrid proteins and SNP's in the promoter region of a blood group gene. Null phenotypes, in which all antigens of a system are absent from the red cells, and are usually rare, may result from homozygosity for inactivating mutations within the gene or deletion of the gene.

Determining blood groups using DNA needs to be approached with a certain amount of caution since there are several instances where genotype does not reflect the phenotype. The most common and valuable application of molecular blood group genotyping is for predicting the fetal D type in pregnant D-negative women with anti-D. Knowledge of the fetal D type reveals whether there is a risk of haemolytic disease of the fetus and newborn and allows appropriate management of the pregnancy. Fetal genotyping is most often carried out for D but sometimes for C, c, E and K and rarely for others such as Fy<sup>a</sup>. Fetal D, C, c, E and K typing can be predicted reliably from cell-free fetal DNA in the maternal plasma, a non-invasive procedure. Other clinical applications include: Extended genotyping of transfusion-dependent patients, who often make multiple antibodies, ideally before they receive their first transfusion; recently transfused patients; patients with a positive DAT; donor screening to supply antigen matched blood; defining RhD variants (weak D and partial D); paternal *RHD* zygosity testing; preimplantation genetic diagnosis and *in vitro* fertilisation; elucidating difficult serological red cell reference investigations.

## New Technologies for Genotyping

### Hyland C<sup>1</sup>

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Traditionally research discoveries herald a lag phase prior to technology development and changes. Over the last 20 years the molecular basis for the 30 defined blood group systems representing over 200 blood group antigens have been defined. The aim of this talk will be to review advances in technologies for molecular typing blood groups. The thesis is that technologies for molecular typing can provide improvement in patient management and provide for more efficient management for blood stocks. The focus will include:

Status for fetal *RHD* blood group gene assessments

Status for Donor and Patient blood group typing in general

It is noted that molecular typing detects a gene sequence but cannot indicate whether the gene is expressed. It is therefore an adjunct to serological techniques which remain the gold standard for assessing the phenotype.

Prenatal fetal *RHD* typing is a model for reviewing developments as molecular typing has shown clinical utility and developed over 17 years ago from:

Invasive sampling to test genomic DNA using PCR methods

Non-invasive prenatal assessment using real time PCR

Potential for high throughput platforms e.g. mass spectrometry

Non-invasive fetal *RHD* using maternal blood samples is in a special category

as the circulating cell free fetal DNA is a small size and in low concentration. The licensing for the IP rights to cfDNA testing also requires consideration. High throughput technologies for screening for fetal *RHD* in Rh D negative women in the future may improve management and usage of anti-D supplies.

For donor and patient typing RhD is also a model for technology roles e.g.

PCR applications for gross detection of *RHD* gene

DNA chip platforms with CE accreditation for defining variants

Sequencing technologies

Specialised laboratories use these platforms to characterize/detect donors and or patients with weak RhD antigen expression difficult to detect by serology but of potential clinical significance.

DNA chip molecular typing platforms are used in combination with sophisticated bio-informatic systems and provide a comprehensive blood group genotyping profile and phenotypic assessment. This can facilitate provision of extended antigen matched blood for patient groups with ongoing transfusion needs such as sickle cell patients.

In conclusion technology developments based on research, defining the molecular basis for the complex array of blood group antigens, have further potential to improve usage and optimise management of blood stocks.

