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Top 2 Abstracts

Novel Web-Based Real-time Dashboard to Optimise Recycling and Use of Red Cell Units at a Large Multi-site Transfusion Service Administration – Oral Plenary

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Don Doiron, Capital District Health Authority

Bryan Crocker, Capital District Health Authority

Craig Winsler, Capital District Health Authority

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Irene Sadek, Capital District Health Authority; Dalhousie University

Calvino Cheng MD, FRCPC, Capital District Health Authority; Dalhousie University

Background: Effective blood inventory management reduces wastage of blood products. At Capital District Health Authority (CDHA), we designed an automated real-time web-based dashboard interfaced to our laboratory information system (LIS) to more effectively recycle units between our sites without requiring addition of human resources. This novel and unique low-overhead methodology has not been described in the literature to date, and has been successful in improving RBC outdate rates.

Methods: The dashboard was deployed August 2011. It is interfaced to the LIS (Cerner Millennium), and is located on the secure and auditable CDHA intranet webserver. It is accessed by a hyperlink/shortcut on the desktop of on all desktop computers in all BTS sites in the CDHA region on a 24/7 basis, refreshed every 10 minutes automatically. Units that are nearing expiry are displayed, along with status, location, other attributes (i.e. CMV, phenotyping) using red, yellow, green color coding. During evaluation, expiry rates and technologist time savings and usage patterns were observed.

Results: Overall RBC outdate rates in the 4 month period following implementation (Sept-Dec 2011) averaged 0.89% (49outdated/5521received), compared to similar periods in 2010 and 2009: 99/5494(1.8%) and 133/4999(2.7%) - 50%/67% reduced vs. 2010/2009 respectively; O negative discards were 0.036%(2/5521) vs. 0.73%(40/5459) and 1.04%(52/4999) - 95%/96% reduced vs. periods in 2010/2009. Annualized projected cost savings ranges from \$67500/\$113400 vs. 2010/2009 rates at \$450/unit. There has been no difference to technologist workflow at RBC issuance; technologists use the dashboard to choose the next oldest unit from their inventory, release crossmatched units prior to them becoming too old to re-allocate, and pre-emptively transfer units near expiry. Serendipitously, it has been used to audit non-returned transfusion tags on issued units, increasing documentation timeliness and improving administrative workflow.

Conclusion: Our dashboard solution to the RBC recycling problem has been a low-overhead and effective method whereby our multi-site institution has significantly reduced RBC wastage rates. It is possible to apply this to other institutions.

Osteoblast Conditioned Media Improves the Expansion of Progenitor Cells Ex Vivo and Subsequent Platelets Recovery
Scientific - Oral Plenary

Nicholas Pineault PhD, Héma-Québec
Lucie Boyer MSc, Héma-Québec
Nellie Dumont MSc, Héma-Québec
Hélène Émond BSc, Héma-Québec
Betül Çelebi PhD, Héma-Québec
Diego Montovani PhD, Laval University
Denis-Claude Roy MD, PhD, HMR

Expansion of hematopoietic stem and progenitor cells (HSPC) is investigated as a cellular therapy to improve the engraftment and recovery of neutrophils and platelets in patients undergoing HSPC transplantation. We showed that co-culture of cord blood CD34+ cells with mesenchymal stem cells (MSC) or osteoblasts (OST) without contact improved the expansion of myeloid and megakaryocyte (Mk) progenitors (Çelebi et al., JIM, 2011). The objectives herein were to determine whether conditioned media (CM) could recapitulate these effects, and determine which between MSC and OST is most advantageous for the expansion of HSPCs and subsequent hematopoietic recovery in vivo. Cell growth was strongly promoted in OST CM. Indeed, the net expansion of nucleated cells (2-fold), CD34+ cells (2.6-fold) and clonogenic myeloid progenitors (2.2-fold) were all found significantly greater ($p < 0.05$) in OST CM than that seen in MSC CM or any other cultures or co-cultures. However, a 30% reduction ($p < 0.05$) in the expansion of Mk progenitors was measured vs control but incubation under mild hyperthermia (MH, 39C) restored their level of expansion ($p < 0.03$). Next, we analysed the levels of human platelets (hPLT) in NSG mice transplanted with CD34+ cells expanded under MH in CMs (OST or MSC) or in control medium. hPLT levels 5 days post-transplant (PT) were 2-fold greater in mice injected with the control cultures ($p < 0.05$). However, hPLT recovery was 3- and 4-fold greater in OST mice 13 and 20 days PT, respectively ($p < 0.01$). In conclusion, OST CM supports greater HSPC expansion ex vivo that translated into improved overall hPLT recovery.

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Administration

A Short Retrospective Review for Washed Red Cells Usage at Capital Health District, Halifax, Nova Scotia

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Robert Liwski MD, PhD, Dalhousie University; Capital District Health Authority (CDHA)
Dietrich Werner MD, Dalhousie University; Capital District Health Authority (CDHA)
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Allam Shawwa MB BCh, Dalhousie University; Capital District Health Authority (CDHA)
Eiad Kahwash MD, Dalhousie University; Capital District Health Authority (CDHA)

Background: Washed and IgA-deficient products have been used in our institution per clinician's request for IgA deficient patients and other indications to prevent anaphylactic reactions. This usage has not been closely monitored by the transfusion medicine services. Severe IgA deficiency is now defined as <0.05 mg/dl which is much lower than the lowest level can be detected by our laboratory of 2-4 mg/dl.

Method: We reviewed our washed red cell usage at CDHA hospitals between January 2009 and March 2011. Then, we compared it to the usage from April 2011 when a new policy for the indications for washed blood products was implemented to Feb 2012. The number of washed units requested and transfused was documented as well as the number of patients and the clinical indications.

Results: During the pre-policy period 95 washed units were ordered for 8 different patients, out of these only 74 were transfused. One patient with beta-thalassemia major and a history of refractory transfusion reactions received 54 units. Only 4 units were transfused to 2 truly IgA-deficient with anti-IgA antibodies. A patient with recurrent transfusion reactions received 2 units. Eleven washed units were transfused to 4 patients, who were identified as IgA-deficient by laboratory. Three other patients received the units to avoid wasting them.

A new policy implemented on April 1st 2011, recommended that those who are identified in our system as IgA deficient by the less sensitive method only with no confirmation of level <0.05 mg/dl and no history of transfusion reactions, will get regular unwashed components. Following the implementation of this policy until February 2012, the number of patients' required washed products was only two with 29 washed units transfused. The chronically transfused patient with beta-thalassemia major consumed 26 units and the rest were transfused to a single new patient with critically low IgA level and with anti- IgA antibodies.

Conclusion: Appropriate monitoring and adherence to clear institutional policy would significantly decrease undesired utilization of washed red cells.

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Comparing C1-Esterase Inhibitor Distribution Between British Columbia and Alberta, and Assessing Impact of Product Licensure

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Purpose: C1-esterase inhibitor concentrate (Berinert®) is a human plasma-derived product, manufactured by CSL Behring and licensed by Health Canada in January 2011 for treatment of moderate/severe attacks of hereditary angioedema (HAE). Before then, it was available through Health Canada’s Special Access Program (SAP). Using Canadian Blood Services (CBS) product distribution data, we compare per capita distribution of Berinert® in Alberta and BC. We also examine the potential impact of Health Canada’s licensure of Berinert® on volume of product distributed by comparing distribution data in BC for periods immediately preceding and following the January 2011 licensure.

Methods: Berinert® distribution data from CBS blood centres in BC and Alberta were obtained from 1 Sep 2010 to 31 Aug 2011. Berinert® distribution data from CBS to hospitals in BC were compared between 1 Apr-31 Dec 2010 and 1 Feb-30 Sep 2011. January 2011, the month of licensure, was excluded. Population data were obtained from provincial Vital Statistics.

Results: Over the study period, BC/Yukon Centre distributed 770 vials (approximately 1 vial per 5,900 population) of Berinert® (500 IU per vial), compared with 5,753 vials (approximately 1 vial per 630 population) distributed from Edmonton and Calgary Centres. The distribution rate in AB was 9.4 times higher than in BC. From Apr-Dec 2010 (pre-licensure), a mean 54.3 units per month of Berinert® were distributed from BC/Yukon Centre, compared with a mean 69.4 units per month Feb –Sep 2011 (post-licensure) - a 27.7% increase.

Discussion: There is a 9-fold difference in per capita distribution of Berinert® between BC and Alberta. Data do not address the number of HAE patients or interprovincial transfer of patients/product. The difference seems unlikely to be explained on the basis of differing population prevalence of HAE. Further investigation is planned to elucidate the reason for the apparent difference. The reasons for the observed 27% average monthly increase in product distribution in BC between periods immediately preceding and following Berinert® licensure in Canada may reflect increased awareness and easier accessibility of the product to physician prescribers.

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Utilization of O-positive Blood in Uncrossmatched Adult Male Transfusion, the Impact on O-negative Blood Conservation

Oral Presentation

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Purpose: To reduce shortages of O Negative blood by stocking both O Negative and O Positive unmatched red cells in an Emergency Room fridge without posing a safety risk to Rh Negative women of child bearing age.

Method: Keeping an adequate supply of O Negative red cells is a constant struggle, which led us to investigate ways to conserve O Negative blood without compromising patient care. In consultation with Human Factors specialists, the inventory of 6 O Negative unmatched red cells stored in an ER fridge was changed to provide 6 units of O Positive uncrossmatched red cells for male trauma patients and 6 O Negative uncrossmatched red cells for female trauma patients. Colour coding of labels, shelves and tracking sheets, as well as a physical separation of the two products, and use of easily recognized gender symbols were utilized to prevent accidental transfusion of Rh Positive units to females during uncrossmatched transfusion.

Results: From implementation in May 2011 to January 2012, 87 patients were transfused 233 units of unmatched red cells. Of these, 56 were males who received a total of 152 units of O Positive blood. One unit of O Positive blood was inadvertently transfused to a female who was of unknown gender at the time of transfusion representing a selection error rate of 0.4%. Of the males who received O Positive blood, 5 were Rh Negative. In follow-up testing, 2 had developed an Anti-D, one was deceased, and one failed to follow up. Review of the patient's transfusion history revealed those who received only one or two units of red cells in total developed anti-D while those who received more than two units did not form an anti-D.

Conclusion: The stocking of both O Negative and O Positive unmatched blood appears to be a successful way of conserving O Negative red cells, allowing us to save over 150 units of O Negative red cells over 9 months. Utilizing colour coding, gender symbols on shelf labels, and shelf separation has been an effective way of decreasing the risk posed to Rh Negative women of child bearing age.

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Notes:

“A Toolkit for the Introduction of a New Blood Product”

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Background: Hospital transfusion services are regularly faced with introducing new blood products. This is a complicated process. For example, at the large centres, hundreds of staff might require training, and at smaller sites, there might not be the resources or expertise to respond to the requirements. Part of the mandate of the Ontario Regional Blood Coordinating Network (ORBCoN) is to provide hospital transfusion services with tangible tools that support best practices in transfusion service delivery.

Methods: A working group was formed; membership included a transfusion medicine physician, a CBS representative, a community hospital pathologist, a blood conservation nurse coordinator, a transfusion safety officer and a senior technologist. The resultant toolkit explains the sequence of events and tasks leading up to introduction of the new product, and included examples of task checklists. The working group provided input by email and by teleconference.

Results: Three sections were created: “What Information Should We Gather Before the Blood Product is Introduced?”, “What Should we Do Before the Blood Product is Introduced?” and “What Should We Do After the Blood Product is Introduced?” to sequentially model the tasks and discussions that should occur when implementing a new blood product. The Implementation Guidance Checklists allow users to track their progress and avoid the inadvertent omission of critical tasks. The importance of linking the toolkit’s aids with the specific organization’s requirements, policies, procedures and governance is stressed. The final toolkit contains thirteen pages of information and nine pages of appendices.

Conclusion: Diverse input was sought in creating this toolkit to provide a multi-discipline perspective. “A Toolkit for the Introduction of a New Blood Product” provides hospitals with valuable information for implementing a new blood product. The toolkit is available in hard copy, as well as on-line. Customizable checklists are posted to the Transfusion Ontario website. User satisfaction will be measured in a future ORBCoN hospital survey.

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Notes:

LEAN Inventory Management - A Saskatchewan Success

Oral Presentation

Kathleen Handford BA, BSW, MEd, Saskatchewan Ministry of Health
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In 2008/09, Saskatchewan had the highest per capita use of red blood cells in Canada - 25% higher than the national average. There are geographic and transportation challenges to maintaining safe and efficient transfusion practice in the province, given that patients are transfused in 78 different facilities, with red cell inventory held in 36 facilities. Many of these sites transfuse less than 50 units per year. However, there were additional concerns about non-standard use (e.g., unique use patterns, inappropriate dosing) and unnecessary wastage (e.g., outdating, improper storage).

LEAN methodology was applied to the blood inventory management process to identify wastage and opportunities for improvement. Red cell inventory levels were established based on Dr. Taher Rad's and Calgary Laboratory Services' Blood Utilization Management Plan. The recommended red cell inventory levels were seven days in the large urban hospitals and ten days in smaller facilities.

The project was piloted in the Saskatoon and Regina Qu'Appelle Health Regions and is currently being rolled out across the province. The methodology continues to evolve with the provincial roll-out, based on feedback from transfusion service managers in the health regions. This has led to the development of a simple, informative data collection tool to review product inventory, transfusion and ABO substitutions. This is of particular benefit to facilities that do not have a Laboratory Information System. In support of continuous improvement, control charts have also been developed to provide health regions with quarterly reports on their outdate rates based on CBS hospital disposition data.

Saskatchewan has seen an 18% reduction in red blood cell outdate rates over the past two years (6.0% to 4.9%), translating into multi-million dollar savings to the provincial health budget. In 2010/11, its per capita use was 15% higher than the national average. Saskatchewan's platelet discard rates dropped by 49%, from 27.9% in 2010 to 14.3% in 2011. It is anticipated that the red cell discard rates will continue to drop as more health regions adopt the new inventory-setting methodology. It is also anticipated that fresher red cell units will be transfused as inventory levels are better aligned with use, reducing outdates and late-date ABO substitutions (i.e., to avoid wastage of O negative units).



Notes:

A light gray rectangular area containing six horizontal lines for taking notes.

Patient Identification Audit (PIA)

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Katerina Pavenski MD FRCPC, Department of Laboratory Medicine, St. Michael's Hospital, Department of Laboratory Medicine and Pathobiology, University of Toronto

Background: The accurate identification of patients is paramount to ensure that the right patient receives the right care. The consequences of missing or incorrect patient identification may be: non-serious (i.e. requirement for repeat venipuncture for laboratory testing), serious (i.e. unnecessary therapeutic intervention triggered by a result belonging to a different patient), and potentially fatal (i.e. incompatible blood transfusion).

A preliminary survey of 11 of 20 in-patient units was conducted at our hospital. Survey results showed that 82% of respondents encountered armband problems. In addition, 36% of respondents encountered patient armband discrepancies in patient demographics. A small number of wrong identification incidents are reported through our hospital's Event Tracking.

Materials & Methods: Our hospital performed an audit of four medical/surgical units and four critical care units from November 18, 2011 to February 26, 2012. Our audit explored the incidence of missing armbands, illegible armbands and armbands with incorrect demographics.

Results: Out of four medical/surgical units, 75 patients were audited. One patient was missing an armband and 6 (8%) patients had an illegible armband. So far, none of the armbands had incorrect patient demographics. Of the illegible armbands, many were damaged by water or were faded. Of the four critical care units, 52 patients were audited. None of the armbands had incorrect patient demographics. 33% of patients had missing armbands. Patient's identity could not be confirmed as per protocol in 54% of cases (e.g. patient was unconscious) Of the patients with missing armbands, an immediate search by an auditor sometimes revealed that an armband was attached to the patient's bed rail, iv pole or window ledge.

Conclusion: The goal of our audit is to have 100% of patients appropriately identified (e.g. armband with correct information). An analysis of data was performed and a feedback report will be given to units comparing them to the units of similar size and acuity of care.

Post audit intentions include focus groups and stakeholder surveys leading to an education and awareness campaign. A follow up audit will be performed one year after to assess compliance and sustainability of education campaign.

Reducing Errors and Improving Human Performance in the Blood Bank Using Interactive e-Learning

Oral Presentation

Karen MacCollum MLT, BSc(MLS), Alberta Health Services

Tanya McKelvey MLT, BSc(MLS), Alberta Health Services

Filling IVIG orders can be a complex and overwhelming procedure. Many questions regarding authorization and eligibility need to be asked before filling a request. Forms for approval may need to be filled out and selecting the appropriate product based on clinical conditions and/or transfusion history adds to the complexity of each request. What questions to ask, where to find the information and when to fill out approval forms can be overwhelming.

To ensure that IVIG orders are filled correctly, the Edmonton Zone Laboratory Services, Alberta Health Services (AHS) implemented an interactive e-learning module which focused on the procedure for evaluating IVIG orders and product selection. As a result of this initiative, IVIG product selection errors were reduced by 21%.

This presentation will cover the construction, implementation and review of the IVIG e-learning module. The principles and techniques used for this learning module can be applied to many other tasks in the Blood Bank. This module is the first of a series of interactive e-learning modules being implemented in the AHS Edmonton Zone Blood Banks.

There will also be a discussion about the software used to create these modules. There are a number of these programs available and are increasingly easy to use. There are no programming or advanced computer skills required. How to use the software to meet the learning needs of the Blood Bank will be covered.

Come and hear how by implementing easy to use authoring tools and following basic e-learning principles can improve your staff's performance.

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Economic Benefit of Subcutaneous Rapid Push versus Intravenous Immunoglobulin Infusion Therapy in Adult Patients with Primary Immune Deficiency

Adriana Martin RN, Providence Health Care

Louis Lavoie PhD, BioMedCom Consultants Inc., Montreal, Canada

Mireille Goetghebeur PhD, BioMedCom Consultants Inc., Montreal, Canada

Robert Schellenberg MD, Department of Medicine, Cardiovascular and Pulmonary Research, University of British Columbia

Background: Primary immune deficiencies (PID) are genetic disorders resulting in recurrent infections. Immunoglobulin replacement therapy in PID patients can be achieved intravenously (IVIG) or subcutaneously (SCIG) with similar efficacy and safety profiles but with different resource use and associated costs.

Objective: The objective of this study is to evaluate the economic benefit of the rapid push method for SCIG compared to IVIG infusion therapy in PID patients from the healthcare system perspective in the context of the Adult SCIG Home Infusion program based at St-Paul's hospital, Vancouver, Canada.

Materials and Methods: SCIG and IVIG options for immunoglobulin replacement therapy in adult PID patients were compared in a cost-minimization model over three years of treatment. The model focused on direct medical costs for infusion supplies and personnel. A three-year budget impact model assessed the economic impact on the healthcare system of switching from IVIG to SCIG for PID patients of the BC Central Transfusion Registry. Sensitivity analyses were performed for both models to measure the effect of different modalities of IVIG treatment and of the proportion of patients switching from IVIG to SCIG.

Results: The cost-minimization model estimated SCIG treatment cost per patient over three years at \$1978 compared to \$7714 for IVIG, resulting in savings to the healthcare system of \$5736, principally due to reduced hospital personnel costs. This figure varied from \$5035 to \$8739 for different modalities of IVIG therapy. Assuming that 50% of patients who received IVIG switched to SCIG, the budget impact model estimated cost savings for the first three years at \$1,307,894 or 37% of the personnel and supply budget. These figures varied from \$1,148,004 to \$2,453,933, or, 36% to 42% with different modalities of IVIG therapy. If 75% of patients switched to SCIG, these figures reached \$1,961,841 or 56% of total budget.

Conclusion: This study demonstrated that rapid push home-based SCIG was less costly than hospital-based IVIG for immunoglobulin replacement therapy. This approach provides not only a beneficial option from the patient perspective but also results in significant savings to the healthcare system for immunoglobulin replacement therapy in adult PID patients in a Canadian context

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Notes:

IVIg Utilization: Results of a clinical consultative screening program in Rheumatology

Oral Presentation

Arjuna Ponnampalam MD, University of British Columbia, Transfusion Resident

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Kam Shojanian MD, University of British Columbia, Division of Rheumatology

Despite its widespread use in clinical practice, the evidence supporting intravenous immunoglobulin (IVIg) therapy is clearly established only for a handful of clinical entities. Unlabelled use of IVIg is contributing a significant portion to the current overall cost of therapy and creating a financial burden on provincial health care budgets. In British Columbia (BC) IVIg utilization trends mirror that of other provinces and in particular growth is commonly seen in specialty areas of Immunology, Rheumatology, Neurology and Hematology.

In this report we describe a novel clinical consultative screening program administered through a partnership between the BC Provincial Blood Coordinating Office and the University of British Columbia - Division of Rheumatology. Initiated in 2008, this project is a clinical service which provides support to physicians across the province in clinical management of adult patients with rheumatologic conditions for which IVIg therapy is being considered. The program links the requesting physicians with on-call screening Rheumatologists through an online interface that is accessible remotely, allowing timely response to urgent requests. The pre-existing Central Transfusion Registry plays a vital role in monitoring the progress of this consultative service by capturing data on IVIg utilization across the province. In addition to providing consultative support, the program promotes appropriate and evidence based use of IVIg with the goal of reducing inappropriate use through sharing clinical expertise and physician education.

Since the program was initiated in 2008 there has been a reduction in annual rate of growth of IVIg use in Rheumatology from 12% between 2005-2008 to 4% between 2008-2011. Mean grams of IVIg per patient has shown a dramatic decline from 17% pre-program to -2% post. The net projected cost savings over three years (2008-2011), despite an increase in the commercial cost per gram of IVIg, is approximately 4 million dollars (CAD). More importantly it has positively impacted the clinical management of patients through the timely provision of clinical expertise by the screening Rheumatologists. The objective of this report is to outline the operational components of this project, with the goal of providing a template for further use in other jurisdictions and in other medical disciplines that rely on IVIg therapy.

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The BloodTechNet Learning Competition: A Networked Approach to Professional Development for Medical Laboratory Technologists

Shanta Rohse MEd, Canadian Blood Services

Background: The Learning Competition is a non-traditional learning program in which content is co-constructed with learners rather than delivered to them. It was designed to address the learning gaps identified by Medical Laboratory Technologists in a 2010 nationwide survey that revealed their need for developing transferable skills in leadership, project management and communication to adapt to their changing workplaces.

Design: The Learning Competition requires MLTs to form their own networks of practice for professional learning. Rooted in social constructivist pedagogy and frameworks like communities of practice, it asks participants not only to submit innovative learning ideas, but also to drive support and vote for unique and worthy learning projects. Over the course of three months, applicants solicit feedback from the community in order to improve and refine their submissions and develop potential partnerships. In the final week, MLTs vote for their favourite projects; the most popular applicants qualify for \$125,000 fund.

Results: In 2011, seven diverse projects were funded. Proposals were submitted from across Canada, involved small, medium and large budgets, included both structured (organized) and informal learning, and were designed to develop both technical/scientific and transferable skills.

Conclusion: The Learning Competition implements an alternative framework to the traditional teacher-lead classroom, one that is based on social theories of learning and focuses on the role participants play in determining their engagement. It highlights the potential the potential for networked learning in the intersection between professional practice and professional development.

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Notes:

Development of a Records Classification and Retention Schedule at Canadian Blood Services

Lucie Sabourin MLT, Canadian Blood Services
Wayne Erskine PM, Canadian Blood Services
Rosanne Dawson LLB, Canadian Blood Services
Donna Tasker, Canadian Blood Services
Stephanie Dawe CRM, Canadian Blood Services

Purpose: It was identified that Canadian Blood Services' records management program needed to include a corporate-wide retention schedule that adequately defines the lifecycle of our many corporate records.

Background: In 2010, the Records and Information Management Department developed a business case to define the work that was required to address retention of records at Canadian Blood Services. A working group was assembled to establish the policy for the management of records, procedures and the development of retention periods.

Methods: In April 2011, the working group concluded that the classification of records was a necessary prerequisite to assigning the retention periods to the classes of records generated and received at Canadian Blood Services. Under the guidance of a Business Advisory Committee, the scope evolved to include record classification, retention and disposition. Definitions for terms commonly used in a records management program were established. Processes and procedures were developed to determine the elements of the lifecycle of records. Retention periods were based on applicable federal / provincial / territorial legislation, standards and industry practice. Three business units within the organization agreed to participate in a pilot project to validate the processes and procedures developed.

Results: Records and Information Management staff and the Business units successfully validated the processes and procedures to classify and define retention for their records. The Records Classification and Retention Schedule was implemented for the first three groups through the development of Records Series Identifiers that were approved by representatives from Legal, Business Unit, Records and Information Management and Quality Assurance groups where applicable. The series allows the grouping of records with the same description of activities and the same retention period.

Conclusion: The schedule will provide a single source where all retention periods are defined and easily managed by staff. It will ensure that records are appropriately retained and disposed when they are no longer needed.

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The 2011 Experience with Red Cell Genotyping at the Immunohematology Reference Laboratory

Robert Skeate MD, MS, Canadian Blood Services
Jacqueline Cote MLT, Canadian Blood Services
Mindy Goldman MD, FRCPC, Canadian Blood Services
Debra Lane MD, FRCPC, Canadian Blood Services
Judith Hannnon MD, FRCPC, Canadian Blood Services

Background: Our reference laboratory started referring samples for red cell genotyping to Progenika (Medford, MA, US) in September 2010. This report summarizes the requests and results for samples sent 2011/01/01 – 2011/12/31.

Methods: Progenika’s BLOODchip testing is requested when RHD genotyping is needed and IDCore+ when RHD genotyping is not needed.

Results: We sent 120 samples for genotyping (96 patients and 24 donors). The average turn-around time (TAT) for customers to receive a final report for BLOODchip testing was 20 days (d) (sd = 9d), and for IDCore+ 13d (sd = 7d). TAT for 2 STAT samples was 2d. Of 96 patient samples, 30 were sent for BLOODchip testing: 24 for patients with discrepant D typings (17 prenatal), 4 for D+ patients with new anti-D, and 2 that were subsequently determined to need IDCore+ testing only. Thirteen weak Ds (7 type 1, 3 type 2, 2 type 3, 1 DEL), 8 partial Ds (4 DAR, 1 each DFR, DHK, DVa, DAU-2), and 1 new variant (exon 07, RHD933C>G) were identified. Sixty six of 96 patient samples went for IDCore+ testing. Predicted phenotypes were obtained for all 50 patients with warm autoantibody or recent transfusion. One of 11 sickle cell patients expressed a variant RHCE gene (d(C)ce*s). The 5 rare patient phenotypes confirmed on genotype. Of 24 donor samples, 16 were sent for BLOODchip testing: 12 for discordant D typing (6 identified as D-, 4 as weak D (type 9, 15, 67, and RHD*46C), and 2 as new variants (one exon 7, 1018G>A, and the other exons 5 and 6, 712G>A, 809T>G)), and 4 for D- donors associated with a new anti-D in a recipient (3 genotyped as D- and 1 as DEL). The 8 donor samples sent for IDCore+ to confirm rare phenotypes confirmed on genotype.

Conclusions: Red cell genotyping was useful for both patient and donors testing as a referred out test, so we have implemented IDCore+ testing in our laboratory.

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Notes:

Platelet Web Application Pilot

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Heather Nesrallah, Ontario Regional Blood Coordinating Network (ORBCoN)
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Background: Platelet redistribution between hospitals is a strategy that may: help alleviate regional platelet shortages; allow for the provision of platelets in a timely manner during emergencies; and reduce the platelet expiration rates. To support platelet redistribution, a web application was created in collaboration with an Information Technology Company, Lixar Inc. A six month pilot was initiated to test the application's functionality and to determine the feasibility of facilitating platelet redistribution using a web-based method.

Methods: During the pilot phase, hospitals were asked to use the web application to post daily, either short-dated platelets (<24 hours until expiry) or all of the platelets the site had available for redistribution. Hospitals requiring a platelet product could log into the application and view inventory available for redistribution within their hospital cluster and arrange delivery of the platelet where appropriate. If the desired platelet product was not available within the application, the product would be ordered directly from Canadian Blood Services.

Results: During the 6 month pilot, a total of 1360 platelet products were entered into the web application with 102 platelet products being transferred to another facility. Hospital feedback was obtained during the pilot phase, with the majority of users finding the web application user friendly and the workload minimal. Additional metrics include platelet outdate rates, estimated workload units and potential cost savings.

Conclusion: The introduction of a web-based application to facilitate the redistribution of platelets between two hospital groups has resulted in the transfer of 102 platelet products which may have otherwise been discarded. The web-based application enables hospitals to view available platelet inventory easily, and with minimal additional workload. The feasibility of expanding the web application to other groups of hospitals within the province will be investigated once data analysis is completed.

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Clinical

Apheresis Platelet Donor with Repeat Positive Culture for an Enteric Organism

Ted Alport MD, Canadian Blood Services
Mindy Goldman MD, Canadian Blood Services

Background: Among the steps taken to reduce the risk of bacterial contamination of blood components has been routine cultures of all platelet components. Most positive cultures have been for organisms consistent with skin flora or environmental contaminants, and no underlying pathology has been found in the donor. Rarely, an enteric organism has been isolated from a platelet concentrate. We report a case of a donor with repeat positive culture of an enteric organism.

Case Report: A 62 year old regular apheresis donor (10 donations in the past year) passed all routine screening procedures (including temperature measurement) had a positive BacT/ALERT aerobic culture, identified as E.coli. The unit had not been transfused. When informed of the culture result, the only symptom the donor recalled with a recent mild upper respiratory infection with a hacking, non-productive cough. He was advised to see his family physician and allowed to continue donating following complete resolution of his symptoms. Following four uneventful donations over the next three months he had a second donation with a positive BacT/ALERT aerobic culture and the same organism was isolated. Again, the unit was identified as a culture positive prior to transfusion. On questioning, he again denied any symptoms suggestive of infection and was referred to his family physician for evaluation. The family physician was specifically advised that his patient appeared to be having episodes of transient bacteremia, most likely from a GI source. A barium enema revealed multiple colonic diverticula, although the donor has remained completely asymptomatic. Based on the history of repeat positive cultures for an enteric organism, the donor has been permanently deferred.

Conclusion: This asymptomatic donor may have intermittent bacteremia from diverticular disease. Isolation of the same enteric organism twice should prompt further investigation and donor deferral.

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Fatal, Acute Hemolytic Transfusion Reaction: Real or coincidence?

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Jacob Pendergrast MD, University Health Network, University of Toronto

Christine Cserti MD, University Health Network, University of Toronto

Background: Hemolysis may be classified in as many ways as its numerous etiologies. Massive intravascular hemolysis requires careful and rapid interpretation of diagnostic tests to determine the exact cause and to initiate appropriate treatment.

Case: We present the case of a 57 year old man in whom embolization of the splenic artery for decompression of gastro-esophageal varices was followed by a fall in hemoglobin from 92 to 69 g/L. Nine hours later, and following the onset of a fever, the transfusion of a unit of packed red blood cells (pRBCs) began. Twenty-two minutes later, he developed hypoxic respiratory distress, rigors, tachycardia, and visible hematuria. After completed pRBC administration, a transfusion reaction was reported to the blood bank. The patient died 7 hours later with the cause of death reported as “acute hemolytic transfusion reaction”.

Blood bank investigations revealed grossly hemolyzed samples with hemoglobin 50 g/L. Pre and post transfusion samples revealed consistent, non-discrepant typing (group O, Rh(D)-negative), negative red cell antibody screens; a negative DAT; and a negative Coomb’s phase crossmatch between post-reaction plasma and erythrocytes from the archived pRBC segment. Taken together, immune hemolytic incompatibility towards the pRBC was ruled out. Hours after sampling, the patient’s blood culture revealed gram positive bacilli, speciated six days later as *Clostridium perfringens*. Microbiologic studies of the retrieved pRBC were negative.

Discussion: *Clostridium perfringens* infection may present as an asymptomatic, transient bacteremia, a classic clostridial myonecrosis of gas gangrene, or with intravascular hemolysis which is a life-threatening feature of *C. perfringens* sepsis. *C. perfringens* septicemia is uncommon but often fatal with mortality rates reported up to 80%. Risk factors for infection include underlying malignancy, diabetes mellitus and recent abdominal procedures. Mechanisms of *C. perfringens* intravascular hemolysis include alpha-toxin induced red cell membrane damage, polyagglutination with exposure of cryptantigen and the acquired B phenomenon with bacterial enzyme polysaccharide modifications.

Conclusion: Septic shock and massive intravascular hemolysis during/despite transfusion support may at first suggest an acute hemolytic transfusion reaction. This case highlights the alternative possibility of *C. perfringens* sepsis, which is uncommon but often fatal. Early recognition and treatment may be life-saving.

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Transfusion Requirements in Elective Cardiac Surgery in a Tertiary Care Center

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Shelley Feenstra RN, Vancouver Coastal Health
Kate Chipperfield MD, Vancouver Coastal Health

Background: Blood product transfusion remains an essential intervention during cardiac surgery (CS). Laboratory workload and product inventory planning require knowledge of average amounts of blood products transfused by type of surgery. Limited international data is published on this topic but no local data is available. We determined the average blood products (PRBC, platelet, plasma) transfused during and within 24 hours after elective cardiac surgery at Vancouver General Hospital (VGH), based on type of procedure. We compared the amount of packed red blood cells (PRBC) issued by slate versus actual transfused PRBC to identify opportunity for efficiency in ordering practice. We questioned correlation of product utilization by fixed and variable pre-operative parameters. Finally, provincial CS product utilization data (BC Cardiac Registry/Central Transfusion Registry Database) were obtained to compare to VGH rates.

Methods: We identified all adult patients who underwent elective cardiac surgeries in VGH over 3 consecutive weeks. A retrospective chart review was carried out, and the following information collected: Demographic data, pre-operative hemoglobin level, type of surgery, time on the cardiopulmonary bypass machine, quantities of PRBC that were requested pre operatively, and the number of transfused units of PRBC, thawed plasma and platelets.

Results: 36 charts were reviewed (mean age was 66.1 years, 75% males). The most frequent type of surgery was isolated coronary artery bypass grafting (CABG), 23/36 (63.9%). During the intra-operative period and within 24 hours post operatively, 19/36 (53%), 13/36 (36%) and 17/36 (47%) patients received PRBC, platelets and plasma transfusions respectively. In 30 cases (83%), more units of PRBC were ordered per slate than what was eventually transfused. Correlation analysis revealed no significant correlation between age, gender, preoperative hemoglobin level, or duration of cardiopulmonary bypass with quantity of transfused PRBC. VGH CS transfusion data will be compared with provincial data upon the availability of the latter.

Conclusion: Although ≥ 2 units PRBC were ordered for every elective cardiac surgery patient in our patient series, only half of the patients required transfusion of PRBC peri-operatively. Age, gender, type of surgery, and pre-operative hemoglobin do not correlate with PRBC transfusion in this small series.

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Timeline for Implemented Changes By Canadian Blood Services to the Blood Products Provided.

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Julie Di Tomasso MLT, BA, Hamilton Health Sciences, Hamilton Regional Laboratory Medicine Program and McMaster University

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Nancy Heddle MSc, FCSMLS(D), McMaster University, Department of Medicine, McMaster Transfusion Research Program

Background: Canadian Blood Services (CBS) was established in September of 1998 after the reorganization of the Canadian blood system. The utmost concern of CBS is the safety of the blood supply and as such has implemented numerous changes from 1998 to present day to improve safety and availability of inventories. When looking back it is often difficult to pinpoint when an initiative was implemented. Knowing such timelines of implementation dates of various initiatives from the blood supplier is valuable when coupled with hospital procedure changes to possibly identify reasons for changes seen at the patient level. This abstract will provide a timeline of the changes over the past 13 years by the blood supplier (phase I of the project).

Method: To prepare this timeline a variety of materials were used including hospital customer letters and updates provided by CBS, literature reviews and bulletins. Each initiative was positioned on a timeline spanning 13 years using the month and year of implementation.

Results: To-date this review has identified 78 initiatives. On a yearly basis the median initiatives was 3.5, with a minimum number of one initiative (years 1998, 2006) with a maximum of 17 initiatives implemented in 2008. The reviewers categorized all initiatives spanning 1998-to date into the following: 6 (7.7%) facility; 10 (12.8%) general; 10 (12.8%) testing; 11 (14.1%) transmissible disease screening; 41 (52.6%) operations related changes.

Conclusion: Just over a quarter of these initiatives are related specifically to testing and over half of the initiatives are categorized as operational improvements of blood manufacturing. Therefore the majority (78.2%) centre on improving the manufacturing and safety of the blood supply consistent with the mission of CBS. 7% have been facility change initiatives. This comprehensive timeline from the blood supplier is phase I of this project. Phase II's objective will be to identify the policy and procedural changes at our local hospitals to assist in understanding utilization patterns and patient outcomes in the clinical setting.

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Transfusion-transmitted S. Aureus Infection from a Platelet Concentrate with “False-negative” Bacterial Culture Results

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Kate Chipperfield MD, Vancouver General Hospital
Arjuna Ponnampalam MD, Canadian Blood Services
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Gershon Grove MD, Canadian Blood Services

This report describes a case of transfusion transmitted (TT)-S. aureus associated with a Buffy Coat Platelet Concentrate (BC PC) which had tested “false negative” by routine donor platelet bacterial culture testing. A 60 year old female patient with leukemia-associated thrombocytopenia was transfused a unit of BC PC at a Vancouver hospital in Oct 2011. The patient experienced a febrile reaction, but no other signs or symptoms of sepsis were reported. Nonetheless, follow-up specimens from the patient and BC PC bag were collected for culture. S. aureus was recovered from both sources. Antibigrams of the 2 isolates were identical and follow-up molecular typing showed an identical A1 pattern. The patient was managed per protocol for febrile neutropenia and discharged 10 days later. A pre-transfusion blood culture on the patient had been negative. Investigation confirmed that bacterial culture test results on the implicated BC PC, using the Bact/ALERT 3D assay, remained negative until the completion of 6 days incubation. All 4 donors associated with the implicated BC PC were followed up. Two donors reported no illness or risk factors for asymptomatic bacteremia, including recent dental work, surgery, or skin injury around the time of donation. One donor reported dental work (a filling replacement) the day prior to donation, although no symptoms of illness. Furthermore, S. aureus is an uncommon constituent of oral flora, dental procedures are rarely associated with S. aureus bacteremia and epidemiologic studies of S. aureus bacteremia do not consider recent prior dental work as a risk factor. The fourth donor reported having a flu-like illness more than 2 weeks prior to donation, for which antibiotics were taken. Follow-up skin and nasal culture tests were obtained on all but the fourth donor, and were negative for S. aureus. The most likely source of S. aureus contaminant was nonetheless still considered to be skin flora from one of the 4 donors at the time of collection. This case highlights a low but ongoing risk of TT-bacterial infections, particularly associated with platelet products, despite existing risk reduction strategies including effective skin disinfection techniques, initial aliquot diversion, and aerobic bacterial culture testing.

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Analysis of Prothrombin Complex Concentrate Beriplex® Usage for the Rapid Reversal of Oral Anticoagulation

Oral Presentation

Hakan Buyukdere MD, The Ottawa Hospital

Philip Berardi, The Ottawa Hospital

Shauna Love, The Ottawa Hospital

Doris Neurath, The Ottawa Hospital

Antonio Giulivi, The Ottawa Hospital

Objectives: The aim of this study is to evaluate the efficacy, dosing and safety of Beriplex® in the setting of major bleeding prevention before any procedure or to achieve adequate haemostasis in acutely bleeding patients. We retrospectively investigated the effect of this product on the International Normalized Ratio (INR) and clinical outcome of 47 patients who were treated at the Ottawa Hospital for urgent anticoagulation reversal.

Methods: We analysed 47 patients who received Beriplex® between September 2011 and December 2011. The collected data included individual features, clinical presentation, why they needed rapid reversal of anticoagulation, pre and post treatment coagulation studies, dose of Beriplex® given, and clinical outcome. While we were reviewing the details of each patient we focused our attention on the dosing, response time, efficacy and safety of this product.

Results: For all eligible patients acute bleeding presentation was the most frequent reason for urgent reversal of anticoagulation (n=27), prevention of bleeding before surgery or necessary procedure constituted the remainder (n=20). Overall normalization of INR to values ≤ 1.5 was achieved for the majority of patients (70 %). We observed successful reversal as early as 15 minutes after the Beriplex® administration. The Beriplex® dose ranged from 25 IU/kg to 50 IU/kg (IU of Factor IX) with a standardized infusion rate of 6 ml/min. Of all eligible patients there was one thromboembolic event reported 10 days after the Beriplex® administration and one reported stroke seen 5 days after the Beriplex® administration while bridging to Warfarin. In both incidences patient's underlying comorbidities were responsible for these adverse events rather than the Beriplex®. Six patients presenting with intracranial bleed showed no significant reduction in morbidity or mortality irrespective of post-Beriplex® INR. Patients with mechanical valves had no thromboembolic event after Beriplex® administration. The group who received the highest dose of Beriplex® (50 IU/kg), total of 7 patients, had no thromboembolic events or mortality.

Conclusions: The use of Beriplex® effectively and quickly reversed anticoagulation and allowed patients to have invasive intervention or surgical procedure without any major bleeding or statistically significant adverse events. In conclusion, this retrospective study shows no clinically significant difference between PCC products used in our hospital. Ongoing studies evaluating the safety and efficacy of various PCCs are underway.

The Effectiveness of Low Dose Prothrombin Complex Concentrates

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Marina Hamilton MScN, RN, NSPBCP, NS Provincial Blood Coordinating Program
David Anderson MD, FRCPC, NS Provincial Blood Coordinating Program

Purpose: In 2008, Health Canada licensed Prothrombin Complex Concentrates (PCC) in Canada. The National Advisory Committee on Blood and Blood Products (NAC) provided recommendations for the indications and dosing of this product. Because this product presents a risk of thromboembolism, the dosing recommendations were lower than the product monograph dosing. In 2011, NAC revised their recommendations for the dosing of PCCs, increasing the dose to 80 mL for an INR of 3.1 – 5.0 and 120 mL for an INR greater than 5.0. In reviewing the provincial data for PCC utilization, the NSPBCP revised Nova Scotia’s guideline based on the provincial utilization data results.

Method: Since 2009, the NSPBCP has collected utilization data for PCCs. This data provides patient information - the initial INR, amount of PCC administered, the INR post infusion of the PCC and if subsequent doses were required. The NSPBCP classified each use as appropriate based on the INR, if the patient was on a vitamin K antagonist and if the patient required urgent surgery/invasive procedure within 6 hours or was actively bleeding.

Results: Between January 2009 and December 2011, 351 patients received PCC. The use in 327 patients (90%) was categorized as appropriate. Of the 327 patients, 178 patients (79%) had an initial INR less than 5.0 and the INR corrected to less than or equal to 1.7 after receiving 40 mL PCC. 67 patients presented with an INR greater than or equal to 5.0. Of these patients, 24 (39%) corrected their INR to less than or equal to 1.7 after receiving 40 mL PCC.

Conclusion: PCC 40 mL provides effective treatment for patients who have an INR between 1.7-5.0 and are bleeding or requiring an urgent invasive/surgical procedure. For patients whose INR is greater than 5.0, a higher dose of PCC (80 mL) is more effective in decreasing the INR to below 1.7. NS has implemented a lower PCC dose as it has been effective in lowering the INR in patients requiring reversal of the anticoagulant effect.

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An Unusual Acute Hemolytic Transfusion Reaction

Gwen Clarke MD, Laboratory Medicine, Royal Alexandra Hospital
Kathleen Wong MD, University of Alberta; Alberta Health Services
Susan Nahirniak MD, University of Alberta; Alberta Health Services

A two unit crossmatch was ordered on an 83 year old female with a pre-transfusion hemoglobin of 98g/L. Pretransfusion testing showed her to be B positive with a negative antibody screen. No prior transfusion history was noted. The first of two immediate spin crossmatch compatible units was transfused uneventfully. After transfusion of approximately 200 mL of the second unit the patient became tachycardic, short of breath and hypotensive. The transfusion was stopped and supportive care provided. She recovered quickly and was asymptomatic with a return to normal vital signs by the next day.

Transfusion reaction investigation at the local and a reference lab revealed the clerical check to be correct. The blood group was confirmed. The post transfusion antibody screen was, however, positive along with a positive polyspecific DAT. (The pretransfusion DAT was negative). Antibody investigation revealed an anti Kell antibody. Testing of an eluate from the post transfusion sample was negative. Additional investigations included a CBC which was normal. A rise in creatinine from 116 umol/L pre transfusion to 184 umol/L post transfusion was identified. PT INR and PTT were unchanged pre and post transfusion and fibrinogen was normal on the post transfusion sample. The serum was noted to be tea colored on examination and her LD level was measured at 10 708 U/L (313 - 618 I/L). The bilirubin on this sample was 23 umol/L (0-19 umol/L) and the haptoglobin was normal. The donor unit which was being transfused when clinical symptoms occurred was Kell antigen positive. Review of the pretransfusion antibody screen at a reference lab suggested that a weak positive result may have been present on the pre transfusion sample.

The reaction was interpreted as an acute hemolytic transfusion reaction secondary to anti Kell antibodies. There are several unusual features. The patient's symptoms were relatively minor and short lived (<24 hours) despite a substantial volume of antigen positive rbc transfused; The post transfusion DAT was positive but the eluate tested in the indirect antiglobulin (IAT) reaction phase, was negative. This suggests the possibility of a significant IgM component to the anti Kell antibody. A predominantly IgM anti Kell antibody may also explain the negative initial antibody screen, carried out as an IAT test with screening cells and patient plasma. The LD rose to an extraordinarily high level, consistent with the tea colored plasma and with acute hemolysis, but the haptoglobin on initial testing was within normal range. The very rapid anamnestic response - with a negative pretransfusion antibody screen and a positive post transfusion antibody screen is also very unusual. In this case, while the mechanism is more akin to a delayed hemolytic transfusion reaction, the time course and laboratory features suggest acute hemolysis.

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Anti-Ata Alloimmunization in a Twin Pregnancy

Jacqueline Cote, National Immunohematology Reference Laboratory, Canadian Blood Services
Sherry Pigeau MLT, Canadian Blood Services
Lisa Campeau-Fleury MLT, Health Sciences North
Mindy Goldman MD, Canadian Blood Services

Background: The first anti-Ata was described in 1967 by Applewhaite et al. Ata is in the 901 series of high incidence antigens (ISBT number 901.003), with a frequency of occurrence of greater than 99% in all populations. The At(a-) phenotype has only been detected in the Black population, and has been associated with rare cases of mild hemolytic disease of the fetus and newborn (HDFN). We report a case of allo anti-Ata in a 33 yr. old multiparous (para 4 gravida 3) Black African female originally from the Congo. She presented at 27 weeks gestation for delivery of twins by caesarean section and was found to have an antibody reactive against all panel cells.

Methods: An antibody identification panel and autocontrol were performed by solid phase PEG-IAT and with ficin, papain and DTT treated cells. A full phenotype of the major clinically significant red cell antigens was performed on the mother's RBCs. Reagent red cells matching her phenotype and negative for high prevalence antigens were tested by the saline indirect antiglobulin test (IAT). DAT testing and acid antibody elution using the Immucor Gamma Elu kit TM Plus were performed on the cord blood samples.

Results: The mother and the babies were all Group O, D positive. The antibody was strongly reactive with all panel RBCs testing by solid phase PEG-IAT, and ficin, papain and DTT treated cells. The autocontrols were negative. An allo anti-Ata was identified in the mother's plasma sample. The DAT on both the cord blood samples was positive (2+). Acid elutions prepared from the cord blood samples demonstrated the presence of anti-Ata on the infants' red cells. Total bilirubin tests performed 48 hours after delivery were 150umol/l and 142umol/l, within the normal range (17-180umol/L). No further treatment was required.

Conclusion: Anti-Ata appeared late in the course of a third pregnancy, and caused a positive DAT but no clinical HDFN in this twin pregnancy.

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The Impact of a Full-Time Transfusion Medicine Medical Director

Karen Dallas BS, MDCM, Saskatoon Health Region
Linda Friesen MLT, Saskatoon Health Region
Lorrie Baryluk BLT, MLT, Saskatoon Health Region

Transfusion Medicine is a complex and cross-cutting area of Medicine which requires both medical and technical expertise; yet until August 2011, Saskatoon Health Region did not have a full-time, dedicated Medical Director for Transfusion Medicine. Since this has changed, both laboratory and clinical knowledge of transfusion medicine is improving and the full spectrum of patient care as it pertains to the use of blood and blood products has been positively affected.

Illustrative examples include

- Education in a variety of settings -- everything from a hospital-wide session on our new Massive Transfusion Protocol which has undoubtedly saved us many thousands of dollars on inappropriate activations to a blood donor advocacy group meeting where certainly many more Saskatonians now understand why donating blood is truly so important.
- Resident physician rotations in Transfusion Medicine have been developed -- both for General Pathology residents and for off-service residents for whom Transfusion Medicine knowledge is indispensable. We now have a waiting list of residents who want to come through the Department!
- "Round-tables" with the Medical Director allowed each technologist to privately express his/her views of and visions for the Department. As a direct result of these roundtables, several individuals' ideas have been acted upon and will benefit the entire Department.
- Our Hospital Transfusion Medicine Committee has been strengthened and now has clinical physicians requesting to be made new members!

In a relatively short time, the Transfusion Medicine Service has transformed itself from a simple provisioner of blood product to an engaging and consultative service within the Hospital. Strong Medical Leadership within the Department has been an instrumental part of this transformation. For hospitals that currently do not have Transfusion Medicine Medical Directors, the case should be made that the addition of this position has the potential to empower and engage, to change practice and save money, and overall, to significantly strengthen the service provided by your Transfusion Medicine Departments.

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Comparative Evaluation of Blood Product Usage & the Design of a Unique Bottom-Up Educational Model to Improve Transfusion Practice in Saskatoon & Saskatchewan

Karen Dallas BS, MDCM, Saskatoon Health Region
Mary Minlock BS, MD, University of Saskatchewan
Heather Panchuk MLT, Saskatoon Health Region

We have examined Saskatoon's Intravenous Immunoglobulin (IVIG) usage data as it compares to National guidelines for IVIG administration and then used this comparative data to develop an Educational Module which focused on a Bottom-Up knowledge dissemination approach and which we hypothesized would serve to change practice as well as provide a foundation for the implementation of IVIG guidelines. We intend to use this approach to educate physicians and implement guidelines for usage of other blood products as well.

In a recent national survey, it was shown that Saskatchewan has significantly higher usage of blood and blood products than other provinces. Blood products are expensive and a reduction in the prescribing of blood products outside of guidelines would result in significant cost-savings. Additionally, transfusion, though seen as safer today than in the past, is not without risk. There are many transfusion reactions/ adverse events which still do occur and can negatively impact patient care. Thus, for both patient safety and economic reasons, this overuse of blood and blood products in Saskatchewan is a major problem that needs to be addressed.

This project sought to analyze the IVIG usage data for the past year in the Saskatoon Hospitals and then to compare that usage data to the Nationally-established guidelines for IVIG indications. We then turned the comparative data into an educational module which we used to provide the groundwork for the hospital-wide implementation of IVIG usage guidelines. In order to work within our particular culture of Medicine here in Saskatchewan, we chose to develop an education module that allowed for a bottom-up approach to knowledge dissemination. Resident physicians and nurses are our primary target audiences for these education modules. During the time period from education to implementation of guidelines, IVIG ordering practice was informally monitored in the blood bank. After the implementation of guidelines, blood bank staff are allowed to release IVIG only for approved indications and for orders falling outside of guidelines, the Medical Director must be called to consult with the ordering physician. IVIG usage data will again be monitored formally to document improvement in practice following implementation of guidelines.

Importantly, while we do not yet have the formal improvement data for IVIG, we do have a unique developed and tested approach to in-house evidence fueled education campaigns which both effects practice change in transfusion medicine and supports the implementation of guidelines. This model will be used to educate physicians and implement guidelines for the use of other blood components both in Saskatoon and in the rest of the province which will result in improved patient care and significant cost-savings. This approach has been a critical piece in quickly engaging physicians from within the system and getting the necessary buy-in for clinical change.

A Case Report of Chagas Disease in a Blood Donor with no Identified Risk

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Purpose: Canadian Blood Services (CBS) began selective blood donor testing for Chagas Disease based on risk questions, in May 2010. Several months later, a Seroprevalence study of ‘no risk’ donors was also undertaken. During the study, one donor was confirmed positive for Chagas Disease.

Case Report: The donor was a 28 year old female who had donated twice prior to this donation. Her sample was confirmed positive by additional ELISA testing and immunoblot carried out by the National Reference Centre for Parasitology (NRCP) at McGill University. PCR (polymerase chain reaction) testing was negative. Additional testing, including the Ortho T.cruzi ELISA test system and RIPA (radioimmuno precipitation) assay (used as the screening and confirmatory tests by most U.S. blood suppliers, was also positive. The donor participated in an extensive questionnaire which identified no risk for Chagas Disease. Subsequently, the donor’s mother who is a nurse, reported to CBS that she had received blood transfusions around the time of her daughter’s delivery, as well as several years earlier. Testing of the donor’s mother confirmed that she too was positive for Chagas Disease. Three of the four donors of the 1983 transfusions to the donor’s mother could be located and they tested negative. Unfortunately there were no records of donors for the five 1978 transfusions. Clinical follow up of donor and mother revealed no signs or symptoms of chronic Chagas Disease. Additional diagnostic testing for Chagas Disease was performed by the NRCP and confirmed the initial results. Follow up testing on the one recipient of this donor (2 donations, 1 RBC issued) was negative.

Conclusions: This is a very interesting case of transfusion transmitted Chagas Disease resulting in vertical transmission to our donor. This case would have probably gone undetected if the patient had not been a blood donor. To date, CBS has tested approximately 70,000 ‘no risk’ donors and only one has confirmed positive for Chagas Disease.



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Hemoglobin Levels and Deferral Rates in CBS Whole Blood Donors

Mindy Goldman MD, Canadian Blood Services, Ottawa
Lori Osmond, Canadian Blood Services, Ottawa
Sheila F O'Brien RN, PhD, Canadian Blood Services, Ottawa

Background: In Sept 2010, CBS implemented a new photometer (DiaSpect, DiaSpect Medical), replacing a 2 step algorithm to measure whole blood donor hb. We assessed the percentage of donors deferred for hb below the minimum requirement of 125g/L, pre and post DiaSpect implementation, and hb distribution post-implementation in 2 CBS regions.

Methods: 1. Deferral rates were extracted from our Progesa database for 12 months pre and post DiaSpect implementation. 2. Post-implementation hb levels in 29,346 NEON and Central Ontario donors were compiled from manual review of donation records. Donation frequency was extracted from the National Epidemiology Database.

Results: 1. Deferral rates decreased from 10.7% to 8.9% for female donors, and from 0.9% to 0.7% for male donors; rates decreased in all regions. 2. Mean hb levels were higher in first time vs repeat donors, particularly for male donors (156.3 vs. 152.1 g/L for males, 137.7 vs. 136.7 g/L for females). For male donors, mean hb levels were 156.3 g/L, 153.6 g/L, and 150.3 g/L in first time donors, donors who had already donated once or twice in the past year, and donors who had donated 3 or more times in the past year, respectively. For female donors, mean hb levels were 137.7 g/L, 137.6 g/L, and 135.6 g/L in first time donors, donors who had already donated once in the past year, and donors who had already donated twice or more in the past year, respectively. Deferral rates are highest in frequent donors, reaching 10.4% in females who had donated twice or more in the past year, compared to 2.3% for first time female donors.

Conclusions: Change in measurement method decreased deferral rates, which may be related to the modified algorithm, new equipment, and/or staff retraining. Preliminary data suggest that hb levels decrease and deferrals increase with intensity of donation. Entry of hb levels in Progesa will permit further analysis and may assist in development of a donor rebooking strategy that would reduce the frequency of development of anemia in donors, leading to decreased deferral rates.

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Safety of INTERCEPT™ Platelet and Plasma Components In Routine Use Assessed by Active Hemovigilance

Melody Holtan, Cerus Corporation
Anne Elliott, Cerus Corporation
Meisa Propst, Cerus Corporation
Laurence Corash MD, Cerus Corporation

Background: The INTERCEPT Blood System™ inactivates pathogens and leukocytes in platelet and plasma components using amotosalen and UVA light. This process has been introduced into routine practice in 100 centers.

Aim: To implement an active hemovigilance program and determine safety outcomes from 41,276 transfusion episodes of INTERCEPT platelet (IBS-PC) and plasma components (IBS-FFP) in routine practice.

Method: 23 Clinical centers in 12 countries using the INTERCEPT Blood System participated in the Cerus active hemovigilance program to determine the frequency of acute transfusion reactions (ATR). Participation required reporting of all transfusions regardless of outcomes. ATR were defined as adverse events “possibly related”, “probably related” or “related” to transfusions. There were no inclusion criteria other than the need for a transfusion.

Results: Data were reported for 13,734 patients during 41,276 transfusion episodes (76,276 components). Transfusion recipients ranged in age from <1-98 years. Primary diagnosis included hematology-oncology (50.5% IBS-PC & 18.0% IBS-FFP); surgery (18.4% IBS-PC+& 36.9% IBS-FFP); and general medical (30.6% IBS-PC & 45.1% IBS-FFP). The mean exposure to IBS-PC was 4.7 components (range 1-156) including 478 more heavily exposed HSCT patients (6.8 PC/ patient). The mean exposure to IBS-PLS was 5.9 components/ patient (range 1-372).

Overall ATR frequency was 0.4% of transfusions and 0.9% of patients. Most ATR were non-serious (Grade 1). The most frequently recorded events were chills, urticaria, rash, pruritus, and pyrexia. ATRs that met the “serious” criteria were infrequent (8 patients; <0.1%) and included allergic, hypotensive, and respiratory symptoms. The “serious” criteria included Grade 2-4 events, classified as fatal, life-threatening, hospitalization, or other important medical events. Two of the cases were considered transfusion associated cardiac overload (TACO). No cases of TRALI, TA-GVHD, or transfusion transmitted infection were reported.

Conclusions: ATR following transfusion of INTERCEPT platelet and plasma components were infrequent and represented the types of events expected following transfusions. Components treated with the INTERCEPT Blood System were well tolerated in broad patient population during routine use.

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Investigating Unlabeled Not Indicated Intravenous Immune Globulin Use in Newfoundland and Labrador

Cheryl Jacobs RN, BN, Provincial Blood Coordinating Program
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Background: The mandate of the Newfoundland and Labrador Provincial Blood Coordinating Program (PBCP) is to provide a safely managed and cost effective Transfusion Medicine Program. In 2010/11 Newfoundland and Labrador was one of the highest users of Intravenous Immune Globulin (IVIG) per capita in Canada. One of the initiatives of the program is to review IVIG utilization practices.

Purpose: To investigate IVIG administered for Unlabeled Not Indicated (UL-N) indications.

Method: The PBCP with the assistance of the Utilization Coordinator and Transfusion Safety Officers in the four Regional Health Authorities reviewed the IVIG request forms to identify UL-N cases to be reviewed. Medical charts were reviewed for additional information regarding the indication and when necessary ordering physicians were contacted to determine and/or verify appropriateness of use.

Results: In 2010/11 there were a total of 223 cases for which IVIG was administered. Twenty -three of these cases were UL-N indications. Further investigation resulted in modification of the initial indication or discontinuation of IVIG, only six of the twenty-three cases remained UL-N.

Conclusion: It is recommended that additional education be provided on IVIG usage and implementation of the Request Approval Process be established throughout the province to facilitate the appropriateness of use.

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Emergency Code for Massive Transfusion

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Background: Mount Sinai Hospital has implemented a hospital wide Emergency Code for Massive Transfusion. The objective is to facilitate and mobilize a coordinated multi-professional team response to a patient who experiences a life threatening hemorrhage requiring rapid access to blood and blood components. The process was developed and implemented by a committee consisting of staff members from the Hospital Risk Management, Blood Transfusion Laboratory, Laboratory Quality Systems Office, the Hospital Communication /Porter Services and interested physicians/practitioners from emergency and critical care areas.

Process: Key elements in this process: (1) A staff physician initiates the emergency code upon recognition of a life-threatening bleeding. (2) The Blood Transfusion Service (BTS) is informed of the code activation before it is announced on the hospital intercom. (2) The charge nurse in the clinical area is assigned to be the communication lead to other departments. (3) A dedicated porter is assigned for the transport of the blood products and specimens between the clinical area and the BTS. (4) Upon receipt of the activation of the code, the BTS will immediately prepare and issue the first pack of pre-determined set of blood/ blood components. The technologist will proactively prepare the next pack and this process will be ongoing until the code is deactivated. (5) The medical head of BTS is informed of the code activation and is available for clinical consultation if required.

Results: The emergency code was implemented in January 2011 and as of December 2011, the code has been activated 18 times (7 times from Obstetrics).

Conclusion: Since the process has been put in practice, the outcomes for the patients experiencing massive bleeding appears to be improved. The attending physicians are more confident in the ongoing management of these patients as the process is well defined. The dedication of the porter significantly reduces the blood products and specimen transport time. The pre-determined set of blood products packs allows the technologists to quickly prepare the products for issue.

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A Protocol to Monitor Hemolysis after Intravenous Immunoglobulin Treatment in Pediatric Patients with Kawasaki Disease

Oral Presentation

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Background: Kawasaki Disease (KD), a childhood disease characterized by high fever, rash and lymphadenopathy, is a systemic vasculitis leading to coronary artery aneurysms in 25% of untreated children. Intravenous immunoglobulin (IVIG) is standard treatment for KD and helps to prevent coronary artery aneurysms. Our hospital used one particular brand of 5% IVIG to treat KD for many years. After the discontinuation of this brand, we sequentially allocated KD patients to one of three 10% IVIG solutions, to assess the safety and efficacy of these preparations.

After observing a number of cases of severe hemolysis after 10% IVIG treatment, we reviewed KD patients treated in the last 4 years for evidence of hemolysis: Hemoglobin (Hb) drop of at least 20g/L, positive Direct Antiglobulin Test (DAT), presence of spherocytes on peripheral blood film. During this review, we discovered that Type and Screen (T&S) was rarely performed on KD patients, and DAT was not done until there was evidence of hemolysis or severe anemia. So in Oct 2011, we implemented a protocol to prospectively monitor the occurrence of hemolysis in KD patients.

Methods: A sample is sent for Type and Screen (T&S) and DAT prior to IVIG infusion. DAT is performed after IVIG infusion. Hb is performed pre and post IVIG infusion.

Results: From Nov 2007 to Oct 2011, T&S was only done in 59/275 (21%) of KD patients. From Oct 2011 to Feb 2012, T&S were performed in 42/43 patients (98%). DAT post-IVIG was performed in 31 patients (10 positive, 21 negative). Mean Hb drop was 15g/L in DAT positive patients and 6.5g/L in DAT negative patients. Positive DAT occurred in 5/8 (62.5%) of Group A patients, 2/2 (100%) of Group AB patients, 3/7 (43%) of Group B patients, and 0 of 14 (0%) of Group O patients. The Hb drop was more pronounced in DAT positive Group AB and B patients than Group A patients (25.5, 16.7, 9.3 g/L respectively).

Conclusions: Non-Group O patients are at risk for IVIG hemolysis. Blood group and DAT results enable physicians to identify those at risk and provide appropriate counseling and post-IVIG monitoring.

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Pediatric Transfusion Related Acute Lung Injury: A case series and review of the literature

Oral Presentation

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Background: Despite the increased recognition of transfusion related acute lung injury (TRALI) as the leading cause of death related to transfusion, information relating to its incidence, pathogenesis as well as diagnosis remains poorly understood. TRALI has been defined as acute respiratory distress with non-cardiogenic pulmonary edema that occurs within six hours of a transfusion. Proposed pathophysiological mechanisms relate to the transfusion of blood components from multiparous or previously transfused blood donors whose plasma contains anti-white blood cell antibodies. The majority of the literature relating to TRALI involves adult patients with only case series or case reports involving pediatric patients. The main objective of this study was to compare the presentation, blood product implicated and outcome between pediatric and adult patients.

Method: A retrospective review of all suspected cases of TRALI submitted to the Canadian Blood Services (CBS) from January 2001 to December 2011 was performed. Data collected included recipient demographics, event details, patient outcomes and type of component transfused. Pediatric patients were defined as children ≤ 18 years of age.

Results: Of the 434 TRALI cases reported to CBS, 155 (36%) were classified as definite or possible TRALI cases. Of these, 145 (94%) were adults and 10 (6%) were children. Regardless of age, surgical or trauma patients were the most likely to experience TRALI. The clinical presentation of TRALI in children was similar to that of adults. The number of TRALI related fatalities was 4 (2.6%) of adults while none of the pediatric patients died. While red blood cells were the most commonly implicated product in adults, mixed products were more commonly implicated in the pediatric cases.

Conclusions: TRALI is a rare diagnosis in pediatric patients. Clinically, children present similarly to adults but may be less likely to die from the illness. TRALI is likely underdiagnosed in children. Pediatricians need to continue to consider this diagnosis in children who experience respiratory distress following a transfusion.

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Notes:

Setting the Age Cut-off for the Transfusion of O Negative Blood for Women in Trauma

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O Rh(D) negative blood, often used in trauma, is a limited resource. Women of childbearing age receive O Rh(D) negative blood if they are Rh(D) negative or of unknown Rh(D) group to prevent alloimmunization and hemolytic disease of the newborn. Our institution defines childbearing potential as an age of less than 45 years, however there is no consensus on what the age cut-off should be. We sought to determine the optimal age cut-off based on institutional data on age of obstetrical delivery and transfusion data for women involved in trauma.

A retrospective audit of women giving birth at Women’s College Hospital and Sunnybrook Health Sciences Centre (SHSC) from 2001 to 2011 was conducted. Age and transfusion data on 203 consecutive women involved in trauma at SHSC from October 30, 2008 to March 31, 2011 was collected. Inclusion criteria was age \geq 17 who received at least one unit of RBCs during hospital admission.

34,734 births occurred between 2001 to 2011. Mean age of delivery was 33.4 years (Range 14-55). 99.4% of women gave birth were less than 45. There was no change in the mean age of delivery between 2001 and 2011.

Data from 203 consecutive women treated for trauma at SHSC from October 2008 to March 2011 were collected. Median age was 48 years (Range, 17-97) . 45% were \leq 45 and 55% were $>$ 45. 10% (21) were Rh(D) negative. 38% of the Rh(D) negative women were \leq 45, while 62% were $>$ 45.

36% of total O negative units were transfused to women involved in trauma above 45 years. No Rh(D) negative women less than 45 years old were transfused Rh(D) positive blood.

At our institution, 99.4% of all births in the past decade occurred in women over the age of 45, validating our age cut-off of 45 for the emergency transfusion of O negative blood for women. 36% of O negative blood was transfused to women above childbearing age involved in trauma, despite our current cut-off of age 45. Further education and investigation is necessary to understand the inappropriate use of O negative blood in trauma patients.

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A Review of “False-reactive” Blood Donors who have Donated at Canadian Blood Services’ Research Clinic

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Purpose: Current Canadian Blood Services (CBS) policy requires permanent deferral of blood donors who have a “false-reactive” transmissible disease (TD) screening test result (i.e. repeat-reactive screening test but negative or indeterminate confirmatory test). Hema Quebec and other blood suppliers have implemented donor re-entry programs, to retest and possibly reinstate such donors. CBS is examining a similar program. A CBS-affiliated clinic, the Network Centre for Applied Development (NetCAD), which opened in Vancouver in 2003, accepts donors with prior false-reactive tests, since blood collected at this site is used strictly for research purposes. Thus, the NetCAD clinic offers a unique source of data about the potential yield of reinstated donors from a future CBS donor re-entry program.

Methods: A standard data collection form was developed. All NetCAD donor files were manually reviewed, and data extracted by a CBS Nurse. Non-nominal data were collected on donors who had been deferred for false-reactive tests involving one of four TD markers: HBsAg; anti-HIV; HIV p24 Antigen; and, anti-HCV. Data collected included dates and results of donor transmissible disease testing, along with basic descriptive epidemiologic data. Data were analyzed using Minitab® 2007 (Minitab State College PA).

Results: 1685 NetCAD donor files were reviewed; 88 donors had been deferred for false-reactive test results. Seventy-five of 88 donors (85%) tested non-reactive to all TD markers and in 74 of 75 (98%) the initial NetCAD donation was 6 months or more after the donation associated with a false-reactive test. Of the 13 of 88 (15%) donors who had at least one false-reactive screening test at NetCAD, 12 reacted to the same marker. Eleven of the 13 donors with false-reactive tests at NetCAD were previous CBS donors and in 5 of these 11 (45%) donors, the initial NetCAD donation was less than 6 months from the donation associated with a false-reactive test result.

Discussion: Most donors (85% in our review) deferred for false-reactive test results could probably be reinstated in a future CBS donor re-entry program if retested at least 6 months later.

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Switching to a 20% SCIG Home Infusion Increases Primary Immunodeficient Patient Satisfaction

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People diagnosed with primary immunodeficiency disorders (PID) face a life-time of immunoglobulin (Ig) replacement therapy to prevent recurrent infections. Replacement by intravenous infusions of purified human polyvalent IgG (IVIG) has been the primary mode of PID treatment, however in recent years subcutaneous formulations (SCIG) have been gaining attention for their cost effectiveness and relative ease of administration affording more freedom to the patient. In fact, SCIG infusion therapy has been shown to be as effective as IVIG therapy in elevating trough IgG levels, preventing infection and enhancing quality of life. Adverse events are generally rare and of mild severity.

Here, we report on the experiences when transitioning patients to 20% SCIG therapy. Results of 10 patients (9 receiving 16% SCIG, 1 receiving 10% SCIG) showed that serum IgG levels were 8.9 ± 2.2 g/L prior to the switch and, 10.3 ± 1.6 g/L 3 months after the transition. A reduction in the number of infusions per week was seen (2.4 ± 0.8 vs 1.3 ± 0.7) as well as a 55% reduction in the total weekly infusion time (154.4 ± 48.9 vs 69.5 ± 21.8 min) mainly due to a lower infusion volume and/or a higher infusion speed. Generally, 20% SCIG was well tolerated with the majority of patients reporting no local injection site reactions. Only 1 AE was noted following the transition to 20% SCIG (local pruritus 2 days post-injection managed with non-drowsy antihistamine). In fact, 2 patients that exhibited adverse events (AEs) with the prior 16% SCIG (painful site reaction and anaphylaxis) did not experience any AEs with the 20% infusions.

These results demonstrate that switching to 20% SCIG is well-tolerated with few minor AEs being observed. The reduced number of infusions required each week allows the patient even greater freedom and flexibility than with other less concentrated IgG products. Initial results indicate that IgG levels are sustained when switching to 20%.

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Platelet Refractoriness: Is it time to abandon the CDC-AHG method or is it still effective?

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Background: Defined as an insufficient platelet rise post transfusion, refractoriness remains a significant issue in transfusion medicine. Outside of the risk of life threatening bleeding, it is a barrier to safe performance of therapeutic and diagnostic procedures. One of the most significant mechanisms is immune platelet destruction from HLA anti-bodies. The scope of this quality assurance study is to examine the adequacy our current protocol before moving ahead with validation studies for more complex, sensitive and expensive Luminex or Flow Cytometric antibody evaluation assays.

Methods: HLA antibodies and their specificities were identified in refractory patients using complement dependant cytotoxicity (CDC-AHG). We then provided apheresis platelet unit were chosen from our local inventory using the donor information sheets avoiding these specific antigens. Thirteen refractory patients with specific antibodies had a total of 67 transfusion episodes (TE) between October 2010 and January 2012. Adequate platelet increments for each TE were assessed using a corrected count increments (CCI) post transfusion, with a 1-3 hour or 18-24 hour post transfusion platelet count. In situations where CCIs could not be calculated, raw platelet count increments were analyzed. Subgroup analysis was also done to see if ABO compatibility of units made a significant difference in increments.

Results: When assessing 1-3 hour post transfusion platelet counts, 80-83% of transfusion episodes were considered adequate. Transfusion episodes for which a 18-24 hour platelet count was available, 25-27.3% were sufficient with a drop or no change in platelet values was seen in 54.2% of instances. When only those units that were ABO identical were examined, 100% of 1-3 hour increments and 37.5-50% of 18-24 hour increments were adequate.

Discussion: Most important in the bleeding patient, or when an invasive procedure is needed, using our current strategy resulted in adequate platelet recovery. As expected improved results were seen when ABO identical units were used. This suggests that for daily management the CDC-AHG method and avoidance of platelet antigen specificity is successful in the clinical management of these patients. Cost effectiveness of more expensive and sensitive methodologies for examination of platelet refractoriness will need to be carefully examined in prospective studies prior to a recommendation to change method.

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Cryoprecipitate Use in On-pump Cardiac Surgery

Oral Presentation

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Background: Cardiopulmonary bypass (CPB) during cardiac surgery is associated with platelet and coagulation defects. In Canada, cardiac surgery is the most common indication for cryoprecipitate (CRYO) transfusion, yet there are no clinical studies to support its use in this setting. Scores to predict RBC transfusion in cardiac surgery have been published (e.g. TRUST). However, the literature on predictors of CRYO transfusion is lacking.

Methods: We evaluated retrospectively the prevalence of CRYO transfusion post cardiac surgery on CPB at St. Michael's Hospital. All adult patients undergoing CPB from January 1, 2010 to June 30, 2010 and who consented to blood transfusion were enrolled in the study.

Results: Charts of 579 patients were reviewed. Baseline characteristics were as follows: males 72.5%, mean age 65 ± 12 years, combined procedure 28.5%, non-elective procedure 42.7%, previous cardiac surgery 4.5%, and mean TRUST score 2.7 ± 1.6 . 47.5% of patients received RBC transfusion, 13.3% received platelets and 11.2% frozen plasma. 28/579 (4.8%) patients received CRYO transfusions within 24 hours of surgery. Among these patients, the mean intra-operative fibrinogen nadir was 1.2 ± 0.8 g/L, and approximately half (53.6%) received CRYO during surgery. The reason for CRYO transfusion was "coagulopathy and bleeding" in all cases. In addition to CRYO, these patients received other blood products. CRYO transfusion correlated univariately with younger age (mean age 61 ± 15 vs. 66 ± 12 , $p=.04$), previous cardiac surgery (17.9 vs. 3.8%, $p=.006$), and combined procedure (78.6 vs. 26.0%, $p<.0001$). There was no difference in sex, non-elective surgery, pre-operative renal or liver disease, or TRUST scores between groups. CRYO transfusion was associated with higher mortality (10.7 vs. 1.3%, $p<.01$), higher re-operation for bleeding (32.1 vs. 2.7%, $p<.0001$), longer ICU stay (5.0 ± 5.2 vs. 1.8 ± 2.9 days, $p<.0001$), and increased post-operative respiratory and renal dysfunction. These findings in part could be attributed to the differences in baseline characteristics. These findings also suggest that CRYO is given to the sickest patients.

Conclusion: About 5% of patients undergoing CPB received CRYO. Patients who received CRYO were younger, had previous cardiac surgery, and were undergoing combined procedures. CRYO transfusion was associated with several adverse clinical outcomes.

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Peanut Hypersensitivity Following Apheresis Platelet Concentrate Transfusion

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In this report we describe emergence of peanut hypersensitivity in a recipient following transfusion of an apheresis platelet concentrate (aPC) from a donor with a history of peanut anaphylaxis. The recipient, a 62 year old female undergoing chemotherapy for acute myeloid leukemia (AML), reported no prior allergic history to food, drugs or environmental allergens. Following her index transfusion of an aPC unit (donor A) she developed pruritis and hives that responded immediately to medical treatment. A second apheresis unit (donor B) transfused 2 days later resulted in more widespread pruritis, hives, hand erythema, and superficial neck and facial edema; she responded well to diphenhydramine and hydrocortisone. Two days afterwards, while at home, she developed facial edema, and epiphora immediately after eating peanut butter; symptoms subsided rapidly following diphenhydramine treatment. A skin test for peanut allergen performed 2 weeks following the attack was reactive. Donor A, on further questioning, had a history of peanut anaphylaxis and carried emergency epinephrine. His serum peanut-specific IgE was markedly elevated at 80 kUA/L [normal < 0.35 kUA/L]. Donor B's history was unremarkable for peanut hypersensitivity, but he recalled consuming peanut-containing food prior to his donation. We suspect donor A was the source of passive transfer of IgE to the recipient leading to the initial mild reaction. During the second aPC transfusion, we hypothesize that peanut allergen from donor B reacted with the passively transferred IgE from donor A, resulting in a more severe allergic reaction. Passive transfer of IgE from donor A would also account for the subsequent reactive peanut-specific skin test. The patient was advised to avoid all peanut containing food for 2 months and all subsequent blood components were plasma reduced. Transfusion is a rarely reported cause of transient, acquired hypersensitivity via passive transfer of donor allergen-specific IgE. A history of severe allergy is not currently a blood donor deferral criterion in Canada and a change would incur significant donor loss, given the relative common frequency of self-reported hypersensitivity in the general population.

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Neutralization Positive but Apparent False Reactive HBsAg Following Influenza Vaccination

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In addition to the negative impact on the donor blood supply, false reactive infectious disease markers can create unnecessary anxiety in blood donors. Biological false reactive reactions to Hepatitis B Surface Antigen (HBsAg) have been documented shortly following routine immunization to Hepatitis B. In this report, we describe a case of a 25 year old repeat blood donor who was found to have repeat reactivity to HBsAg 2 days after she received the seasonal influenza vaccine.

She had been a donor since 2003 and on the day of the index donation did not report any symptomatic illness or recent high risk activity for transmissible infections. She had been immunized against Hepatitis B a number of years ago and 2 days prior to the donation, she received the 2011-12 seasonal trivalent (H1N1, H3N2 and Influenza B) inactivated split virion influenza vaccine (Fluviral®) at her physician’s clinic without any reported reactions. The ABBOTT PRISM® HBsAg chemiluminescent (ChLIA) assay performed on the donated sample was found to be repeatedly reactive. The neutralization confirmatory test demonstrated 53.55% neutralization and the donor was deemed positive for HBsAg. Hepatitis B surface antibody (anti-HBs) was documented at 55 IU/L and both HBV DNA and Hepatitis B core antibody (anti-HBc) were negative. Repeat testing 2 weeks following the donation by her family physician demonstrated negativity for both HBsAg and anti-HBc. Anti-HBs on re-testing was 38.7 IU/L.

We postulate that split virions in the influenza vaccine may present one or more epitopes that immunogenically resemble HBsAg binding sites, to produce transient cross-reactivity in both the HBsAg ChLIA screening assay and confirmatory neutralization test. Although influenza vaccine has been associated with false-reactive antibody tests to HIV, hepatitis C and Human T Lymphotropic Virus, to our knowledge, this report represents the first reported case where influenza vaccine may have caused repeat-reactive HBsAg screening and positive confirmatory neutralization test results. If so, the phenomenon is very rare and does not necessarily indicate the need of changing the current 2 day donor deferral following immunization with inactivated influenza vaccine; such rare instances might be addressed through a donor re-entry program.

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Waste not, Want not: A look at the rate of BacT/ALERT false positive cultures at Canadian Blood Services

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Background: Implementation of platelet screening for bacterial contamination with the BacT/ALERT culture system has positively impacted platelet safety in Canada. This system identifies potentially-contaminated units and therefore prevents transfusion reactions. Besides true positive results, false positives are also obtained during platelet screening. A false positive refers to a culture with an initial positive result which results negative during confirmatory testing, due to either machine failure or user contamination during sampling. Herein we summarized the false positive results obtained since 2004 at Canadian Blood Services.

Methods: False positive results were obtained through our Quality Monitoring Program which documents results obtained during platelet screening with aerobic BacT/ALERT culture bottles. Quality control (QC) sterility testing of platelet concentrates (PCs) is performed on expired units that have initially tested negative using both aerobic (BPA) and anaerobic (BPN) BacT/ALERT culture bottles.

Results: As of February 2012, 223,846 apheresis PCs have been tested with 331 initial positive results. Out of these, 187 (57%) and 53 (16%) were machine failures and user false positives, respectively. A total of 265,596 buffy coat PCs have been screened with 158 initial positive results. From those, 58 (37%) and 39 (25%) have been machine failures and false positives due to contamination, respectively. During QC monitoring, a total of 59 and 29 false positives have been obtained for apheresis and buffy coat PCs, respectively. The majority of these false positives (62% and 69%, correspondingly) were machine failures with BPN bottles.

Discussion: Although the occurrence of false positives does not impact patient safety, it results in unnecessary wastage of blood products. Noticeably, the rate of machine failures at Canadian Blood Services during platelet screening is very high (57%) compared to other blood suppliers such as the Welsh Blood Services (28%). However, significant reduction in this rate has been recently observed due to active surveillance and consultation with the vendor. Results from QC monitoring on expired PCs suggest that implementation of anaerobic cultures in routine platelet screening would result in significant product waste. It is important to continue tracking data and further reduce false positives for a more cost-effective practice at Canadian Blood Services.

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The Ones That Got Away: A look at the rate of BacT/ALERT false negatives cultures at Canadian Blood Services

Oral Presentation

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Background: The incidence of bacterial contamination in platelet concentrates (PCs) at Canadian Blood Services is very low (0.1%) in comparison to other blood transfusion services. This is due to the implementation of several interventions including donor skin disinfection, first aliquot diversion and platelet bacterial screen testing with the BacT/ALERT culture system. Despite these measures, false negative cultures, resulting sometimes in adverse transfusion reactions (ATRs), continue to be reported. A test is considered a false negative if an initial culture is negative but found to be positive on re-culture following an ATR or as part of quality control (QC) monitoring. Here we summarize false negative cases documented at Canadian Blood Services since 2004.

Methods: ATRs have been summarized from information provided by Regulatory Affairs. Furthermore, data obtained through QC sterility testing, which has been performed on a monthly basis since 2009 on expired PCs, have been included.

Results: As of February 2012, 540,593 PCs have been tested and a total of seven ATRs have been reported for a rate of 0.01/1,000. Four ATRs were associated with apheresis PCs, one with buffy coat (BC) pools, and two with platelet-rich-plasma PCs. Microorganisms isolated included virulent species such as *Staphylococcus aureus* (3) and *Serratia marcescens* (1); the later resulted in a fatality. The common skin/mucosa contaminants coagulase negative *Staphylococcus* (CoNS, 2) and Group A *Streptococcus* (1) were also identified. QC monitoring revealed six additional false negative cultures involving units that were not transfused: three apheresis PCs were contaminated with *Staphylococcus aureus* (1) and CoNS (2) while three BC pools grew CoNS (1) and the anaerobe skin contaminant *Propionibacterium acnes* (2).

Discussion: Our results show that bacterially-contaminated units still escape detection during initial culture screening. Although some contaminated units are not transfused as demonstrated by our QC data, other units cause ATRs and even fatalities. An analysis of published reports revealed that the rate of false negative BacT/ALERT cultures causing ATRs vary from 0.01/1,000 (Canadian Blood Services) to 0.18/1,000 (German Red Cross). It is therefore important to adopt new measures such as pathogen reduction technologies to decrease the risk of ATRs involving contaminated PCs.

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Trimming the Fat with an IVIG Approval Process

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Purpose: In an effort to optimize the use of IVIG at Sunnybrook Health Sciences Centre, an approval process for IVIG was implemented in March 2011, which included a dosing weight calculator based on ideal body weight and formal approval by a transfusion medicine physician. IVIG is an expensive blood product and has the potential to cause significant transfusion reactions, particularly hemolytic reactions, when the dose exceeds 2 gm/kg of ideal body weight. The purpose of this audit was to determine the impact of the IVIG approval process on the amount of IVIG saved in grams and in dollars.

Methods: A retrospective nine-month audit of the IVIG approval forms was conducted. The amount of IVIG saved was based on the amount ordered by the physician on the approval form compared to the amount of IVIG issued. Because the dosing weight calculator was available to physicians at the time of ordering, savings obtained using the dosing weight calculator may have been underestimated. Savings attributable to the dosing weight calculator were calculated comparing the IVIG amount that would have been ordered based on actual weight compared to the amount using the dosing weight calculator.

Results: 88 IVIG forms submitted between March and November 2011 were reviewed. 86 requisitions for 70 unique patients were included in the final analysis. The average BMI was 26.4 +/- 5.3 kg/m². The total amount of IVIG ordered was 12,075g. Dose modification occurred in 34% of the approval forms, the majority being for weight (20/28 forms). There was 708.5g of IVIG difference (6% decrease) between the amounts ordered and approved. At a product cost of \$63/gram, this translates to a potential savings of \$44,635.50. Use of the dosing weight calculator alone may have resulted in a total theoretical savings of 1,100 g of IVIG (\$69,300.00). No hemolytic transfusion reactions were reported in this time period. No concerns were raised by clinicians regarding lack of effect or loss of effect with the IVIG dose reductions.

Conclusion: The IVIG approval process resulted in significant cost-savings. In particular, the dosing weight calculator was a simple and effective tool in decreasing IVIG use.

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Is this a RhIG failure?

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Background: We are presenting a case of Rh negative woman whose alloimmunization pattern resemble anti-D & anti-C. She was subsequently shown to have Anti-G.

Case Report: A 31 year old G5 A3 P1 with a history:

- 2004, 1st pregnancy, ectopic, Antibody Screen Test (AST) negative, received RhIG
- 2005, 2nd pregnancy, term delivery, AST negative, received RhIG pre and post delivery
- July 2005, 3rd pregnancy, D&C for therapeutic termination of 7 week pregnancy, AST negative, received RhIG
- 2008, 4th pregnancy, spontaneous miscarriage in 1st trimester, AST negative, received RhIG
- 2008, received 4u of Rh negative RBC transfusion for hemorrhagic ovarian cyst
- 2011, 5th pregnancy, 7 weeks gestation, AST positive, anti-D & anti-C detected in patient's plasma, titre of anti-D was 8 and anti-C was 32
- All pregnancies with the same partner. Rh phenotype of the father of the fetus was D+C+E-c+e+.

Results of anti-D and anti-C titre were suggestive of anti-G rather than anti-D and anti-C. Patient's specimen sent to Reference Laboratory for confirmation. Further investigation revealed anti-C and anti-G in patient's plasma but not anti-D. Patient was a candidate for RhIG. Titre was monitored until 29 weeks. Patient received RhIG at 29 weeks. February 2012, patient had full term delivery of male infant with Hemoglobin 216 g/L, Billirubin level 98 umol/L, DAT 4 + positive. Cord red cell phenotyped as D+ C- E- c+e+ (R0r). Only Anti-G eluted from Cord cells. Infant's Bilirubin level peaked on 2nd day. Infant was treated with light therapy and discharged on 6th day.

Conclusion: Not a RhIG failure. This case presents a number of interesting facts:

1. In spite of 4+ DAT, baby's hemoglobin was normal.
2. Anti-G developed secondary to pregnancy with G+ partner or transfusion of Rh negative G+ (D-C+G+) unit.
3. Suspect anti-G, when anti-D titre is less than anti-C and patient never received Rh positive blood products.

This case poses a question: Should we issue Rh negative C negative RCC to all Rh negative female, child bearing age to prevent alloimmunization to G antigen

HLA- and HPA-Alloimmunization Rate In the Setting of Refractoriness to Platelet Transfusion: A Single Center Study

Oral Presentation

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Background: Refractoriness to platelet transfusion is a common clinical problem in hematology/oncology patients. While often non-immunologic in etiology, alloimmunization to human leukocyte antigens (HLA) or human platelet antigens (HPA) may contribute to this phenomenon. At our institution, platelet refractoriness is defined as a platelet count rise of $<10 \times 10^9/L$ measured within 24 hours post-transfusion of ABO-matched single-donor (SD) platelets on at least two occasions. The goal of this study was to measure the HLA- and HPA-alloimmunization rates in adult patients at our institution who were refractory to SD platelet transfusions.

Methods: All refractory patients to SD platelet transfusion between January 2006 and June 2011 were included. HLA and HPA antibody testing was performed at the Canadian Blood Services Platelet Immunology Laboratory in Winnipeg, Manitoba. For patients with HLA antibodies, panel reactive antibody (PRA), which estimates the percentage of potential reactivity against donors in the population, was calculated using cPRA software from the Organ Procurement Transplantation Network (OPTN).

Results: Thirty-three patients (18 male and 15 female) were tested and most patients (85%; 28/33) had a hematologic malignancy. No HPA antibodies were identified in any patient. HLA antibodies were found in 42% of patients (14/33) and differed according to gender: 22% for males (4/18) vs 67% for females (10/15). Males ($n=4$) had an average PRA of 33% (range 11-62%) while females (9/10) were highly sensitized to HLA with PRA values between 95 and 100%. There was no association between the presence of HLA antibodies and either patient diagnosis or a history of allogeneic stem cell transplantation.

Conclusion: The HLA alloimmunization rate was 42% among patients refractory to SD platelets while HPA antibodies did not contribute to platelet refractoriness. Patient diagnosis and/or history of allogeneic stem cell transplantation did not appear to influence the rate of HLA alloimmunization. Most refractory patients with HLA-antibodies and high HLA sensitization ($PRA \geq 95\%$) were female (64%; 9/14) which implicates pregnancy as a contributor to platelet transfusion refractoriness. PRA values of $\geq 60\%$ seen in most HLA-sensitized patients (71%, 10/14) argues against using platelet crossmatching for identification of HLA-matched platelets.

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Mixed Field Reactions in ABO and Rh(D) Typing Due To Chimerism Resulting From Twin Hematopoiesis

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Background: Difficulties in ABO and Rh(D) typing due to mixed field reactions may be encountered in the transfusion medicine laboratory. Obtaining relevant historical information from the patient and performing additional laboratory investigations may be required in order to provide resolution. We report a case of a 70 year old female who was found to have mixed field reactions in both ABO forward typing and Rh(D) typing prior to an operative procedure. She had no history of transfusion or transplantation but further questioning revealed that she had a twin brother who died a few months after birth.

Results: Tube testing and the MTS gel method demonstrated mixed field reactions on forward grouping using Ortho monoclonal anti-A and anti-A,B while monoclonal anti-B reagents were non-reactive. Reverse grouping revealed only the presence of anti-B (3+). Macroscopic mixed-field reactivity was observed with DBL monoclonal anti-D and with Ortho anti-E reagents on immediate spin. Flow cytometric analysis demonstrated that anti-A was non-reactive with 88% of red blood cells (RBC) and reactive with 12%. Similar results were seen with anti-A+B. All RBC were non-reactive with anti-B. Anti-H testing was strongly positive suggesting a majority group O population. Analysis of peripheral blood by PCR-ASP and PCR-RFLP revealed an ABO genotype of A2O1 (A201/O01) only while a buccal swab showed a genotype of A2O1 and the presence of a chimera with a minority population representing between 2-12% of the patient’s cells. Human Leukocyte Antigen (HLA) typing revealed the same result for both a buccal swab (somatic cells) and a peripheral blood sample (hematopoietic cells).

Conclusion: This case illustrates the possibility of an immunologically-immature twin receiving hematopoietic stem cells in utero from the other twin through placental blood vessel anastomoses, with subsequent production of a natural permanent genetic chimera resulting in mixed field typing for ABO and Rh(D). The A2O1 genotype found on DNA analysis of somatic cells and hematopoietic cells from the patient was likely acquired from the twin in utero and masks an O1O1 genotype underlying the majority of the patient’s RBC.

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Appropriateness of Transfusing Two Units of Red Blood Cells in Stable Anemic Patients

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Background: Hemoglobin ≥ 70 g/L is considered acceptable in most clinically stable anemic patients. The dogma to “transfuse two units of red blood cells (RBC) or don’t transfuse at all” potentially exposes patients to unnecessary transfusion when one unit of RBC would be sufficient. In addition to adverse transfusion reaction risk resulting from over-transfusion, suboptimal utilization of health care resources is associated with this practice.

Methods: RBC transfusion data from June and July 2011 was reviewed at Capital District Health Authority in Halifax, Nova Scotia to determine if there was clinical justification for two-unit RBC transfusions. This was accomplished by comparing the clinical findings and the hemoglobin level of patients before and after transfusion events. During the study period, 126 stable, non-bleeding patients who received a two-unit RBC transfusion were included; 81 were medical patients and 45 were surgical patients. Patients transfused during surgery, in Medical Day Unit, or on a hematology/oncology floor were excluded due to potentially exceptional transfusion needs.

Results: Pre-transfusion hemoglobin levels ranged from 52 to 122 g/L, and averaged 73 ± 10 g/L. Post-transfusion hemoglobin levels ranged from 62 to 132 g/L, averaging 94 ± 11 g/L. Two-unit RBC transfusion was considered justified if there was a history of acute coronary syndrome or peripheral vascular disease. No medical or surgical patient transfused to a hemoglobin level of 80-90 g/L had justification for receiving two units of RBC. Transfusion of medical and surgical patients to a haemoglobin level 90-100 g/L was justified 27% of the time and 17% of the time, respectively. Eight percent of medical and surgical patients transfused to a hemoglobin >100 g/L had justification. Overall, a two-unit RBC transfusion was clinically justified in only 9% of the patients reviewed.

Conclusions: Our findings indicate that it is reasonable to recommend a one-unit RBC transfusion in stable anemic patients and to measure the hemoglobin level thereafter to assess subsequent transfusion needs. Adherence to these recommendations will improve RBC utilization while saving financial resources and nursing time. In addition, the potentially harmful effects of unnecessary transfusion will be avoided with no detriment to the clinical outcomes of patients.

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Septic Transfusion Reaction due to Staphylococcus Aureus Contamination in One of Two Units from a Split Apheresis Platelet Donation

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Background: Our blood system continues to experience instances of bacterial contamination of platelets despite donor skin disinfection, use of a diversion technique to isolate the first portion of the donation from the product, and pre-release sampling for culture (BacT/ALERT, bioMérieux). Here we report a case of bacterial contamination in one of two units from a split apheresis platelet product.

Case Report: 50-year-old male, status-post recent allogeneic stem cell infusion for therapy-related myelodysplastic syndrome/myeloproliferative disorder with significant marrow fibrosis was infused with one half of a split apheresis product. During the infusion the patient developed a mild rash, hip pain, and nausea and vomiting. After the infusion, the patient became febrile, experienced rigors, and was hypotensive. The suspected septic shock was stabilized with crystalloid boluses, and broad spectrum antibiotics were empirically initiated. The remainder of the platelet product was sent for culture, and a blood culture was drawn from the patient. Both cultures grew Staphylococcus aureus with identical antibiotic sensitivity and metabolic profiles. Antibiotic therapy was switched to vancomycin and then cefazolin, and the patient subsequently recovered from the reaction. The blood centre retrieved the other portion of the split donation from a different hospital prior to use. Culture of that unit was negative. The original, pre-release culture obtained from the product prior to splitting was negative throughout the 6-day culture period

Conclusions: Several interventions have improved safety but do not completely remove the risk of septic transfusion reactions from platelets. One hypothesis as to the source of false-negative pre-release cultures is extremely small initial inoculum with failure to obtain organism in the sample drawn for culture (ie, sampling error). Small initial inoculum is also a potential explanation for contamination of only one of two platelet products from a split production donation. The BacT/ALERT system can detect approximately 50% of contaminated platelets when used early during the platelet lifespan. This case highlights the need for implementation of further methods to improve platelet safety such as pathogen reduction technologies or platelet re-testing prior to transfusion.

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A Trend Analysis in the Reporting of Adverse Events following Blood Products to Health Canada

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Carole Légaré BSc, MD, CCFP, Health Canada
Duc Vu PhD, Health Canada

Background: Manufacturers are required under the Food and Drug Regulations to report to Health Canada, all serious adverse drug reactions that have occurred in Canada. Reports of serious adverse drug reactions to blood products are assessed in the Marketed Health Products Directorate and are reconciled with those received by the Transfusion Transmitted Injury Surveillance System (TTISS) for inclusion in the TTISS data.

TTISS is a national surveillance system for reporting of adverse events (AEs) to blood, blood components or blood products (plasma protein products). TTISS currently involves the participation of all Provinces and Territories, representing 83% of transfusion activity. Adverse events captured through TTISS are compiled, analysed and reported by the Public Health Agency of Canada.

Purpose: To report a trend analysis of adverse events reported to Health Canada following administration of blood products (2004-2011)

Methods: Line listings of reports of adverse events following any blood product were requested from Canada Vigilance for the years 2004-2011. These reports were analysed for trends.

Results: The numbers of reports received by Health Canada from 2004 to 2011 has grown. In 2007-8, 80 serious reports sent to TTISS were not received by Health Canada. The most frequently reported serious AE overall was hemolysis following IVIg. Hemolysis following IVIG represents a growing portion of all serious events. This increase follows the increase in utilization of IVIGs. The profile of reported adverse events is consistent with the Canadian labelling of these products.

Conclusions: In 2007 and 2008 serious reports were sent to TTISS and not received in Health Canada. For the ongoing monitoring of the safety of blood products, it is important that all adverse events be also reported to the manufacturer.

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A Review of Serious Adverse Events for Blood Products, Reported to Health Canada

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Background: Manufacturers are required to report to Health Canada, all serious adverse drug reactions that have occurred in Canada. The Marketed Health Products Directorate of Health Canada assesses these serious adverse events according to the Transfusion Transmitted Injury Surveillance System (TTISS) definitions and participates in data reconciliation with the Public Health Agency of Canada.

Purpose: To review Canadian reports of blood product adverse events with onset in 2010, reported to Health Canada between January 1, 2010 and June 30, 2011.

Methods: Line listings of reports of adverse events following any blood product were requested from Canada Vigilance for reports received from January 1, 2010 to June 30, 2011. Serious reports with onset in 2010 were assessed for adverse event, relationship of adverse event to transfusion, severity of adverse event and outcome of the report, using the TTISS definitions.

Results: Of the 307 reports received January 1, 2010, – June 30, 2011, 76 reports (with 78 adverse events [AEs]), having an onset in 2010 were assessed as serious. The majority (69%) of serious events were grade 2/severe. Outcome of the reports was minor or no sequelae in 33 (43%), not determined in 31 (41%), major or long term sequelae in 3 (4%) and death in 9 (12%) reports. For the fatal reports, a relationship of the transfusion to death was ruled out in 4, not determined in 3, doubtful in 1 and possible in 1 report. 77% of reports were assessed as at least possibly related to transfusion. The majority (81%) of serious AEs assessed as at least possibly related to transfusion followed administration of IVIGs; 61% of these were hemolytic reactions. The most frequently reported serious AE overall and in the pediatric age group was hemolysis following IVIg.

Conclusions: The most frequent adverse event following a blood product, reported to Health Canada in 2010 was hemolysis following IVIg. The profile of reported adverse events is consistent with the Canadian labelling of these products.

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The Value of Measuring Reticulocyte Hemoglobin Levels For The Management of Pre-operative Iron Deficient States

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Antonio Giulivi MD, FRCP(C), The Ottawa Hospital

Purpose: Efficient treatment of iron deficiency anemia prior to surgery is an important component of pre-operative blood conservation. Standard laboratory tests used to assess iron deficiency include serum ferritin, serum iron, total iron binding capacity, percent iron saturation, and transferrin. Several of these biochemical tests are considered to be indirect markers and acute phase reactants which may provide inaccurate information. Reticulocyte hemoglobin content can provide a measure of the iron status at the level of newly formed erythrocytes. The reticulocyte hemoglobin content test is considered to be a stable and sensitive clinical marker for assessing status. The purpose of this study is to observe the relationship between the results of the standard diagnostic iron studies and the reticulocyte hemoglobin content.

Methods: The study design is prospective observational. The study sample includes eighteen consecutive surgical patients referred to the Ottawa Hospital Blood Conservation Program diagnosed with iron deficiency anemia and received intravenous iron therapy prior to surgery. The results of the serum iron studies and reticulocyte hemoglobin content were observed pre and post intravenous iron therapy.

Results: In approximately one third of the pre-treatment cases (33%) the baseline reticulocyte hemoglobin content suggested adequate available iron in contrast to the standardized indices which showed significant iron deficiency. The majority of cases in the post treatment group (76%) showed normal iron status confirmed by both standardized indices and the reticulocyte hemoglobin content. In the remaining post treatment cases the results of both iron studies and reticulocyte hemoglobin content suggested ongoing iron requirements and functional iron deficiency (24%).

Conclusions: Serum iron, total iron binding capacity, percent iron saturation, and serum ferritin remain the laboratory gold standard to confirm low iron stores in our pre-operative patient population. However the availability of reticulocyte hemoglobin content can provide additional information for the management of functional iron deficiency anemia and may help to streamline intravenous iron treatment during the pre-operative period. We need to further assess the cutoff for the measurement of reticulocyte hemoglobin content at our center.

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Blood Transfusion Education the E-learning Way

Peggy Wilson RN BScN, Nova Scotia Provincial Blood Coordinating Program

Marina Hamilton RN, MScN, Nova Scotia Provincial Blood Coordinating Program

Nova Scotia's Department of Health and Wellness, Health Information Technology Services - Nova Scotia (HITS -NS) and the Nova Scotia Nurse Educators worked collaboratively to develop a provincial e-learning educational system that offers competency training for registered and licensed practical nurses throughout the province. A provincial group of nurse educators identified fifty modules for development with blood transfusion being one of the top ten priorities. The e-learning mode of education allows nurses to access and complete evidence - based competency training learning twenty-four hours a day. The educational modules also achieve Accreditation Canada's Required Organizational Practices by focusing on client safety as a strategic priority.

The modules are made available through an online learning management system (LMS) using MEDworxx. With the use of Articulate, a content authoring tool, the modules are made interactive. The blood administration modules were developed by the Nova Scotia Provincial Blood Coordinating Program with input from two of its working groups; the Transfusion Medicine Quality Specialists Working Group and the Provincial Nurses Transfusion Practice Working Group.

Three e-learning modules were developed for blood transfusion. Blood Basics provides an overview of the basic functions of blood, ABO and Rh typing, compatibility, the indications for use and storage. The Administration module focuses on informed consent, physician orders, IV access, infusion devices and assessment and documentation of the transfusion.

The third and final module is on Adverse Reactions. This module presents information on the types and signs and symptoms of transfusion reactions as well as the clinical management, reporting and investigation of reactions. The content of the modules is reviewed a minimum of every 3 years.

The three modules have questions throughout and a test at the completion of all the modules. There are assigned pass marks that must be obtained to be considered competent. The system has compliance tracking capabilities allowing direct managers and nurse educators access on the progress and test scores of the learners.

The provincial e-learning educational system is an avenue that allows nurses to stay up to date on current best practices in blood transfusion. It provides them with a method to maintain their competency at a time that is convenient for them.

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Notes:

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Immune Complex Formation Following Addition of IVIg to Human Plasma

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The increase in plasma viscosity observed following infusion of high doses of IVIg to patients was suggested to be a consequence of immune complex formation between IVIg and plasma proteins. Indeed, the presence of IgG reactive with human plasma proteins in IVIg preparations was demonstrated previously. In the present work, we determined the amount of immune complexes produced after incubating IVIg with human plasma. The Raji cell assay, which identifies the presence of complement-bearing immune complexes, was first used. As control, ovalbumin (OVA)-anti-OVA complexes were used. While a dose of 100 ng of OVA-anti-OVA complexes was readily detected by flow cytometry, analysis of a mixture containing a therapeutic dose of IVIg (15 mg/ml) with human plasma did not reveal the presence of complexes bound to Raji cells, suggesting that either the amount of complexes present was below detection limit or that IVIg/plasma protein complexes do not bind complement, and therefore cannot be detected using the Raji cell assay. We thus set up a procedure using size-exclusion chromatography and immunoprecipitation techniques to quantify the amount of complexes in the IVIg/human plasma mixture. The results obtained showed the presence of about 120 mg of complexes per ml of plasma. We therefore conclude that measurable amounts of complexes are formed in human plasma in presence of therapeutic doses of IVIg, but that these complexes do not activate complement since they were not identified using the Raji cell assay. The role of these complexes in the therapeutic effects of IVIg is under investigation.

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IgG Dimers are Dispensable for the Therapeutic Effect of IVIg in ITP

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TIVIg has been used for many years for the treatment of immune thrombocytopenia (ITP). Several years ago, it has been reported that IgG dimers present in IVIg preparations were responsible for the increase in platelet counts in ITP (Teeling et al, Blood 2001; 98:1095). In our laboratory, we are studying the effect of IVIg preparations devoid of autoreactive IgG using human polyclonal IgG isolated from pools of plasma from 10 to 30 donors using non-denaturing conditions (St-Amour et al, Clin Immunol 2009; 133:52). These preparations do not contain detectable amounts of IgG dimers, as determined by size-exclusion gel chromatography. These “in-house” IVIg preparations were compared to commercial IVIg preparations for their ability to prevent thrombocytopenia in a mouse model of experimental immune thrombocytopenia. The results obtained showed a similar therapeutic efficacy for both preparations, therefore questioning the role of IgG dimers in the prevention of thrombocytopenia in this assay. We thus revisited the role of IgG dimers using dimer-enriched IVIg (about 15% dimers) prepared by dialysis against PBS and incubation at 4oC for two weeks, and monomeric fractions of IVIg prepared by size-exclusion chromatography on Superdex 200. The results obtained showed that dimer-enriched IVIg was not more efficient than IVIg to prevent thrombocytopenia (tested at 0.25 and 1 g/Kg). In addition, the monomeric IVIg fraction was as efficient as IVIg used at the same concentration (1 g/Kg). We thus conclude that IgG dimers are dispensable for the therapeutic effect of IVIg in ITP.

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Microvesicle Phospholipids in SAGM Stored Red Blood Cells Supernatants

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In packed red blood cells (RBC) stored under blood banking conditions, RBC microvesicles (MV) increase in the product supernatant over the storage time. These vesicles are shed in response to biochemical changes in the RBC and physical stresses encountered during whole blood production and manifest as reorganization of the RBC membrane lipids resulting in an explosion of phosphatidylserines (PS) on the surface of the RBCs. Many clinical studies have suggested that RBC MV accumulated in hypothermally stored RBCs are associated with post-transfusion complications, which are thought to be likely mediated at some extent by proinflammatory lipids. It has been well established that the RBC membrane lipids are about 50% phospholipids and 50% cholesterol. This present work investigated the phospholipid composition of microvesicles shed in SAGM preserved leukoreduced RBC units stored at 1-6°C. Using Electro Spray Ionization (ESI) and Fourier-Transformed Mass Spectrometry (FTMS) MV pellets collected (3,200 x g, 30 min, 4°C; 50,000 x g, 60 min, 4°C) from RBC product supernatants (n = 6) were analyzed within the context of high sample throughput (1 min/analyses) "shotgun lipidomics". Phospholipids were attributed on the basis of elemental compositions calculated (error < 2 ppm) to suit mass-over-charge (m/z) peak values. Several phospholipids including 16 species of phosphatidylcholines, 15 sphingomyelins, 14 phosphoethanolamines and 11 phosphatidylserines were characterized in all the MV and RBC lipid extracts, and the difference in their relative distributions in the samples were statistically significant for the phosphatidylserines. In accordance, mass spectrometry data subjected to principal component analysis did return MV and RBC group separation on the basis of phosphatidylserine analysis. In conclusion, the ESI-FTMS methodology adopted proved its value for enabling the construction of large lipid databases that can be feasibly applied for quality assessment of blood products and contribute with relevant information to RBC storage / preservation research.

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Sterility and Coagulation Factor Validation of Thawed Apheresis and Cryosupernatant Plasma Stored for 120 Hours

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Background and Purpose: In an effort to reduce waste of blood products, and following changes to CSA standards for storage of Frozen Plasma, some blood banks have recently extended the expiration of all thawed plasma products from 24 hours to 120h (aka 5 day plasma). Questions remain as to the effect of storage on coagulation factor levels during storage at 1-6°C, as well as the effect on sterility for apheresis and cryosupernatant plasma which are processed in an open system at time of collection. The aim of this study was to evaluate the integrity of thawed apheresis (AFFP) and cryosupernatant plasma (CSP) units after 120 hours storage at 1-6°C by measuring the levels of coagulation factors V, VII, and VIII in addition to confirming the sterility of the units. Methods: Ten units of apheresis and ten units of cryosupernatant plasma were aliquoted at 0, 24, 48, 72, 96, and 120 hours after thawing. After 120 hours storage at 1-6°C, sterility testing was performed by culturing an aliquot of the plasma.

Results: Our results indicate that there was a significant decrease in the levels of coagulation factors V, VII, and VIII after 120 hours storage at 1-6°C. Factors V and VII decreased by 16% and 29% respectively, on average. However, levels remained above 0.5 IU/mL, with the exception of one apheresis plasma unit. Factor VIII levels in the apheresis plasma units decreased 42% on average, but were maintained above 0.4 IU/mL. No bacterial contamination was found in any of the plasma units.

Conclusion: Our results indicate that although there is a reduction in the levels of coagulation factor levels during 120 hours storage at 1-6°C, the apheresis and cryosupernatant plasma units will provide similar coagulation factor replacement as other 5 day plasma. Although initially created as “open” systems, our testing revealed no evidence of bacterial contamination in the AFFP or CSP units. The freezing and thawing process may render growth of potentially contaminating bacteria less likely despite the open system processing.

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Flow Cytometric Analysis of Residual Red Blood Cells and Microparticles in Platelet Concentrates

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Purpose: In Canada, platelet concentrates (PCs) are produced either by single-donor apheresis (AP) or, more recently, by the pooling of 4 to 5 buffy coats (BC). The amount of residual red blood cells (rRBCs) in PCs might increase the risk of alloimmunization to Rh antigens in transfused patients. In this work, we have characterized rRBCs in terms of size, RhD expression and Annexin V binding capacity.

Methods: Antigen expression was monitored by flow cytometry. PCs were stained with anti-CD41a (platelets) and anti-CD235a (rRBCs). CD235a-positive events were reanalyzed by size and granularity to distinguish “intact” RBCs from MPs (microparticles). The size of CD235a-positive events was evaluated by comparing the forward size scatter intensity to that of latex beads of well-defined sizes. Anti-RhD or Annexin V was added to estimate the number of RhD antigen and phosphatidylserine sites, respectively.

Results: Blood product volumes were lower for AP-derived PCs than BC-derived PCs (223 ± 17 mL vs. 349 ± 16 mL). The average size of CD235a-positive events was $8,3 \pm 0,3$ μ m for intact RBCs, while the size of MPs was $3,1 \pm 0,2$ μ m in BC-derived PCs and $2,2 \pm 0,2$ μ m in AP products. The mean number of RhD antigen sites was $13\ 245 \pm 7\ 396$ for intact RBCs, $1\ 759 \pm 493$ for MPs in BC-derived PCs and $3\ 077 \pm 2\ 314$ for MPs in AP-derived PCs. No Annexin V binding was observed on intact RBCs; however, $68 \pm 33\%$ of MPs from BC-derived PCs and $61 \pm 14\%$ of MPs from AP-derived PCs were Annexin V-positive.

Conclusions: The determination of rRBCs in PCs, generally based on intact RBC measurements by microscopy or flow cytometry, probably underestimates the alloimmunization risk in transfused patients. A better understanding of the clinical importance of the immunogenic potential of MPs in blood products might guide clinicians in determining the relevance of a prophylactic treatment of patients at risk of alloimmunization against the RhD antigen.

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Notes:

Validation of a Novel in-House Dombrock Genotyping Test

Oral Presentation

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Purpose: Blood group genotyping is now widely used to identify compatible blood units for transfused patients, and the demand for this specialized testing is growing rapidly. For small hospital laboratories, validating these tests to meet regulatory standards can be challenging. In this work, we report the validation strategy used in our laboratory to validate a novel in-house Dombrock (DO*01 and DO*02) genotyping test.

Methods: Primer sequences were designed according to Wu et al. (2001, *Vox Sang.* 81, 49-51) and GenBank sequence AF290204. Their concentrations were adjusted so as to give bands of comparable intensities with either allele on agarose gel. Primers for the human growth hormone gene were also included as an internal amplification control. Testing was performed in 8-well strips prefilled with 19 μ l reaction buffer (AmpliAq DNA polymerase, buffer, dNTPs and water) to which were added 1 μ l DNA and 5 μ l primer mix. The stability of three lots of primer mixes was evaluated at three-month intervals. Test specificity was confirmed using DNA samples representing the three possible genotypes (DO*01/DO*02, DO*01/DO*01 and DO*02/DO*02). A repeatability study was carried out on six replicates from each genotype. Assay precision was determined on two PCR instruments from distinct vendors and was done by two operators working on different days. Tests were repeated by another laboratory to determine interlaboratory reproducibility. The limit of detection and the impact of subjecting reagents to freeze-thaw cycles were also evaluated. The validation protocol was approved by our Quality Assurance Department.

Results: Completion of the validation protocol lasted about four weeks, and involved two laboratory technologists. Both DO*01 and DO*02 tests showed excellent repeatability, with strong intensities for both DO-specific bands and the internal control on agarose gels. Primer mixes correctly identified blood donors representing the three genotypes. The assay was not influenced by operators, PCR instruments or freeze-thaw cycles. Stored frozen at -35°C , primer mixes were stable for up to six months.

Conclusion: This validation confirmed that our in-house Dombrock genotyping assay complies with regulatory requirements. This achievement facilitated the rapid transfer of the assay to the routine operations of our Immunohematology Reference Laboratory.

Efficacy of IVIg Treatment in a Murine Model of Autoimmune Hemolytic Anemia

Oral Presentation

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Intravenous immunoglobulin (IVIg) is used to effectively treat autoimmune diseases including immune thrombocytopenia (ITP), yet its use in the treatment of autoimmune hemolytic anemia (AIHA) remains controversial due to inconsistent efficacy, with no established explanation as to why some patients respond while others do not. AIHA is characterized by red blood cell (RBC) destruction by antibodies against RBC-specific antigens, and the current paradigm suggests that RBC clearance is primarily caused by macrophage/complement-mediated phagocytosis and/or lysis. In this study, we tested the efficacy of IVIg therapy in a murine model of AIHA induced by 4 IgG anti-mouse RBC antibodies: anti-GPA, -Kell, -band 3, and -CD24. We then evaluated the degree of RBC destruction attributable to FcγR- vs complement-dependent mechanisms using mice genetically deficient for the Fcγ chain (γ -/-) or complement C3 (C3-/-), respectively. Consistent with previous reports, we found a minimal role for complement-mediated mechanism in anemia caused by the antibodies. The γ -/- mice were found to develop 58% and 62% less anemia when injected with the anti-band 3 and anti-CD24 antibody, respectively, compared to WT mice, suggesting a role of FcγR-mediated RBC destructive mechanism induced by these 2 antibodies. In contrast, RBC destruction caused by the anti-GPA and anti-Kell antibody was not significantly different between γ -/- and WT mice. When IVIg (4g/kg total, 3 injections over 5 days) was infused into AIHA mice, significant response was only seen when anemia was caused by anti-band 3 or anti-CD24 antibody, where IVIg was associated with a 56% and 38% increase in RBC counts, respectively. Interestingly, a lower dose IVIg regime (2.5g/kg single injection), which effectively ameliorates thrombocytopenia in mouse models of ITP, did not increase RBC counts in anti-band 3-treated mice. In conclusion, similar to observations in human patients, our findings demonstrate a varied efficacy of high dose IVIg treatment in a mouse model of AIHA. With the use of 4 anti-mouse RBC antibodies we found 1) an association between response to IVIg treatment and presence of FcγR-dependent RBC destructive mechanism(s); and 2) treatment of AIHA may require a different (or more intense) IVIg regime than in other autoimmune cytopenias.

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Notes:

Effect of Room Temperature Exposure On Red Blood Cells Contamination

Oral Presentation

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Purpose: According to Canadian and to other international standards, red blood cell (RBC) units must be stored between 1 to 6°C and never exceed 10°C during their transport. During their storage, RBC units may be repeatedly exposed to ambient temperature and blood banks frequently refer to a 30-minute time frame to maintain units at the appropriate temperature. Interestingly, the 30-minute rule was never validated. In this study, we investigated the effect of repeated exposures at ambient temperature of 30 and 60 minutes on the growth of bacterial contamination.

Methods: SAGM RBCs and AS-3 RBCs (n=6 par arm) were split in three 150 mL PVC transfer bags. Each bag was spiked with 1 colony-forming unit (CFU)/mL of either *Serratia liquefaciens* (ATCC35551), *Serratia marcescens* (ATCC43862) or *Staphylococcus epidermidis* (ATCC49134). Each bag was then subdivided in three fractions in empty bags. Split RBC units were inserted between two 600 mL PVC bags filled with 100 mL of saline in order to simulate the temperature variation of routine RBC units. Temperature profiles were measured with temperature probes placed inside the bags. Contaminated RBC units were stored at 1-6°C for 42 days. On days 2, 7, 14, 21 and 42, RBC were exposed at 20-24°C for a period of 0 (control), 30 or 60 minutes at RT. Following exposure, bags were sampled and the CFU number was determined on blood agar.

Results: After being exposed at 20-24°C, the temperature of RBC bags reached $11.8 \pm 0.5^\circ\text{C}$ and $15.1 \pm 0.2^\circ\text{C}$ after 30 and 60 minutes respectively. As expected, *S. epidermidis* did not grow in RBC in any condition. At Day 42, for *S. liquefaciens*, the number of CFU was similar in bags exposed for 30 or 60 minutes ($2.8 \pm 1.1\text{E}9$ CFU/mL vs. $3.2 \pm 0.4\text{E}9$ CFU/mL in SAGM RBCs and $2.1 \pm 0.5\text{E}9$ CFU/mL vs. $2.0 \pm 0.7\text{E}9$ CFU/mL in AS-3 RBCs). *S. marcescens* did not grow in RBC/AS-3 while a 2 log increase in CFU was observed between day 21 and 42 in SAGM RBCs exposed for either 30 or 60 minutes.

Conclusion: Repeated exposure of RBC units for up to 60 minutes at 20-24°C has not significantly influenced the growth of bacteria in voluntarily contaminated RBC units. Additional experiments are required to validate these results on a larger number of microorganism species.

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Effect of Transitory Exposure of Red Blood Cell Units on their In Vitro Parameters

Oral Presentation

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Purpose: In Canada, red blood cell (RBC) units must be stored between 1 to 6°C and never exceed 10°C during transport. During storage, RBC units may be repeatedly exposed to ambient temperature and blood banks frequently refer to a 30-minute time frame to maintain units at the appropriate temperature. In this study, we investigated the in vitro parameters of RBC units exposed repeatedly at room temperature (RT) for 30 or 60 minutes at various time during storage.

Methods: SAGM RBCs and AS-3 RBCs (n=6 par arm) were split in three fractions in 150 mL PVC transfer bags. RBC units were then inserted between two 600 mL PVC bags filled with 50 mL of saline in order to simulate the temperature variation of routine RBC units. Temperature profiles were measured with temperature probes placed inside the bags. Split RBC units were stored at 1-6°C for 42 days. At days 2, 7, 14, 21 and 42, the RBC units were exposed at RT for 30 or 60 minutes. A series of control bags were kept at 4°C. Before returning the units to 4°C, samples were taken for in vitro measurements (complete blood count, ATP, 2,3-DPG, hemolysis, pCO₂, pO₂, potassium, sodium, glucose, lactate, pH and mechanical fragility).

Results: After being exposed for 30 and 60 minutes at 20-24°C, the temperature of RBC bags reached 11.8 ± 0.5°C and 15.1 ± 0.4°C, respectively. No statistically significant differences (p>0,05) were observed between in vitro parameters of RBC units exposed at RT for 30 or 60 minutes and those of the control units that remained at 4°C.

Conclusion: This work suggests that transitory interruptions of cold storage of RBC units do not measurably impair the blood product quality. On the basis of our results, it appears that the 30-minute limit could be extended to 1 hour without increasing risk for patients. Additional experiments are required to validate these results.

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Red Blood Cell Hemolysis: Comparing Quality Management Data To The New Canadian Standard

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Purpose: Hemolysis is the most apparent red blood cell (RBC) storage lesion. In Canada, the recently revised CSA standards on blood and blood components now specify that hemolysis of all RBC units tested must be less than 0.8% at the end of the shelf life. This standard is stricter than requirements in Europe and in the US. We evaluated the significance of this standard by reviewing hemolysis data obtained through our quality control program.

Methods: Data were collected between February 2010 and April 2011. RBCs derived from three methods of collection and processing were tested. Apheresis RBC units were prepared using Trima Accel (CaridianBCT) and stored in AS-3 (n=241). Whole blood-derived RBC units were produced from CP2D WB and stored in AS-3 (n=2655) (Pall Medical Corp.), or prepared using the Atreus system (CaridianBCT) from CPD WB and stored in SAGM (n=335). RBC units were all prestorage leukoreduced. Supernatant Hb concentration was measured after 42 days of storage using the HemoCue Low Hemoglobin system. The percentage of hemolysis was defined as follows: $[\text{Supernatant Hb}] \div [\text{Total Hb}] \times (1 - \text{Hematocrit})$.

Results: Overall, 1.2% (n=39) of all tested RBC units did not meet the hemolysis standard, despite adequate collection, processing and storage conditions. The mean percentage of hemolysis was 0.26 ± 0.22 (range: 0.07 - 6.47). The percentage of hemolysis was higher in SAGM (0.33 ± 0.15 ; range: 0.11 - 0.89) than in AS-3 RBCs (0.26 ± 0.23 ; range: 0.07 - 6.47; $p < 0.0001$). Percentage of hemolysis was 0.33 ± 0.15 (range: 0.11 - 0.89) with the Atreus system, 0.33 ± 0.13 (range: 0.14 - 1.04) with the Trima Accel and 0.25 ± 0.24 (range: 0.07 - 6.47) with the manual WB collection.

Conclusion: The results suggest a high degree of variability in hemolysis that may depend on certain donor factors, making the recently adopted Canadian standard difficult to meet. This information could contribute to the establishment of an adequate and realistic hemolysis standard.

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Notes:

Overnight Storage of Whole Blood: Validation of a New System to Rapidly Cool and Transport Whole Blood Units at 20-24°C

Oral Presentation

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Purpose: Overnight storage of whole blood (WB) prior to processing is generally performed with butane-1,4-diol cooling plates. Unfortunately, these cooling plates have not been designed to operate under extreme temperatures that may occur during transport of WB units. This study aimed to validate a new cooling system based on the Phase 22 material (TCP Reliable Inc.).

Methods: We investigated two extreme temperature scenarios that may occur during transport of WB bags from remote blood collection sites to the processing facility (winter scenario: -30°C; summer scenario: +40°C). The specially designed Phase 22 packs were conditioned at 18-22°C for at least 24 hours before use. The resistance of preconditioned Phase 22 packs to extreme temperatures during their transport to remote sites was tested for a period of 24 hours. Cooling efficiency was challenged with Atreus blood collection sets (CaridianBCT) filled with 495 mL of warm (~37°C) saline packed in the VIP (vacuum insulated panel) transportation boxes exposed for 12 hours at -30°C and +40°C. To mimic the transport of WB units to the processing facilities, warm saline Atreus collection sets (405 mL) were rapidly cooled with Phase 22 packs and stored at -30°C or +40°C for a 24-hour simulated transport.

Results: Phase 22 packs must be shipped to blood collection sites within 11h45 min for the winter scenario and within 19h20 min for the summer scenario respectively. Phase 22 packs were efficient to rapidly cool the temperature of bags filled with 495 mL of warm saline within 2h10min for the winter scenario, and within 2h55min for the summer scenario. Used with VIP boxes, Phase 22 packs are able to cool and maintain the saline bags' temperature between 18-24°C for up to 24 hours for the summer condition and for about 8 hours in the winter condition.

Conclusions: This validation study confirms the operational advantages of the new Phase 22 packs used with VIP transport boxes. This system is simple to operate, does not require temperature-controlled trucks for use at remote blood collection sites, and its operational range is superior to that of other commercial cooling systems.

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Residual Plasma Content of Red Blood Cells Units

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Purpose: In recent years, Héma-Québec has introduced several changes to automate its blood processing operations. We recently introduced automated plasma extractors (MacoPress, MacoPharma) and the Atreus/OrbiSac system (CaridianBCT) for whole blood (WB) derived platelet production. We wanted to characterize the content of residual plasma as well as the volume of red blood cell (RBC) units prepared with these different technologies. Despite several microbiological and biochemical standards, there are no national or international standards for residual plasma and volume for RBC units. In this work, we measured the content of plasma in three types of RBC products.

Methods: AS-3 RBC units (n=52) were produced using apheresis with Trima Accel instruments (CaridianBCT). AS-3 RBC units (n=60) were also produced from CP2D WB after plasma extraction with the MacoPress extractors. Finally, SAGM RBC units (n=60) were produced using the Atreus system from CPD WB. RBC units were weighted to calculate their volume. The amount of residual plasma in RBC units was determined based on the ratio of IgG, measured with a validated ELISA, in the RBC supernatant and in their respective plasma.

Results: The mean volume of RBC units was 278 ± 16 mL, 316 ± 24 mL and 327 ± 10 mL for units processed with the Atreus system, the MacoPress extractors and by apheresis. The volume of residual plasma was lower in RBC units produced with the Atreus system (23 ± 5 mL; 13 to 40 mL) than in RBC units processed with the MacoPress extractors (39 ± 12 mL; 14 to 71 mL) or by apheresis (38 ± 9 mL; 22 to 55 mL) representing about 8%, 12% and 12% of the total volume of RBCs, respectively. We found a direct correlation between the volume of RBC units and the amount of residual plasma. However, the volume of WB collection has no effect on the quantity of residual plasma in RBC units.

Conclusion: These results will be useful to review our blood processing specifications for RBC units. Currently, Canadian standards for RBC units do not specify quality control requirements for RBC volume and residual plasma.

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A Simple Device for Rapid Cooling and Transport of Whole Blood Under Extreme Temperature Conditions

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Purpose: Butane-1,4-diol cooling plates, used by blood banks for overnight storage of whole blood (WB), fail to preserve WB temperature when shipped from blood drives under extreme temperatures. Our group developed a unique system, based on a phase change material (Phase 22 from TCP Reliable Inc.) that rapidly cools WB to 20-24°C and better maintains its temperature when exposed to extreme conditions. In this work, the performance of this new system and of the CompoCool WB (Fresenius HemoCare) is compared.

Methods: Phase 22 packs were conditioned at 18-22°C for ≥ 12 hours while the CompoCool WB was used according to manufacturer's instructions. For temperature profiling studies, Atreus collection sets (CaridianBCT) were filled with 450 mL of 25% glycerol ($35^{\circ}\text{C} \pm 2^{\circ}\text{C}$) and chilled using Phase 22 or CompoCool WB systems. Cooling profiles were evaluated at their maximum capacity (6 bags) at 35°C, 24°C and 39°C. To study the impact of cooling systems on blood product quality, WB bags (n=24 per arm) were processed into leukoreduced blood products with the Atreus and OrbiSac instruments after an overnight hold. Blood product parameters were measured during storage.

Results: It took 1.7 ± 0.2 hours and 0.9 ± 0.2 hours to chill 25% glycerol bags to 20-24°C with Phase 22 packs and CompoCool WB, respectively. At -35°C, Phase 22 packs maintained the bags at a temperature of 20-24°C for 15.4 ± 2.7 hours, compared to 2.3 ± 0.3 hours with CompoCool WB. At 39°C, WB temperature increased gradually to exceed 24°C after 11.0 ± 0.9 hours with CompoCool WB, while it remained between 20-24°C with Phase 22 packs. No differences were observed for the in vitro quality of platelet concentrates and plasma. On Day 42, average RBC hemolysis was slightly higher (0.3 ± 0.2 % vs. 0.2 ± 0.1 %; $p < 0.05$) in units prepared from WB cooled with Phase 22 packs.

Conclusions: The new Phase 22 system has several operational advantages. The system is simple to use and reusable. Its implementation has facilitated our logistics of blood collection and transport operations as well as improving the use of blood collected at remote blood drive sites.

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Notes:

The Effect of Hemoglobin Concentration on RHD Genotyping Performed Directly from Whole Blood

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Complex and time-consuming procedures contribute to the lack of blood group genotyping in routine transfusion medicine practice despite its value in resolving serological discrepancies. Performing PCR directly from whole blood samples could reduce complexity and improve turnaround times by eliminating the need for DNA extraction procedures. However, the composition of whole blood is highly variable and may affect PCR results, as heme is known to inhibit the reaction.

To demonstrate whole blood RHD PCR, samples (n=6) with known RhD phenotypes were tested using previously published primers that amplify exon 5 and exon 7 of RHD and Omni Klentaq polymerase, an enzyme designed to be resistant to inhibitors when used with a PCR enhancement cocktail. Dilutions of D-positive whole blood in D-positive plasma were used to investigate the effects of hemoglobin concentration on whole blood PCR. Hemoglobin concentration of the D-positive whole blood sample was measured on an automated hematology analyser and used to calculate hemoglobin concentrations for the dilutions. Serial dilutions of genomic DNA in D-negative whole blood were used to create a standard curve for determination of reaction efficiencies. PCR with melt curve analysis was performed on an ABI 7500 fast PCR system using SYBR Green.

Exon 5 and exon 7 of D-positive samples were successfully amplified. No amplification was seen for D-negative samples. Both melting temperature (T_m) and fluorescence intensity are reduced as hemoglobin concentration increases. For an increase in hemoglobin concentration from 46 g/L to 155 g/L, T_m shifted from 81 °C to 79 °C for exon 5 and from 85 °C to 83 °C for exon 7. Over the same hemoglobin range, fluorescence intensity dropped by 78% for exon 5 and 77% for exon 7. Reaction efficiencies calculated by the ABI 7500 software from the standard curves for exon 5 and exon 7 targets were 88.2% and 87.6% respectively.

Although melting temperature and fluorescence intensity are affected by hemoglobin concentration, RHD genotyping can be performed directly from whole blood. This may facilitate the development of a point-of-care style platform and shift blood group genotyping from reference labs to routine transfusion services.

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Notes:

Rare Blood Donors Identified: A Success Story

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Case: A 27 year old O+ woman of Moroccan origin was referred to our Immunohematology Reference Laboratory (LRCS) for antibody identification. She suffers from sickle cell disease and was recently transfused (<3 months). Her obstetrical history was unknown to us.

Methodology and Results: The phenotype done at the hospital was the following: D+C-c+E-e+, K-, Fy(a-b-), Jk(a+b+), S-s+. The expected phenotype resulting from the genotype done by the LRCS confirmed this phenotype. The FY GATA box was mutated. When her serum was tested on a first red blood cell panel, all cells were reactive, except the autocontrol which was negative in gel LISS but positive in gel ficin. Stronger reactions were noted with some cells (D2, 1, 2, 3, 7) in gel ficin indicating an anti-C. The gel LISS eluate gave two negative cells (D1, 4) pointing at an anti-e. Differential alloadsorptions were performed and again the anti-C and anti-e seemed real. The RHCE mRNA was fully sequenced. A polymorphism was found at 733C>G in homozygous form. This particular polymorphism is known to cause the partial expression of c and e antigens, as well as a reduced expression of hrB (hrBw/-). The reason why this patient developed an anti-e was not elucidated. It could be the result of incompatible previous transfusions (true e+) or it could be an anti-e like which is really an anti-hrB.

Compatible blood: This patient is transfusion-dependant, compatible units will be needed. Since December 2008, Héma-Québec has been recruiting donors among cultural minorities. The phenotype and genotype of these new donors is established. So far, close to a thousand such donors have been screened and entered in our database. We were able to find 57 donors with the same polymorphism, 20 were ABO and D compatible. Nine red blood cell units were actually available in the frozen rare units.

Conclusion: An increasing number of transfusion-dependant patients with rare phenotype has been noted. The laborious screening work to identify rare donors is starting to pay off and will be continued to fulfill the increasing demand.

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Notes:

Impact of Reformulation of Ortho Clinical Diagnostic 0.8% Reagent Red Cells on Antibody Detection

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Background: In April 2004, Ortho Clinical Diagnostics (OCD) advised customers of assay limitations with the 0.8% reagent red cell product line. In January 2006, OCD reported the outcome of their investigations performed in response to concerns identified by customers that some weakly reactive antibodies (particularly, anti E and anti K) were going undetected. OCD determined that a component of the preservative used in the 0.8% diluent formulation interfered with some antibody detection. At that time, OCD notified customers that they were investigating options for product improvement. In April 2007, OCD began distribution of a reformulated 0.8% reagent red cell product. Post-implementation of the reformulated product, our staff identified concerns around increased incidences of cold reactive antibodies and unidentified antibodies which were impacting our ability to provide red cells in a timely manner.

Methods: A retrospective review of the reported positive antibody screen findings for 6 months pre reformulation (November 2006 to April 2007) and post reformulation (May 2007 to October 2007) of the 0.8% reagent red cells was performed. The frequency of overall reported positive antibody screens was identified. In addition, the reported frequencies of unidentified antibodies or cold reacting antibodies and reported anti K and anti E (identified individually or in combination with other antibodies) were determined.

Results: The total number of antibody screens performed, as well as the overall frequency of positive antibody screen findings (Pre 7.7% vs. Post 7.6%) , were comparable for the time periods reviewed. The frequency of reports identifying anti K and/or anti E demonstrated no statistical difference pre and post reformulation (Pre 31.4 %vs. 29.1%). In contrast, the frequencies of unidentified antibodies, as well as cold reactive or non-specific antibodies, were significantly increased post reformulation. Unidentified antibodies, pre reformulation 2.8 % vs. Post reformulation 5.4%, cold antibodies 7.0 % pre reformulation and 10.8% post reformulation.

Conclusion: The reformulated red cell product did not result in an increase in detection of weak reactive anti K and anti E antibodies. However, the frequencies of unidentified antibodies, cold reactive antibodies, and antibodies of no specificity were increased, supporting staff observations of non-value added additional investigations and product issue delays.

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Notes:

Using Fluorescent Cell Barcoding to Monitor the Effect of Blood Products on Mononuclear Cells

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Purpose: Red blood cell (RBC) transfusion is a life-saving treatment for patients suffering from anemia, but these blood transfusions are not without risks. The goal of this project is to establish a multiplex flow cytometry method to study the effect of the RBC supernatant on Peripheral Blood Mononuclear Cells (PBMCs). In order to do this, fluorescent cell barcoding (FCB) in flow cytometry, reported by Krutzik and Nolan (Nat. Methods, 2006), was adapted to monitor the phosphorylation level of signaling pathways, which can be directly related to cellular activation status.

Methods: PBMCs from healthy individuals were incubated in a serum free media for 4 hours in the presence or absence of stimulus enabling strong activation of PBMCs. A mix of phorbol myristate acetate, anti-CD3/CD28 beads and LPS was used to activate B cells, T cells and monocytes, respectively. In a second step, PBMCs were incubated for 24 hours either with storage solution or with supernatant of RBCs stored for 1, 7, 14, 21, 28, 35, and 42 days. Antibodies were used to measure phosphorylation of STAT1, STAT3, STAT5 and ERK1/2 and antibodies specific to CD3 and CD22 were used to target T and B cells specifically as well as monocytes, which are negative for both surface markers. Pacific blue and Dylight 800 fluorescent dyes were used to barcode our samples. All analyses were done by flow cytometry using a CyFlow ML cytometer with 10 parameters.

Results: A 3 X 5 matrix was developed to enable us to analyze the phosphorylation status of intracellular phospho-proteins of three independent PBMCs samples for five tested conditions simultaneously, namely 15 analyses in one tube. Furthermore, by combining phosphorylation status with extracellular staining for CD3+ T cells and CD22+ B cell populations, we were also able to differentiate each population within PBMCs. In the end, over one hundred analyses were combined into only four tubes. Also, the fact that the samples are combined prior to antibody staining reduces antibody consumption considerably and eliminates staining variability between samples.

Conclusions: We have adapted with success a simple, inexpensive and rapid method based on FCB, to monitor simultaneously the response of independent PBMC samples to several blood products.

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Notes:

Prevalence and Pattern of Hepatitis B Viral Marker Detection in Blood Donors at Canadian Blood Services Halifax/PEI

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Background: Current Canadian Blood Services (CBS) testing for the presence of the Hepatitis B virus (HBV) in blood donors involves universal screening of HBV DNA, Hepatitis B surface antigen (HBsAg) and Hepatitis B core antibody (anti-HBc). Supplementary testing includes Hepatitis B surface antibody (anti-HBs).

Methods: A retrospective review of donor files testing positive for a HBV marker at CBS Halifax/PEI from January 1, 2011 to February 1, 2012 was undertaken to characterize the test result pattern. All donors denied prior hepatitis and vaccination within the past three months on their Record of Donation. Information about previous HBV vaccination was obtained from donors who contacted CBS about their HBV-positive test results.

Results: Thirty-six donors tested negative for HBV DNA but reactive to one or more HBV seromarkers during the study period. Six donors tested anti-HBc repeat-reactive (RR) only. Twenty-seven donors tested RR for both anti-HBc and anti-HBs (17 new and 10 returning donors). Of the 10 returning donors who tested RR for anti-HBc and anti-HBs, 4 were documented to be HBsAg-negative less than 3 months prior to the reactive donation. Another four out of the 27 RR anti-HBc and anti-HBs reactive donors were documented to have had HBV vaccination remotely. Three scenarios were observed for three donors who tested HBsAg-RR: HBsAg neutralization negative with reactive anti-HBs; HBsAg neutralization positive with non-reactive anti-HBs; and HBsAg neutralization negative with anti-HBs not tested. The donor testing RR for HBsAg (HBsAg neutralization positive) and anti-HBs non-reactive received a vaccination within days of donation.

Conclusions: The majority of donors testing positive for a HBV seromarker were found to be RR for anti-HBc and reactive for anti-HBs which in conjunction with a non-reactive HBsAg and negative HBV DNA, most likely reflects previous, resolved acute HBV infection. An isolated RR anti-HBc result among 6 donors likely reflects either a false-reactive anti-HBc or a remote, resolved acute HBV infection with loss of anti-HBs. Recent HBV vaccination may cause a transient reactive HBsAg. Acquiring information from donors on the status of prior HBV vaccination may be of use in interpreting HBV test results.

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Intrauterine Fetal Transfusion in Severe Red Cell Alloimmunization

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Background: Poor perinatal outcome is associated with early severe Rh alloimmunization. Percutaneous umbilical cord blood sampling (PUBS) prior to 22 weeks is challenging due to difficulty in gaining fetal intravascular access. Many fetuses at this gestational age cannot tolerate transfusion because of acute hemodynamic changes.

Objective: To determine the outcome in 30 pregnancies less than or equal to 22 weeks.

Methods: Retrospective analysis of 30 patients requiring their first intrauterine transfusion (IUT) <22 weeks gestation from 1991 to 2011. Either the development of ascites or performing Doppler evaluation of the middle cerebral artery peak systolic velocity (MCA-PSV) was used to base the timing of the first IUT.

Results: Thirty three per cent of the patients had experienced a previous intrauterine fetal death as a result of red cells alloimmunization. Ten of these alloimmunized pregnancies were associated with Anti-D, 4 with Anti-K and 16 had more than one antibody involved. The antibody titres before the first IUT ranged from 1:32 to 1: 4096. All fetuses were severely anemic before the first IUT. The median gestational age at the first IUT was 20.4 (16-22 range) weeks and between 1 and 9 transfusions were needed during pregnancy. Transfusion was done via intraperitoneal 3%, intracardiac 7%, umbilical artery 7%, umbilical vein 20% or intrahepatic vein 63%.

Conclusions: Fetuses can be severely anemic without hydrops and prognosis cannot be predicted by the presence or absence of hydrops in early severe red cell alloimmunization. Early intrauterine transfusion followed by serial transfusions results in 80% perinatal survival.

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Notes:

Tattoo and Piercing Deferral

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In Canada donors are directly asked about history of tattoo, ear or body piercing during pre-donation screening and are deferred if they engaged in these behaviours in the last 6 months, but the safety benefit in modern times is questionable. We examined trends in these behaviours among donors by age and gender both with an anonymous donor survey and deferral records.

Methods: An anonymous questionnaire was mailed to 80,000 donors (40,000 first time and 40,000 repeat), with a 48% response rate. Donor records were accessed for deferral reasons.

Results: In the anonymous survey, 18% of female and 10% male donors had ever had a tattoo (20% of females <25, 8% of males under 25). 92% of females (13% of males) had ever had their ears pierced (90% of females under 25, 14% of males under 25). 18% of females (3% of males) had ever had body piercing (35% of females under 25, 8% of males under 25). For these behaviours occurring in the past 6 months, the percentage was highest in females under 25 years: 1.2% for tattoo, 2.4% ear piercing, 1.4% body piercing, and for males under 25: 0.7%, 0.6% 0.6%. For females under 25, the percentage of donating donors with these behaviours in the last 6 months was higher than those actually deferred (for behaviours in the last 6 months) 0.5%, 0.7%, 1.0% for females under 25 ($p < 0.05$), and for males under 25 it was similar between donating donors and those deferred 0.5% 0.4% 0.3% ($p > 0.05$).

Conclusion: Tattoos, ear and body piercing are very common behaviours among young donors, especially young women, the lowest risk segment of the population. As at least as many donors fail to disclose deferrable risk as do, the utility of this deferral criteria is questionable.

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ABO Confirmation of Pre-transfusion Patients and Donors

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Judith Hannon MD, FRCPC, Alberta Laboratory Quality Enhancement Program

Background: Canadian transfusion medicine standards require confirmation of the ABO group of pre-transfusion patients and donor red cell units only when an electronic crossmatch is performed. In that situation, the transfusion service(TS) must have two determinations of the patient ABO group and confirmation of the donor red cell ABO group. However, ABO confirmation is advocated by many laboratorians as one measure to ensure ABO compatible transfusion.

Methods: All western Canadian TS (n=138) participate in an Alberta based proficiency testing program. 25% of participants perform complex serologic testing and 75% perform basic testing only. Biannually, facilities complete a comprehensive pattern of practice profile, including questions related to ABO confirmation.

Results: 29% of participants perform electronic crossmatch and require two determinations of the patient ABO group and confirmatory donor ABO typing. All of these facilities perform the second patient typing if there is no acceptable historical ABO group, with the exception of one facility that performs the confirmation on all patients. Of the 98 facilities performing serologic crossmatch, 39 (40%) perform a second patient typing - 37 when there is no historical group, and two on all patients. Of all participating laboratories that perform a confirmatory typing, only six facilities require the confirmation be performed on an independent sample collected at a different time from the original sample. 87 (89%) of the facilities performing serologic crossmatch perform a confirmation of donor ABO groups – 75 on all units and 12 on selected units.

Conclusion: All western Canadian laboratories performing electronic crossmatch perform the required ABO patient and donor confirmation. Although not a mandated test for TS performing serologic crossmatch, 40% of those laboratories require a second ABO typing of pre-transfusion patients. A variety of testing schemes are employed but the majority of participants perform the confirmatory test on the blood sample used for the initial test. In these cases, the test is a control on laboratory procedures but does not confirm correct sample identification. As well, 89% of TS utilizing serologic crossmatch perform a donor ABO confirmation.

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Trends in Pre-transfusion Testing in Western Canada

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Background: Laboratory practices for pre-transfusion serologic testing vary as deemed appropriate by individual facilities. Review of peer practices is one criteria used by laboratories when evaluating local test schemes. Methods: All transfusion service laboratories in western Canada (n=138) participate in an Alberta based proficiency testing program. 25% of program participants perform complex serologic testing and 75% perform basic testing only. Biannually, facilities complete a comprehensive pattern of practice profile. The data is analyzed to determine current pre-transfusion testing practices and to evaluate testing trends for program strategic planning. The aggregate data is distributed to program participants.

Results: 2011 data has been compiled and compared to previous data to determine where significant changes have occurred. 72% of facilities utilize a tube test for ABO/Rh typing. 17% use a gel technique and 11% perform solid phase testing. There has been a steady decrease in the use of tube techniques for ABO/Rh typing in the previous decade from 92% in 2001. In 2011, 59% of program participants utilize gel technology for antibody screening, followed by PEG IAT (21%) and solid phase methods (18%). Since 2001, the rate of gel use has increased from 22% and solid phase from 2%. The most commonly used technique for crossmatch in 2011 is an immediate spin (41%) and an additional 29% perform an electronic crossmatch. This is a significant increase from the 2001 rates of 20% for immediate spin and 16% for electronic crossmatch. As well, the use of gel crossmatches has also increased from 10% in 2001 to 19% in 2011. Consequently, the use of tube IAT crossmatch methods has decreased from 53% in 2001 to 11% in 2011.

Conclusion: Although tube technique continues to be the method of choice for ABO/Rh(D) typing for western Canadian transfusion services, there has been a significant shift to the use of gel and solid phase technologies for ABO/Rh typing and antibody screening. The use of automated instruments also continues to increase. As well, the 2011 data indicates that the majority of facilities now utilize a crossmatch method designed to detect ABO incompatibility only.

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Resistance of Biofilm-forming Staphylococcus Epidermidis to the Skin Disinfectant Chlorhexidine

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Background: Canadian Blood Services implemented a one-step 2% chlorhexidine/70% isopropyl alcohol skin disinfection kit in 2009. Although the use of this kit has not impacted the true positive rate obtained during platelet screening, it does not eliminate all bacteria since contaminated units are still captured. We have shown that a variant of the skin contaminant Staphylococcus epidermidis carrying the icaADBC operon, which confers the ability to form surface-attached aggregates (biofilms), is distributed in the “healthy” donor community. This suggests resistance of the virulent biofilm phenotype to standard skin disinfection. The goals of this study were to compare susceptibilities of ica+ (biofilm-positive) and ica- (biofilm-negative) strains to chlorhexidine, and to evaluate the efficacy of 2% chlorhexidine in eradicating staphylococcal biofilms.

Experimental Approach: Minimum inhibitory concentrations (MICs) of chlorhexidine were established in Mueller Hinton broth for planktonic (non-biofilm) and biofilm cells of an ica+ Staphylococcus epidermidis strain, and its isogenic ica- mutant using the MBEC high-throughput assay (Innovotech). Each assay was repeated at least four times, each time with eight replicates. Additionally, $\sim 10^6$ CFU/ml of planktonic or biofilm cells of the ica+ strain were exposed to 2% chlorhexidine for 30 seconds simulating donor skin disinfection conditions. The survival of the cells post-chlorhexidine treatment was evaluated by serial dilutions and plating.

Results: The MIC of chlorhexidine for the ica- strain and ica+ planktonic cells was 1 mg/L while the MIC for ica+ biofilm cells was >32 mg/L. The ica+ planktonic cells did not survive the standard chlorhexidine treatment (2%), whereas exposure of ica+ biofilm cells to the same concentration only caused a reduction in cell count from $\sim 10^6$ to $\sim 10^3$ CFU/ml .

Discussion: It is known that biofilms present increased antibiotic resistance in comparison to their planktonic counterparts. Our results suggest that biofilm-forming isolates of the predominant skin contaminant Staphylococcus epidermidis, which are distributed in the otherwise healthy donor community, are more likely to resist killing by 2% chlorhexidine. Future studies will investigate the survival of treated biofilms in platelet preparations and whether combining 2% chlorhexidine/70% isopropyl alcohol would be more effective in destroying staphylococcal biofilms than 2% chlorhexidine alone.

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S-303 Treatment System for Pathogen Inactivation of Red Blood Cell Components (RBCs)

Oral Presentation

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Background: The S-303 Treatment System for RBCs uses S 303 to crosslink nucleic acids and prevent replication of contaminating pathogens and leukocytes. Glutathione (GSH) is included to quench non-specific reactions. A second generation S-303 treatment process has been developed, evaluated successfully in a Phase 1 clinical study, and clinical evaluations in patients with acute and chronic anemia are planned.

Methods: RBCs in SAGM were processed from CPD WB (450mL) held at 4°C or controlled ambient temperature prior to separation. On D (Day) 1 WB units were leukocyte-filtered, separated into platelet poor plasma and RBC suspended in SAGM. For RBC function studies, each replicate utilized ABO matched pairs which were combined and split into units of 274-344mL (n=6 per WB hold condition). Each replicate consisted of a Control (C) that was stored at 4°C and Test (T) that was processed with the S-303 system. T and C RBCs were stored at 4°C for 35D. For pathogen inactivation (PI) studies, RBCs (ambient WB hold) were spiked with pathogens (*S. aureus*, *Y. enterocolitica*, *E. coli*, *S. marcescens*, Ad5, CA-HIV, BV, BVDV; n=4) and then processed. The units were incubated for 3 hours at ambient temperature before sampling for residual viable organisms by growth on rich agar plates (bacteria) or cell culture (viruses).

Results: Post-treatment, the Hb content of T was $55.7 \pm 4.3g$ (n=12), with a process loss of $2.5 \pm 1.6\%$. On D35 hemolysis was lower in T units (0.19%) compared to C units (0.27% 4°C WB hold, 0.29% ambient WB hold; $p < 0.05$). Some attributes were statistically different between T and C (Hct, pH, K+, lactate, ATP) however, results were within physiological ranges. Viruses were inactivated to or below the limit of detection (> 4 log) and inactivation of all bacterial species was greater than 4 log.

Conclusions: The Second Generation S-303 Treatment System is compatible with RBCs derived from WB with 4°C, or ambient overnight hold condition. There was minimal loss in hemoglobin due to the S-303 treatment process. S-303 treated RBCs met current EU and US guidelines for leukocyte-reduced RBCs in additive solution. Pathogen inactivation efficacy was demonstrated across a range of bacteria and viruses.

Notes:

Inactivation of Transfusion-Transmitted Tick-Borne Pathogens

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Background: Several blood-borne pathogens of particular significance in north eastern North America are primarily transmitted to humans by tick vectors, specifically Ixodes species. These pathogens are problematic in transfusion medicine because donors may be repeatedly exposed to the vectors and infections are frequently asymptomatic, rendering both donor screening questions and physical findings insensitive.

The INTERCEPT Blood System™ for pathogen inactivation of platelet and plasma components was developed to prevent transfusion-transmitted infections. This proactive approach inactivates high levels of the commonly tested pathogens (HIV, HBV, HCV, HTLV-I/II, West Nile virus and *T. pallidum*), as well as a broad spectrum of cell-free and cell-associated, enveloped and non-enveloped viruses, gram negative and gram positive bacteria, protozoa parasites and residual leukocytes in platelet and plasma components. Treated components have demonstrated retention of therapeutic capacity in randomized controlled clinical trials and post-marketing surveillance studies.

Aim: To determine the effectiveness of the INTERCEPT Blood System for inactivation of important tick-borne pathogens in platelet and/or plasma components.

Methods: In these studies plasma (~600 mL) and platelet components (2.5 – 6.0 x10¹¹ platelets in ~300 mL of 35% plasma/65% InterSol™) were inoculated with pathogens to a target titer of 10⁶ viable pathogens/mL, or the highest titer feasible in the assay system. Inoculated units were treated with 150 μM amotosalen and a 3 J/cm² UVA treatment. Infectious titers were measured before and after treatment. The viability of *Anaplasma phagocytophilum* and *Babesia microti* was determined in mouse infectivity assays and viability of *Borrelia burgdorferi* was assessed by growth in culture medium.

Results: Several pathogens that are transmitted to humans by Ixodes ticks were effectively inactivated in platelets and plasma. Inactivation achieved in plasma: *A. phagocytophilum* >4.2 logs, *B. burgdorferi* >10.6 logs^a and *B. microti* >5.3 logs^b (all n=4). Inactivation achieved in platelets *B. burgdorferi* >6.8 logs^c (n=4), *B. microti* >5.3 logs^b (n=2). Inactivation of *A. phagocytophilum* was not evaluated in platelets.

Conclusion: The INTERCEPT Blood System is highly effective for inactivation of a spectrum of transfusion transmitted tick-borne pathogens. All pathogens tested were inactivated to or below the limit of detection by INTERCEPT treatment.

^a Singh, et al. *Transfusion*. 2006. 46:1168

^b Grellier, et al. *Transfusion*. 2008. 48:1676

^c Lin, et al. *Transfusion*. 2004. 44:1496

Stability of Cryosupernatant Plasma Thawed and Refrigerated for up to 120 Hours

Oral Presentation

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Background: Frozen plasma (FP) for transfusion can be further processed into cryosupernatant plasma (CSP) and cryoprecipitate by slow thawing and centrifugation. CSP is partially depleted of von Willebrand factor (VWF), FVIII, FXIII, and fibrinogen, but retains other factors such as ADAMTS13 and vitamin K-dependent factors such as FVII. It is currently indicated in the management of patients with thrombotic thrombocytopenia purpura (TTP) requiring plasma exchange, or in urgent reversal of warfarin anticoagulation. Both the current CSA standards and the CBS Circular of Information require that CSP be discarded after 24 hours of refrigerated storage. FP can also be employed for both CSP indications, and can be refrigerated for up to 120 hours prior to transfusion.

Objective: To determine if the period of CSP refrigerated storage could be extended from 24 to 120 hours to reduce product wastage at outdate. **Methods:** Thirty-six CSP units (18 type O, 18 type non-O) were thawed and refrigerated for 120 hours. Samples were aseptically obtained at thaw, 24, 48, and 120 hours post-thaw. Units were tested for bacterial contamination using the BacT/ALERT system at the end of the study period. Samples were tested for FV, FVII, FVIII, and fibrinogen activities using an automated coagulation analyzer, and for ADAMTS13 and VWF activities using ELISA-type assays.

Results: All units were negative for bacterial contamination. No significant differences were observed between values at thaw or those measured 120 hours later for fibrinogen, VWF, or FVIII. Mean ADAMTS13 activity levels did not differ from Day 0 values after 48 hours of refrigerated storage, exhibited a decline of only 2.6% by Day 5, and remained above the theoretical 1.0 IU/mL normal value. FV and FVII activity losses by 120 hours were 7.7 and 11.9% of at thaw values, respectively; the FVII reduction resembled that seen in FP refrigerated for 120 hours (-14%) and was greatest in the first 24 hours of refrigerated storage.

Conclusion: Plasma protein factors relevant to both indications for CSP transfusion were well maintained after 120 hours of refrigerated storage, suggesting that the outdate for CSP could be increased from 24 to 120 hours.

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Fluorescent Cell Bar Coding: A simple method to help in multiple myeloma diagnostics and prognostics

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Multiple myeloma (MM) is a complex disease defined as malignant growth of plasma cells in the bone marrow, leading to diverse clinical symptoms. Unfortunately the disease remains incurable and a survival of 3 to 7 years is expected for treated patients. Currently, MM diagnosis is mainly based on examination of bone marrow biopsy along with the presence of monoclonal immunoglobulin and diseases-related organ dysfunction. The clinical criteria for diagnosis and prognosis are however relatively subjective. To help better characterize MM, we propose here a flow cytometry approach, using the frozen bone marrow aspirates from patients to probe the physiological responses of primary MM plasma cells to cytokines. We have been using the fluorescent cell barcoding (FCB) methods as reported by Krutzik and Nolan (Nature Methods, 2006) and combined phospho-specific flow cytometry to extracellular phenotyping using antibody specific to CD38 and CD138. Flow cytometry analyses were done on seven human cell lines, mononuclear cells from healthy participant as well as one MM bone marrow aspirate. Our observations demonstrated that it is possible to analyze the specific response of plasma cells to a cytokine-panel without further purification or culture of the cells. This information may then be used to define an activation profile of the plasma cells, which could help clinicians to provide a more precise diagnosis or prognostic. Such method could also be of interest for researchers in the MM field, to study the cell physiology of primary MM cells even with a relatively limited amount of material, and without culturing.

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Bacterial Contamination of Platelet Concentrates: Implication of Negative Culture When Retesting the Blood Product After a Positive Result With The BacT/ALERT 3D

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Purpose: Bacterial screening of platelet concentrates (PCs) is now routinely done in several countries. Due to a high rate of false-positives results, it is a general practice for blood banks to inoculate the positive culture in a fresh bottle and repeat the test on a second sample of the suspected systems. If positive results are repeated, the contamination of PCs is confirmed. Recently, the presence of *Bordetella holmesii* was detected in the primary culture of two PCs with the BacT/ALERT 3D system (BioMérieux). PFGE analysis showed that the two bacterial strains were not related. Repeat culture of the implicated PCs after a 3-day storage failed to identify the presence of bacteria, making us suspect a low bacteremia in the donor’s blood and the inactivation of *B. holmesii* during storage. Interestingly, *B. holmesii* is a rare cause of human infections and have been reported to cause bacteremia in splenectomized patients. In this work, we investigated the growth of these *B. holmesii* strains in PCs.

Methods: Pool and split PCs from 3 donors were spiked with 1 and 10 CFU/mL of *B. holmesii* and *Serratia marcescens* (ATCC 43862) (control) (n=3 per condition). After spiking, PCs were stored at 20-24°C with agitation for 7 days. Samples were collected on days 2, 3, 4 and 7 to determine the number of CFU on blood agar. Samples were also used to inoculate BPA bottles which were incubated in the BacT/ALERT 3D system.

Results: On blood agar, no significant growth was observed for two strains of *B. holmesii* spiked at 1 or 10 CFU/mL in PCs during storage. *S. marcescens* grew rapidly to reach up to 6E18 CFU/mL at day 7 in PCs. The BacT/ALERT 3D system detected sporadically the presence of *B. holmesii* in spiked PCs on days 3, 4 or 7 while the all PCs spiked with *S. marcescens* were positives.

Conclusions: *B. holmesii* is probably surviving in PCs during storage, but at a very low number, making its detection difficult with the BacT/ALERT 3D, especially after prolonged incubation in the platelet concentrate. Using a negative result upon retesting of the positive product to exclude bacterial contamination of the product may not be appropriate for certain microorganisms.



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Validation of Buffy Coat Platelet Production with the Atreus and OrbiSac Blood Processing Devices

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Purpose: The need for platelet concentrates (PCs) increases about 10% yearly. To maintain an adequate inventory of PCs, Héma-Québec has converted its production of whole blood (WB)-derived PCs from the Plasma-Rich Platelet production method to buffy coat-derived platelets with the automated Atreus/OrbiSac system (CaridianBCT) that allows preparation of blood products from overnight-stored WB. In this work, the validation study of the Atreus/OrbiSac system implementation in our routine operations is reported.

Methods: WB (450 mL) was collected with Atreus collection sets containing 63 mL CPD. Within 30 minutes from collection, WB was rapidly chilled and stored at 20-24°C using Phase 22 cooling packs (TCP Reliable Inc.) until processing into blood products. The 2C+Fresh (n=160) and 2C+Overnight (n=164) Atreus programs were used to prepare leukoreduced RBC units, plasma and BCs from WB stored for either 2-16 hours or 14-24 hours, respectively. A total of 60 PCs were prepared by pooling 5 BCs, either from fresh or overnight-stored WB, using the OrbiSac device. Blood products were tested against the CAN/CAS-Z902-10 and the AABB standards.

Results: For RBCs, no difference was observed between blood products processed with fresh WB (2C+Fresh) and overnight-hold WB (2C+Overnight) for all QC tested parameters (Hematocrit, Hemoglobin, RBC recovery and residual leukocytes). The mean end of storage hemolysis was slightly higher in RBC produced from overnight-stored WB ($0.3 \pm 0.2\%$ vs. $0.4 \pm 0.2\%$) and 11 units exceeded the 0.8% acceptable limit. The volume of plasma units was similar with 264 ± 20 mL and 278 ± 19 mL for fresh and overnight-hold WB. Residual leukocytes content, pH at day 5 and bacterial contamination results of PCs were also comparable. The platelet yield was, however, higher in PCs produced after overnight hold ($3.7 \pm 0.7E11$ /unit vs. $4.5 \pm 0.5E11$ /unit).

Conclusion: Blood products produced with the Atreus/OrbiSac system satisfy the CSA standards, except for the recently introduced end-of-storage hemolysis limit of $<0.8\%$ for all tested units. Since excess hemolysis in a limited number of units might be associated with unknown donor-related factors, this result was considered insufficient to prevent implementation of the Atreus/OrbiSac processing system.

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Antibody Mediated Immune Suppression(AMIS) to Foreign Erythrocytes in a Murine Model: Timing of AMIS induction

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It is well-known that administration of anti-D to prevent hemolytic disease of the fetus and newborn (HDFN) should be given within 72 hours of delivery to an Rh-negative woman delivering an Rh-positive infant. In some cases anti-D may still have some residual protection when is given beyond the three-day time period although a full understanding of the phenomenon has not yet been achieved. Anti-D can also prevent immunization to transfused red cells. To evaluate the effectiveness of delayed antibody administration on immune responses, we employed immunization of normal mice with murine erythrocytes from mice expressing a foreign antigen. HOD mice express a transgene which directs the erythroid expression of a well characterized antigen denoted as HOD (Hen egg lysozyme (HEL) in sequence with ovalbumin and the human duffy antigen). Transfusion of normal mice with HOD erythrocytes induces the production of IgG to the HOD antigen. In the present study, we investigated if the AMIS effect could be induced in mice challenged with HOD-RBC. Initially, we transfused mice with different numbers of HOD-RBCs, and IgM and IgG of anti-HEL and anti-OVA in the serum were detected by ELISA. In addition, anti-Duffy IgM and IgG was determined by flow cytometry using Human Duffy positive RBC. We observed that an immune response only to HEL occurred. To determine if IgG anti-HEL antibody could be used to produce an AMIS effect (similar to the use of anti-D to prevent immunization to the D antigen), mice were challenged intravenously with 10E7 HOD-RBC followed by IgG anti-HEL (clone, 4B7) at a dosage of 2ug/mouse concurrently as well as at -24hr, 5min, 1hr, 24hr, 48hr, and 72hr after transfusion. The immune response to HEL was significantly inhibited by IgG anti-HEL at all-time points except 72 hrs. These data are the first experiments to show an AMIS effect in a fully murine model. They also demonstrate (using this model antigen) that the timing of administration of an anti-RBC antibody in suppression of the murine immune response to red blood cells can be an important parameter in successful AMIS induction.

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The Density of an Antigen on Erythrocytes can be Important in Mediating an Immune Response

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Erythrocytes possess many antigens, and some are highly immunogenic, such as the D-antigen. Although there appears to be a relationship of antigen density to immune hemolysis, less is known about antigen density on erythrocytes and the induction of IgM and IgG immune responses. We developed a model of erythrocyte immunization and have assessed antigen density by using mouse RBCs (mRBCs) coupled with a well-known antigen termed Hen Egg Lysozyme (HEL) at both high and low antigen density. Coupling of HEL to the erythrocytes was accomplished using 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide HCl (EDAC). The HEL density on the mRBCs was assessed by flow cytometry. C57BL/6 mice were challenged intravenously with nothing (Nil), or up to 10^9 erythrocytes (~ 1 unit) per mouse in a single transfusion. Erythrocytes were injected as either low or high antigen density RBC. Anti-HEL IgM and IgG antibody in the serum were assessed by an antigen specific ELISA on day 6, 12 and 20 post-transfusion. The immune response to low or high antigen density RBC were both quantitatively and qualitatively different. A high antigen density RBC did not induce an IgM immune response at any dose of red cells tested. The IgG immune response to these RBCs was more pronounced as the number of erythrocytes was decreased. The predominant IgG isotype produced was IgG1 but all the other murine isotypes (IgG2a, IgG2b and IgG3) were also produced. The immune response to low antigen density RBC did surprisingly lead to the production of IgM observable only on day 6. There was a direct positive relationship between the number of these RBCs transfused and the magnitude of the IgM response. The predominant IgG isotype produced to low antigen density RBC was IgG2a with only a minimal amount of IgG1 produced. Low antigen density RBC also stimulated the production of the other two IgG isotype's (IgG2b and IgG3). These data suggest that the density of an antigen on an erythrocyte may have an important impact on both the qualitative and quantitative aspects of the humoral immune response.

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