

THE COLLECTION EXPERIENCE FROM A PILOT CORD BLOOD PROGRAM IN WESTERN AUSTRALIA

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Cord blood has been recognised as a valuable source of stem and progenitor cells since the first successful related cord blood transplant in 1988 in Paris. In 1998 the Australian Red Cross Blood Service - North West Region, in collaboration with King Edward Memorial Hospital, embarked on a Cord Blood Pilot Study in order to establish and optimise collection, processing and storage procedures with a long term view to banking cord blood in Western Australia.

During the pilot study, information regarding the donation of cord blood was made available to potential donors via the Pre-admission Clinic for Caesarean Section. Informed consent to both collect and process cord blood was obtained. The time of delivery was pre-determined. Cord blood was collected from delivered placentae. The delivery process was not altered in any way to facilitate the collection of cord blood, therefore there was no risk to the infant or mother. Cord blood was collected aseptically into CPDA- 1 anticoagulant by cannulation of the umbilical vein and gravity.

A total of 40 deliveries were attended. In 17% (n=7) of these, cord blood was not collected due to delivery circumstances or the condition of the placenta. Of the 33 collections attempted, 63% (n=21) were potentially suitable for banking, based on a total nucleated cell yield of greater than 500×10^6 . This represents 52% of the total deliveries attended. The remaining 37% (n=12) had insufficient nucleated cell yields. Of the potentially bankable units, a further 62% (n=13) were bankable after a manual buffy coat separation technique. This equates to 32% of the total deliveries attended. The post processing total nucleated cell recoveries ranged from 57% to 89.3% with a mean of 73%.

The aseptic collection technique was validated by examining microbial contamination. Samples of each collection were tested at Princess Margaret Hospital, Department of Microbiology. Results showed no growth for both aerobic and anaerobic bacteria in 100% of units tested. The success of collection was dependent on procedures adopted in theatre, experience of collectors and the condition of the placentae. Attempts have been made to address factors which have adversely affected collection outcome.

The cord blood pilot study has provided important information regarding successful cord blood collection rates from women delivering by caesarean section. This has implications for human and financial resources required for establishing a cord blood bank. A method of distributing information to women for donating cord blood has been established collection techniques

have been validated and the collection rate from caesarean sections determined. In the event that funds become available, it is hoped that this activity will expand to include all women who deliver at King Edward Memorial Hospital in the future. We acknowledge that there will be a greater risk of bacterial contamination in collections from vaginal deliveries but potentially a greater success in obtaining bankable units.

A NEW MONO-COMPONENT FACTOR IX CONCENTRATE: A PHARMACOKINETIC STUDY AND SINGLE CENTRE EXPERIENCE

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MonoFIX-VF is a new mono-component, plasma derived factor IX concentrate with two viral inactivation steps in the manufacturing process to enhance safety. A clinical trial of the pharmacokinetics of MonoFIX-VF was conducted in 12 subjects with severe haemophilia B. In the post marketing setting, a single centre experience administering MonoFIX-VF via continuous infusion is reported. Methods: Subjects were administered a single bolus infusion of 50IU/kg of MonoFIX-VF in the pharmacokinetic study. Blood samples to determine factor IX levels were collected prior to the infusion and 10, 30 and 60 minutes and 3, 6, 9, 12, 24, 30, 36 and 50 hours post-infusion. The half-life, recovery, total body clearance, volume of distribution at steady-state, mean residence time and area-under-the curve were assessed. # The post marketing experience relates to subjects who were administered a bolus infusion of MonoFIX-VF to achieve a desired therapeutic level of factor IX: 0.5-1IU/mL in surgical subjects and >0.3IU/mL for subjects with haemarthroses. MonoFIX-VF was then reconstituted according to the manufacturer's instructions and then transferred into a polyvinylchloride (PVC) bag for administration. The continuous infusion was commenced within four hours of the bolus infusion being administered. Factor IX levels were measured four to six hours after the infusion commencing and then daily thereafter. A one stage clotting assay was used to perform factor IX levels, with plasma factor IX standard used as a control. Results: The pharmacokinetic study of MonoFIX-VF following a single bolus infusion of 50IU/kg of MonoFIX-VF shows MonoFIX-VF to have a half-life of 23.9+/-3.3 hours and a recovery of 60.7+/-16%. Four male subjects were treated for ten bleeding episodes, including five surgical procedures with MonoFIX-VF in this single centre experience. The surgical procedures included three arthroscopies (one arthroscopic washout), removal of an infected knee prosthesis and the revision of a total knee reconstruction. One subject required MonoFIX-VF to treat a thigh bleed, while another required treatment for two hip bleeds and a knee bleed. MonoFIX-VF was reconstituted at 50IU/mL and infused continuously with a piggy-back normal saline drip, to maintain factor IX levels above 60% (normal range 50-150). Surgical haemostasis was achieved and haemarthroses settled in all subjects. Conclusion: The pharmacokinetic data gained from the pharmacokinetic study revealed a half-life and recovery similar to other high purity factor IX concentrates in the literature. It has been demonstrated in this small cohort of subjects that MonoFIX-VF can be administered as a continuous infusion with predictable recovery and clearance. # Acknowledgment – Professor Kevin Richard The Royal Prince Alfred Hospital, Dr Ross Baker Royal Perth Hospital, Dr John Rowell The Royal Brisbane Hospital, Dr John Lloyd Royal Adelaide Hospital

THE PHARMACOKINETICS, SAFETY, TOLERABILITY AND EFFICACY OF A HIGHLY PURIFIED FACTOR VIII CONCENTRATE IN THE TREATMENT OF HAEMOPHILIA A.

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Biostate is a new, highly purified plasma derived factor VIII concentrate with two virus inactivation steps in the manufacturing process to enhance safety. A clinical trial to investigate the safety, tolerability and efficacy in 30 subjects with haemophilia A over a six month period in Australia and New Zealand#. A subset of subjects undertook an initial pharmacokinetic assessment prior to entry into the safety, tolerability and efficacy study. A repeat assessment was conducted between three and six months of the initial pharmacokinetic study. The aim of the study was to assess the safety, tolerability and efficacy of Biostate in the treatment of severe haemophilia A. The aim of the pharmacokinetic study was to assess subjects for a change in pharmacokinetic parameters of greater than 20% in the initial and repeat studies. A change of this magnitude would have been considered clinically significant. Results: The half-life of Biostate was 12.4 hours in the initial study and 14.1 hours in the repeat study. The recovery of Biostate was 107% and 110% in the initial and repeat studies respectively. There were no clinically or statistically significant changes in the pharmacokinetic parameters in subjects who participated in the initial and repeat pharmacokinetic study. A total of 1416550IU were administered during the study. The mean dose of Biostate administered to each subject was 1390IU per infusion. The average responses to treatment with Biostate were excellent (13%), good (60%), moderate (27%) and poor (0%). There were no product-related viral seroconversions during the safety, tolerability and efficacy study. There was one serious adverse event during the study that was not related to Biostate. The most commonly reported adverse events that were related or possibly related to Biostate were headaches (8 episodes, 0.8% of infusions) and back pain (3 episodes, 0.3% of infusions). These events occurred in two subjects. The other adverse events considered to be related or possibly related to Biostate included; mild chest pain and anxiety (0.2% of infusions), skeletal pain, arthralgia, flushing and dizziness (0.1% of infusions). All episodes resolved without sequelae. Conclusions: The results of this study assessing the safety, tolerability and efficacy of Biostate in subjects with haemophilia A indicate that Biostate is well tolerated. Biostate is a safe product with no product-related viral seroconversions or factor VIII inhibitor development. The initial and repeat pharmacokinetic parameters are in close agreement. # Acknowledgment – Professor Kevin Richard The Royal Prince Alfred Hospital, Professor Alison Street The Alfred, Dr Ellen Maxwell The Alfred, Dr Ross Baker Royal Perth Hospital, Dr John Rowell The Royal Brisbane Hospital, Dr Paul Ockelford Auckland Hospital, Dr John Carter Wellington Hospital, Alison Pritchard CSL Bioplasma Division.

IgA Levels in Chromatographically Purified IgG

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The potential for adverse reactions following the administration of IgA containing commercial preparations of IgG represents a serious problem for IgA deficient patients. At present most available commercial preparations are manufactured using classical Cohn fractionation procedures. Although Cohn fractionation techniques are capable of providing IgG in a relative pure form, literature reports have indicated that some preparations are contaminated with IgA in levels greater than 100 μ g/ml. It is for this reason that most manufactures of IgG preparations warn against their use in IgA hypersensitive individuals. CSL, Australia has recently replaced its Cohn fractionated IgG product Intragam $\text{\textcircled{R}}$ with the chromatographically purified product Intragam $\text{\textcircled{P}}$. The chromatographic procedure which also incorporates two viral inactivation stages generates a highly purified IgG preparation. We were thus interested in comparing the IgA content of chromatographically purified IgG with that obtained by Cohn fractionation. This was achieved following the development of a highly specific ELISA technique capable of detecting IgA to levels less than 50ng/ml. The results obtained indicate that the IgA content in the chromatographically purified product Intragam $\text{\textcircled{P}}$ is below 2.5 μ g/ml while Cohn fractionated material contains IgA levels between 25-185 μ g/ml. It is expected that the significantly lower levels of IgA in the product Intragam $\text{\textcircled{P}}$ will pose a reduced risk of anaphylaxis following administration in IgA sensitive patients.

DEVELOPMENT OF A BIOSENSOR ASSAY FOR ROUTINE TESTING OF Fc FUNCTION OF HUMAN IMMUNOGLOBULIN PRODUCTS.

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The efficacy of immunoglobulin preparations as immunotherapeutic agents is dependent on retainment of Fc and Fab biological activity during manufacture and storage. The current British Pharmacopoeia (BP) assay for measuring Fc function in intravenous immunoglobulin G (IVIg) preparations involves assessment of haemolysis of rubella virus antigen coated human red blood cells. This assay is time consuming, labour intensive, insensitive and is reproducible only when performed by an experienced operator. The development of an alternative assay, which is rapid, robust and capable of being automated for routine testing of IgG products, is therefore desirable.

In this study we utilised the Biacore, which allows the monitoring of surface plasmon resonance changes to quantitate the interaction of the Fc region of the immunoglobulin molecule with the FcRII receptor as a means of assessing Fc integrity. This interaction occurs within seconds and therefore has the potential to form the basis of a rapid assay. Specifically the methodology involved immobilising human FcRII receptor onto the dextran matrix of a Biacore CM5 sensor chip and then passing IgG preparations over the immobilised receptor and monitoring the optical changes associated with the interaction between ligand and receptor. The specificity of the interaction was established by showing decreased binding following exposure of IgG to the enzyme pepsin, which is known to compromise Fc through proteolytic cleavage. The results obtained in these studies correlated with values obtained using the recommended BP, Fc method. Therefore these data indicate that the Biacore utilising an immobilised Fc receptor can be utilised to develop a specific, rapid method for measuring the Fc function of IgG solutions.

TRANSFUSION REVIEW NURSE

Board Number: 106

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Current management practices acknowledge the value of assessment and evaluation in the delivery of quality service. The American Association of Blood Banks recommends the monitoring of transfusion practice and peer review to promote efficiency and excellence in the practice of transfusion medicine. Hospitals in other countries have found it advantageous to employ a registered nurse with specific responsibility in this area.

With the support of the hospital transfusion committee, the Blood Bank at Middlemore Hospital appointed a Transfusion Review Nurse in June 1997. The job description for this position included responsibility for monitoring of transfusion practices, promote staff awareness of policies and protocols related to the use of blood and blood products and response to queries from patients and their families related to this area of treatment.

This role has provided an interface between the laboratory and clinical staff and become a means to identify and eliminate problems which undermine good clinical practice. It has improved standards of documentation, provided staff education about transfusion medicine and been a tool to provide information and support for patients receiving blood products.

"HAEMOGLOBIN OF 2 - WHAT DO WE DO?" OPTIMISING PERIOPERATIVE CARE OF SEVERELY ANAEMIC PATIENTS WHO DECLINE BLOOD PRODUCTS, WITH EMPHASIS ON ANAEMIA PREVENTION

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Introduction: A small but significant proportion of patients will elect *not* to receive blood products for religious or other reasons. Insufficient haematological reserve to cope with traumatic or surgical blood loss can be life-threatening, particularly if Hb falls below 5g/dL¹ (50g/L). Workup for elective surgery may be inadequate due to lack of awareness on the part of patients or health care providers, or a belief that "it won't happen to me". Presentation of two anaemic patients "*in extremis*" to RPH (nadir Hb 16 and 18g/L, respectively) prompted development of a clinical protocol and information package designed to: 1) facilitate immediate aggressive management of acute anaemia, including optimal delivery of oxygen, drugs and nutrition, and 2) increase awareness of anaemia prevention by means of patient education, preoperative consultation, haematological workup, Hb maximisation and intraoperative, bedside and laboratory blood conservation techniques.

Method: Literature review and multidisciplinary clinical experience were combined to develop a guideline for bloodless perioperative management of acute anaemia in critically ill adult patients. Particular emphasis was placed on provision of drug information, so that initiation of therapy would not be delayed by uncertainties concerning IV iron and epoetin (erythropoietin) formulations, cost, efficacy, after-hours availability, dosing, administration, monitoring and adverse effect potential. This guideline is kept with relevant clinical papers in the "Jehovah's Witness" file in the RPH ICU.

Results: Usual haemoglobin maximisation "rescue" regimen for severe anaemia:

Day 1: Iron polymaltose IV infusion (see Ferrum-H® product information; do not exceed "6g/dL" dose) with epoetin (albumin-free) 300units/kg slow IV, hydroxocobalamin 1000mcg IV infusion, folic acid 15mg IV

Then: Repeat epoetin 300 units/kg IV daily OR alternate days OR 3 times per week, according to clinical condition and continue folic acid 15mg IV daily

Plasma volume expansion is achieved using crystalloid and/or succinylated gelatin (Gelofusine®, B. Braun) solutions. Pentastarch 10% (imported via Therapeutic Goods Administration Special Access Scheme) may occasionally be needed if crystalloid and succinylated gelatin solutions provide inadequate plasma volume expansion.

This team approach has been successful for patients with intraoperative haemorrhage or multiple trauma and Hb 30-80 g/L on transfer to ICU. Use of the "rescue" regimen in 5 severely anaemic patients resulted in clinically significant Hb rise: nadir Hb range 32-53g/L; post-nadir Hb 49-81g/L after 4-6 days; 87-106g/L after 10-14 days (overall post-nadir Hb rise 3.9-5.8g/L/day). An informal multidisciplinary "Anaemia Team" has developed, facilitating preoperative identification and preparation of "at-risk" patients. Consultation, Hb maximisation and blood conservation techniques are being combined to prevent critical Hb drops in patients who do not accept blood products. Education of patients and health care professionals in anaemia prevention is continuing by means of public lectures, conference and other presentations, individual consultations and through the JW Hospital Liaison Network. Application to the wider community, with potential for reductions in transfusion requirements and hospital stay, is being investigated. In a recent series, hip replacement was performed without use of epoetin or blood products in 42 of 43 patients (98%)².

Conclusion: Aggressive treatment of severe anaemia should *not* be delayed until Hb falls to critical levels. Relatively rapid Hb rise is achievable, and immediate availability of appropriate drugs and management guidelines may be lifesaving. Anaemia prevention strategies are even more likely to improve patient outcome, by enabling clinicians to "do what we can do, *before* the Hb gets to 2".

Acknowledgment: We thank Janssen-Cilag and B. Braun Australia for assistance with compassionate supplies of epoetin and pentastarch.

References: 1) Viele MK, Weiskopf RB. Transfusion 1994; 34:396-401. 2) Martyn V. Bloodless Surgery Update 2000; 4(1). Centre for Bloodless Medicine & Surgery, Fremantle Kaleeya Hospital, Western Australia. In press.

A RETROSPECTIVE STUDY OF PRETRANSFUSION PATIENTS WITH POSITIVE DIRECT COOMBS TEST IN SOUTH WESTERN SYDNEY

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In this retrospective study 41,603 pretransfusion antibody screens were processed by South Western Area Pathology Service (SWAPS) in the 2 year period from Jan 1998 to Dec 1999. The SWAPS pretransfusion testing protocol routinely includes performance of a Direct Coombs Test (DCT) using the Diamed LISS/Coombs card. The aim of performing the routine DCT is to detect those patients who have been recently transfused or pregnant, who may have developed alloantibodies not yet detectable by Indirect Coombs screening. Hopefully avoiding potential haemolytic transfusion reactions. The majority of all pretransfusion samples (26,340 - 63%) had not recently been transfused/pregnant. Of these 1045 had a positive DCT (4.0%). Most samples (1021) were DCT positive due to IgG. The largest group represented was Emergency Department patients. Although the incidence of a positive DCT in this group of Emergency Department samples was only 3.6%, there was a strong bias toward samples from patients who were more than 60 years of age (72% of the 323 A&E patients with a positive DCT). The incidence of a positive DCT in the smaller Haematology/Oncology patient group was 6.9% (54 out of 777 Haematology/Oncology samples from patients not recently transfused/pregnant). The minority of samples (15,263 i.e. 37% of the total), comprised the group of patients who were recently transfused /pregnant. Of those recently transfused/pregnant 1160 had a positive DCT (7.6%). Of these 1112 were DCT positive due to IgG. The largest group represented was Haematology/Oncology patients. The incidence of a positive DCT in this group of Haematology/Oncology patients was 16.9% (401 out of 2376 Haematology/Oncology samples from patients recently transfused/pregnant). Eluates were performed on all recently transfused/pregnant patients with a positive DCT, except where the patient had a recent sample with a similar Direct Coombs result. The elution method used Gamma Elukit II tested by the Diamed LISS/IDC method,. After performing 41,603 Direct Coombs Tests only 3 of the resulting eluates yielded an antibody which was not detectable in the patient's plasma. The eluted antibodies were anti-c+E, ant-K+E, and anti-e. As a result of eluting these antibodies, antigen negative blood could be provided and three potential haemolytic transfusion reactions avoided.

CAUTION IN INTERPRETING HEPATITIS C TRANSMISSION DATA OF BLOOD TRANSFUSION FROM IMPLICATED HEPATITIS C REACTIVE BLOOD DONORS

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An analysis of 100 blood transfusion recipients tested as a result of the ARCBS-NSW HCV (Hepatitis C Virus) Donor Triggered Lookback Program reveals different transmission rates depending on the testing used on the donors. We differentiated the transmission rates on those donors found to be reactive on first generation testing with no subsequent testing performed against those found to be confirmed positive on second generation testing. The transmission rates were 53% and 75 % respectively as shown in the table below.

	1 st generation HCV positive donor	2 nd generation HCV positive donor	Total
Total number of positive transfusion recipients	18	50	68
Total number of transfusion recipients tested	34	66	100
Percentage of recipients infected	53%	75%	68%

TEST RESULTS OF THE NEW AUTOTRANSFUSION DEVICE 'ELECTRA' AND OF A 55ML-BOWL FOR SMALL BLOOD VOLUMES

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Modern autotransfusion equipment allows preparation of washed red blood cell concentrates from blood shed during surgery at a high technical standard. Still, new technical developments in intraoperative blood salvage can improve safety and handling, and extend application. „Electra“ is a new autotransfusion device for cell washing. A cassette system allows easy and fast set-up. Multiple detectors for hemoglobin concentration and hematocrit improve process control and facilitate quality control. A 55ml-Latham centrifugation bowl has been developed for processing smaller blood volumes like in pediatric surgery. Methods: In 20 patients undergoing surgery with transfusion-relevant blood loss blood was collected from the surgical field, anticoagulated with heparin, filtrated and the red blood cells (RBC) washed with the autotransfusion device “Electra” (Sorin; Mirandola, Italy) (125 ml bowl, filling rate 250 ml/min, washing rate 200 ml/min, wash volume 900 ml). In 10 patients intraoperative blood salvage was performed with a COMPACT A apparatus equipped with the 55ml-bowl (Sorin; Mirandola, Italy) (filling rate 200 ml/min, washing rate 200 ml/min, wash volume 500 ml). Samples were drawn from the reservoir (after disconnection of suction and mixing), and analyzed for hematocrit and hemoglobin concentration. Plasma hemoglobin, total protein and potassium concentrations were measured in the supernatants. Recovery was calculated from the hematocrit and blood volumes in the reservoir and the transfusion bag. Calculation of elimination rates of plasma and contaminating substances was based on comparison of total contents, derived from the concentrations of total protein, albumin, plasma hemoglobin (phb), heparin, potassium, and from the blood volumes, since elimination takes place first with elimination of supernatant (cell concentration) and only second by dilution during cell washing. Cell damage was measured by microscopic evaluation, determination of osmotic resistance, and by comparing parameters that are dependent on wash-out and hemolysis (phb, K⁺, GOT) and those that change only by wash-out (protein, heparin, albumin). Results: Cell recovery with “Electra” was 91.4%. The final product had a mean hematocrit of 55.3 ± 2.3%. The elimination rate, as measured by total protein, was 98.8%. According to the microscopic evaluation, a normal osmotic resistance, normal 2,3DPG and ATP levels, and with 96.0% only slightly lower elimination rate of plasma hemoglobin, cell damage was only minimal. RBC preparation with the 55ml-bowl resulted in a mean hematocrit of 53%, with a recovery exceeding 90%. Elimination rates for total protein, albumin, heparin and plasma hemoglobin were 98.3%, 98.5%, 96.8%, and 98.2%, respectively. Conclusion: With “Electra” a new generation of autotransfusion devices is available with improvements in handling, safety and data management. The tested quality parameters gave excellent results. The 55ml-bowl allows preparation of washed RBC from small blood volumes for application in pediatric surgery. It is also useful for cell washing of blood from postoperative drainage, or for the preparation of small quantities of platelet gel. Compared to the usual centrifugation bowls instead of miniaturisation cell quality and efficacy of elimination of plasma and its contaminants is well preserved. The observed cell recovery, cell integrity, and elimination rates show that both new equipments allow intraoperative blood salvage at a high quality standard.

MANNOSE-BINDING LECTIN POLYMORPHISMS IN THE AUSTRALIAN POPULATION

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Mannose-binding lectin (MBL) is a serum lectin that participates in the innate immune response. By binding to sugar moieties on the cell surface of microorganisms, MBL facilitates phagocytosis and activates complement. MBL deficiency is a relatively common genetic disorder in humans, which has been linked to increased susceptibility to bacterial, fungal, viral infections and autoimmune disorders. The human MBL gene lies on chromosome 10. Three single nucleotide polymorphisms in exon 1 have been identified at codon 52 (Arginine -> Cysteine), 54 (Glycine -> Aspartic acid) and 57 (Glycine -> Glutamic acid). These polymorphisms disrupt the assembly of MBL peptides into a polymeric structure or accelerate MBL degradation and result in profoundly reduced serum levels of functional MBL. Polymorphisms in the promoter and 5'untranslated region of MBL gene also influence serum MBL levels. These polymorphisms are located at nucleotide -550 g/c (alleles H/L), -220 c/g (X/Y) and +4 c/t (P/Q). When in cis with a wild type coding region, the promoter haplotypes HY, LY and LX are associated with high, intermediate and low MBL levels respectively. As part of a number of studies investigating the association of MBL genotype and disease occurrence or outcome we have genotyped individuals with Sjogren's syndrome (n = 96), Cystic Fibrosis (n = 64), bone marrow allograft recipients and donors (n = 188), pregnant women involved in a study of preterm labour (n = 96) and a normal control group (n = 109). Cis combinations of MBL alleles were directly amplified using PCR-SSP (polymerase chain reaction - sequence specific primers). Forward and reverse allele specific primers were used to directly amplify the haplotypes. The proportion of individuals positive for a given allele was established for each group. Allele frequency of both control and clinical groups were comparable to results previously published for Caucasian populations. These findings confirm that MBL genotypes which predict low MBL serum levels are present at a significant frequency in a variety of patient groups and normal controls. The data is currently being linked to clinical findings in the study cohorts.

COMPARATIVE ANALYSIS OF PLATELET PRODUCTS MANUFACTURED BY 3 DIFFERENT METHODOLOGIES

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The aim of this study was to evaluate platelet products manufactured by three methodologies in use in the Australian Red Cross Blood Service, South Australia. Random single donor platelet concentrates (PC), buffy coat pooled platelet concentrates (BCP) and apheresis platelets (AP) were compared according to the following parameters at day one and day five post-collection: pH; platelet count by an automated haematology analyser; flow cytometric analysis of contaminating leucocytes using Propidium Iodide; proportion of platelets expressing the platelet activation marker CD62P; erythrocyte contamination; platelet swirling and clumping as a measure of platelet viability; platelet response to the aggregation stimulator adenosine diphosphate (ADP) by aggregometry. Unlike PC or AP, the pH of BCP increased over the storage period. The decline in pH observed in AP was markedly greater than that seen in PC. The low pH observed at day 1 in BCP may be attributed to storage prior to processing. Total platelet yield was significantly lower in AP compared to PC and BP, this was attributable in part to the reduced volume obtained with the apheresis procedure, however the concentration of platelets was also lower than that obtained in the other two products. Translocation of CD62P to the platelet surface is a consequence of platelet activation thus the proportion of platelets expressing this marker is an indicator of the extent of activation of the platelets. Less than 10% of the platelets were activated at day 1 in all products with no significant difference between groups. At day 5 all products contained a greater proportion of activated platelets than at day 1, with AP showing the most significant increase. The increase in activation correlated with the decrease in pH seen in this product over the storage period. Upon activation platelets undergo a change in shape which can be induced with an aggregating agent such as ADP. The proportion of platelets which respond to the aggregating agent can be measured in relative terms by an aggregometer. All platelet groups responded optimally following a 2 hour recovery period at 37°C on day 1 suggesting that all products retained a capacity to recover functionally. BCP gave a markedly higher response index than other products. As expected all products responded significantly less by day 5. The extent of recovery following 37°C incubation at day 5 was similar in all groups but the response index was markedly lower in apheresis platelets. Since completion of this trial a new generation of blood cell separators have been introduced which offer improved platelet product and the added advantage of in process mechanisms for production of leukodepleted apheresis platelets products.

PLASMAPHERESIS AND CONTINUOUS HEMODIAFILTRATION (CHDF) IN THE TREATMENT OF MULTIPLE ORGAN FAILURE (MOF)

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MOF still carries high mortality despite recent advances in critical care. Blood purifications such as plasmapheresis and CHDF have been claimed to play an important role in the treatment of MOF. The present study was undertaken to investigate the efficacy of plasmapheresis and CHDF in the treatment of MOF. During past 10 years 141 patients with MOF were treated in our ICU and 62 patients survived (44.0%). Of those 141 patients, 13 patients (9.2%) received plasmapheresis and 98 patients (69.5%) received CHDF. Therefore, CHDF was far more often performed on the patients with MOF compared to plasmapheresis. The main purpose to perform plasmapheresis on MOF patients was as artificial liver support. On the other hand, variety of efficacy can be expected with CHDF when performed on the patients with MOF.. The main purpose to perform CHDF in the management of the patients with MOF is the maintenance of water, electrolytes and acid-base homeostasis and the removal of metabolic waste products in the patients with impaired renal function. Besides those efficacies, we apply CHDF on MOF patients with the expectation that CHDF can be effective as a countermeasure against the pathophysiologic causes of MOF such as humoral mediators . The clearance of TNF, IL-6, IL-8 and lipid peroxide with CHDF significantly and positively correlated with the blood level of those humoral mediators. Those results indicate that CHDF should be very effective as humoral mediator removers in the treatment of MOF. The blood levels of those humoral mediators decreased significantly with 3 consecutive days of CHDF among the patients with high blood level of those humoral mediators. On the other hand, the blood level of those humoral mediators did not change significantly with 3 consecutive days of HCDF when the blood levels of those humoral mediators were not elevated before the initiation of CHDF. The degree of the decrease of those humoral mediators with CHDF and the degree of the improvement in pulmonary oxygenation, and the degree of decrease of those huomoral mediators with CHDF and the improvement in cellular injury evaluated with the change in cellular injury score correlated positively and significantly. The degree of the decrease in humoral mediators with CHDF and the degree of the improvement in general condition evaluated with APACHE H, modified from APAHCE II, also correlated significantly and positively. Those results clearly indicate that the removal of those humoral mediators with CHDF has clinical benefit. We compared the survival of the MOF patients who were treated with CHDF or without CHDF. There was a significant improvement in the survival with CHDF among the patients with moderate severity. Therefore, we conclude that CHDF is an inevitable therapeutic tool in the management of MOF and plasmapheresis can play a role as an artificial liver support when MOF patients complicated liver failure.

TRANSFUSION-RELATED ACUTE LUNG INJURY (TRALI) DURING EXCHANGE WITH CRYOSUPERNATANT PLASMA

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Transfusion related acute lung injury (TRALI) is a rare complication of blood component transfusion. We describe one patient in whom TRALI occurred during exchange with cryosupernatant plasma. The patient was a 36 year old woman with a history of postpartum microangiopathic thrombocytopenia. She had been transfused on several occasions with packed red cells (neither irradiated nor filtered) for symptomatic anemia and was in treatment with daily plasma-exchange with cryodepleted fresh frozen plasma. At the beginning of the last procedure the patient developed clinical signs and symptoms of noncardiogenic pulmonary edema. She was treated with corticosteroids, oxygen and mechanical ventilatory support. Chest-X rays revealed bilateral pulmonary infiltrates. She improved rapidly within 24 hrs. The plasma unit causing the reaction had been collected by apheresis from a multiparous woman. Laboratory tests were performed. The crossmatch of the patient's WBC toward the donor plasma was positive; but the lymphocyte-toxicity assay and the apoptosis test were negative. The Immucor Capture_P Ready Screen assay showed the presence in the donor plasma of antibodies anti A2, A23, B17, B18, B60. Previous units of plasma and platelets concentrates collected from the same donor did not cause any side effect to other transfused patients. Actually the units collected by this donor are reserved for the plasma derivatives. Conclusion: TRALI seems to be the result of concomitant events: The granulocyte antibodies in the donor plasma cause clinical complications if the patients transfused present some predisposing conditions.

THE LONG TERM EFFECTS OF A SHORT TERM BLOOD DONOR RECRUITING PROGRAM

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Background: The Geelong Blood Centre is a 7-couch whole blood collection and 4-couch apheresis collection static blood centre. A theme-focused, short term blood donor recruitment campaign, based on the potential for the Y2K 'bug' to interfere with critical systems and negatively impact on blood supply was undertaken from November 1999 to January 2000.

Aim: The aim of this project was to double the number of whole blood units collected over a period of three months through a concentrated, major focus of resources, in both donor collection personnel and marketing activity.

Method: A temporary increase in staff occurred to cope with the projected increase in activity. In the first two months a concentrated, strategically planned marketing effort was put in place, using various tools (such as e-mail and fax advertising), incentives for new donors and encouraging existing donors to recruit new donors. The program was specifically designed to have maximum effect over a short time frame.

Results:

Month	All WB Donors			New Donors		
	1998-1999	1999-2000	Percentage Increase on Previous Year	1998-1999	1999-2000	Percentage Increase on Previous year
November	667	1226	84	109	328	201
December	792	1393	76	125	368	194
January	600	664	11	95	113	19
February	637	722	13	89	81	-9
March	716	924	29	86	94	9

Conclusion: This project dramatically demonstrates the powerful effects of targeted marketing in increasing donor numbers and collections. The number of donors dropped significantly in January, coinciding with a cessation of marketing. In March, a secondary rise in collections was seen as the new donors recruited in November and December 1999 returned to donate again. This would suggest that a short-term targeted donor recruiting program has the potential to result in long term benefits in terms of increased blood donations.

CLINICAL EXPERIENCE WITH INTRAGAM®;P, A CHROMATOGRAPHICALLY PURIFIED INTRAVENOUS IMMUNOGLOBULIN, IN THE TREATMENT OF IDIOPATHIC THROMBOCYTOPENIA PURPURA AND PRIMARY IMMUNE DEFICIENCY.

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Background: Intragam®P is a 6% chromatographically purified intravenous immunoglobulin (IVIg) with two viral inactivation steps (pasteurisation and low pH incubation) included in the manufacturing process. In two recent clinical studies the safety and efficacy of Intragam®;P was assessed in patients with chronic and acute ITP and primary immune deficiency (PID). The purpose of this presentation is to discuss the safety and efficacy of Intragam®;P in the treatment of these diseases. Method: Both clinical trials were open, multicentre studies based on the Committee for Proprietary Medicinal Products (CPMP) guidelines (1). A total of 17 ITP patients received up to 2g/kg bodyweight of Intragam®;P over a period of 2 to 5 days and 35 PID patients received 0.4-0.6g/kg bodyweight per infusion. The ITP study required that the magnitude and duration of the platelet response be measured to determine the effect of Intragam®;P on platelet levels, which were categorised as excellent, good or poor. All patients returned to the clinic 1, 4, 7, 14, 21, 28, 35, 84 and 182 days after their final infusion. The design of the PID study required patients receive replacement Intragam®;P therapy at intervals of 28+/-3 days. IgG concentrations were determined by nephelometry prior to and 5 minutes after each infusion. Patients received 6 infusions and attended a follow-up visit 28 days after the final infusion. In both studies the safety of Intragam®;P was assessed by monitoring adverse events, haematology, biochemistry and virology profiles. Results: In the ITP study, a total of 41 infusions of Intragam®;P were administered a dose of 1.70g/kg over an average of 2 days. A total of 13 (76.5%) patients achieved good or excellent platelet count responses after Intragam®;P treatment. In the PID study the mean Intragam®P dose administered was 0.42 g/kg body weight. The mean IgG trough level was 8.47 g/L, significantly greater than the limit of 4 to 5 g/L which is reported in the literature to be associated with a reduction in the incidence of infections. There were no serious adverse events that were considered by the investigator to be related to Intragam®P in either study. There were no transfusion related viral infections (HIV-1, HBV, HCV and Parvovirus B19) associated with this IVIG preparation. The most frequently reported adverse event was headache. Conclusion: Both studies showed that Intragam®;P is an effective and clinically safe treatment for patients with either ITP or PID

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1. Committee for Proprietary Medicinal Products; Guidelines to Assess Efficacy and Safety of Normal Intravenous Immunoglobulin Products for Marketing Authorisations. London, 13 February 1996; CPMP/388/95 Final.

ANTI-D QUANTITATION QAP IN AUSTRALASIA

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ARCBS supplies plasma to CSL for the production of Rh D immunoglobulin. Plasma is tested for the level of anti-D prior to dispatching to CSL. The amount of anti-D in plasma has traditionally been determined by the auto analyser method, but other methods including flow cytometry and ELISA have recently been developed. The objective of the QAP originally was to establish a network of contributing centres for comparing results and to resolve issues relating to anti-D quantitation. In March 1999, the first QAP samples were distributed to eight centres throughout Australia and New Zealand. Since then a further two QAP exercises have been completed. Each QAP exercise consists of three different serum or plasma samples containing different levels of anti-D. An information sheet, requesting details of test methods and standards used to calculate results, is sent with each QAP exercise. Each participating centre also tests the CSL (anti-D) Plasma Control in parallel.

Centre	Method	Standard used
ARCBS-NSW	Autoanalyser	British Standard for anti-D antibodies (Human), 73/515
ARCBS-QLD	Autoanalyser	First International Standard for anti-D Ig 68/419
ARCBS-VIC	Flow cytometry	British Standard for anti-D antibodies (Human), 73/515
NZ Blood Service (Southern)	Flow cytometry	First International Standard for anti-D Ig 68/419
NZ Blood Service (Northern)	EIA	WHO/in-house

Table 1. Methods and standards used by the eight participating centres. Table 1. shows the different methods and standards used by the eight centres. When comparing the results of the anti-D quantitation on each of the QAP samples irrespective of the method used, there was a large variation in the level of anti-D. However, when results were compared within the same method, they were found to be within statistical limits (since there was only one centre using EIA to complete all three QAP exercise, these results were not included in the statistical analysis) Within the same method all results fall within 2 standard deviations from the mean. No statistically significant deviation was found. There was large variation within the same method, CV % for autoanalyser varies from 4-44 %, while CV % for flow cytometry varies from 15-40%. Results of the QAP exercises to date are in agreement with other studies, where the comparison of anti-D quantitation by autanalyser versus flow cytometer may be inappropriate (1). 1. Kumpel BM (2000). Transfusion 40: 6-9. (Editorial)

FREQUENCY OF CLINICALLY SIGNIFICANT RED CELL ANTIBODIES IN PATIENTS PRESENTING TO THE EMERGENCY DEPARTMENT IN SOUTH WESTERN SYDNEY

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Liverpool Hospital provides a tertiary (level 3) level trauma service to South Western Sydney and there is a frequent need for the rapid provision of uncrossmatched blood in patients admitted with trauma. The purpose of this study was to define the incidence of alloantibodies in different demographic groups of patients. 64,606 antibody screens in 39,260 patients were analysed between January 1998 and December 1999 for all the hospitals in South Western Sydney Area Health Service. The overall incidence of positive antibody screens was 2.6%. Of all the antibody screens, 6,523 originated from the Emergency Departments of which 178 screens (2.7%) detected an antibody. The antibodies were classified as 1) capable of causing an immediate transfusion reaction 2) capable of causing delayed transfusion reaction 3) doubtful significance 4) not significant. Of the 178 detected antibodies, 38(0.6%) were judged capable of causing immediate transfusion reactions (Kell or Kidd antibodies detected on this or a previous screen), and 93(1.4%) capable of causing delayed transfusion reactions (mainly anti-Duffy or Rh antibodies). The incidence of alloantibodies in other subgroups will also be analysed. We conclude that uncrossmatched blood has a high degree of safety in the demographic group presenting to an Emergency Department in South Western Sydney.

LIFE THREATENING HAEMOLYTIC TRANSFUSION REACTIONS IN HAEMOGLOBINOPATHY PATIENTS

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Background: Three cases from a total of 164 haemoglobinopathy transfusion dependant patients attending Monash Medical Centre (MMC) were investigated after displaying serious life threatening haemolytic transfusion reactions (HTR). One of the cases had been diagnosed as sickle cell disease (SCD) and two as homozygous β thalassaemia. Life threatening acute and delayed HTR have been reported previously in sickle cell disease patients, however the HTR in the three MMC haemoglobinopathy cases have all been acute reactions. Two of the patients demonstrated multiple red cell antibodies and the third patient had no detectable antibodies initially but developed acute intravascular haemolysis post transfusion. Results: After transfusion, the 3 patients showed signs of acute haemolytic crisis with rapid destruction of the transfused red cells. Antibody screening tests detected multiple red cell antibodies of various specificities. However, in all cases another antibody with unknown specificity was eventually detected. Blood matched to multiple red cell antigens proved to be unsuccessful. In one case, it was suspected that this antibody was of haemolytic anti-H specificity. Strategies : These three patients at MMC are untransfusable and one has subsequently died. Hydroxyurea is recommended for the management of SCD as it stimulates production of haemoglobin F and total haemoglobin levels in some patients, reducing the requirement for transfusion. In future cases, the use of phenotyped designated donor red cells may be an additional strategy for consideration. Conclusion: Chronic blood transfusions in haemoglobinopathy patients may lead to development of unidentifiable haemolytic antibodies which render the patients untransfusable indefinitely. It is impossible to predict which patients will develop this phenomenon.

PREOPERATIVE AUTOLOGOUS AND DIRECTED DONATIONS—THE ARCBS-QLD EXPERIENCE

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Predeposited autologous blood has been regarded as a standard of care in many elective surgical settings over the last decade. However, the ever-increasing safety of allogeneic blood and the expense of providing autologous and directed donations have raised questions regarding their place in transfusion practice. A recent Commonwealth Review (April 1999) into autologous blood transfusion concluded that an autologous service cannot be justified either on clinical or cost-benefit grounds, given the current quality and safety of Australia's blood supply.

The objectives of this study were

To examine the pattern of requests for autologous and directed donations and assess their appropriateness.

To identify problems encountered during the collection process

To review the proportion of collections which were actually transfused

A review of all autologous and directed donations collected at ARCBS-QLD in 1999 was undertaken. The ARCBS-QLD database was examined to obtain information regarding patient demographics, the surgical procedures for which blood was requested, the number of collections requested and those actually collected and whether there were any adverse events during the collection period. A total of 1095 autologous units were collected from 557 donors, and 25 directed units from 27 donors. For the autologous collections, procedures for which blood was requested included orthopaedic (64%), urological (9%), gynaecological (6%), maxillofacial (3%), general surgery (3%), bone marrow harvest (3%), vascular surgery (3%), breast reconstruction/mastectomy (3%) and other miscellaneous indications (6%). The age of donors ranged from 16 to 81, with a peak in the 60-69 year group. For directed donations, conditions for which blood was requested included orthopaedic (30%), anaemia (30%), neurosurgery (15%), renal surgery (7%), leukaemia (7%) and other cases (11%). Donor ages ranged from 20 to 38.

Each request was assessed regarding its appropriateness using the 1999 ASBT Guidelines for Pretransfusion Testing. A collection was only deemed appropriate if it was requested for a procedure where a crossmatch is normally required.

Information was obtained as to whether the collected autologous units, as well as any additional homologous units, were transfused and whether any other blood conservation strategies were utilised.

BLOOD TRANSFUSION INFRASTRUCTURE

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This presentation reports the results of a survey of blood transfusion infrastructure within NSW Hospitals. A minority of hospitals have blood bank policies for the issue of blood products and policies for Doctors on the administration of blood products. Less than 50% of hospitals had a transfusion committee and a minority of hospitals measured the expiry rate of red blood cells, platelets and fresh frozen plasma. Lack of such transfusion infrastructure makes it difficult to monitor the appropriateness of blood product transfusion and wastage. These findings are discussed in the context of studies that have shown a lack of transfusion infrastucture may lead to sub-optimal patient care, additional health cost and wastage of a scarce resource.

FRESH FROZEN PLASMA: A THREE MONTH PROSPECTIVE AUDIT

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Fresh Frozen Plasma (FFP) is a valuable resource. It's availability as a source of coagulation factors has led to increased use over recent decades. In common with all blood products, FFP carries a potential viral risk. Good clinical practice would therefore avoid unjustified use of this product. Although international guidelines have been developed for the use of FFP, audit in other centres has revealed wide variation in the rationale for the use of this product. It was therefore decided to review clinical use of this product at Middlemore Hospital, which is a 750 bed tertiary referral hospital in South Auckland, New Zealand. This audit examined criteria for use (compared against local printed guidelines), volumes transfused and product waste. Data was collected on patients aged between 1 day and 95 years. Indications for the orders of FFP fell into three main groups: bleeding and/or coagulopathy, reversal of anticoagulation prior to invasive procedure or reversal of over-anticoagulation. Smaller numbers of patients were septic or had impaired liver or renal function. Results of pre and post transfusion INR (international normalised ratio) and APTT (activated partial thromboplastin time) were examined as was the use of vitamin K. Although 97% of the transfusion events monitored were justified in terms of the local consensus guidelines, in some cases there could be some debate about whether treatment with FFP was best clinical management. By the nature of donation all blood products are precious and costly to process. They should be used only in specific situations when this is the optimal treatment for the individuals concerned. This paper therefore examines how well this is being achieved in respect of FFP.

PROSPECTIVE STUDY OF HEMOLYSIS INDUCED BY IGIV USED AS TREATMENT OF GUILLAIN BARRE SYNDROM (GBS) AND MYASTHENIA GRAVIS (MG).

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Even rare, hemolytic anemia has been reported after high dose of IgIV. Residual anti A and anti B IgG hemagglutinins (<1:64) found in IgIV preparations and identification of such antibodies on patients (pt) red blood cells (RBC) by elution test are in favor of an ABO conflict. Our objectives were to study prospectively the clinical and immunohematological consequences of IgIV administration in an homogeneous population of O and non O pts. Methods: 1.2 to 2.8 gr/kg of IVIg (Tegeline LFB) were administrated to 30 pts with GBS and 14 pts with MG. ABO groups, hematocrit (ht), direct Coombs test (DCT) before, after and day +7 after treatment (TO-T1-T2) were performed. A Sol-Elisa technic was used for the quantitative dosage of in vivo adsorbed IgG. Results: 19 pts were group O, 13 group A, 10 group B and 2 group AB. Clinical hemolysis or need of RBC transfusions were not observed even through a * ht To-T2 of 16% to 22% was present in 5 pts with GBS (4 pts group B and 1 pt group O with DCT IgG positive at TO). The biological parameters of hemolysis were not significantly modified. DCT was found positive (1 IgG, 2C') in 3 pts at TO and in 31 pts (IgG) at T1. The frequency of DCT positive was not significantly different in group O or group non O pts. Anemia determined by * ht T1-T2 was significantly more important in non O compared to O pt group, DCT positive pts, group B pts and after IVIg administration >132gr. Conclusion: DCT was found positive in 70% of pts including those with group O after IVIg administration. The frequency of ht fall was more frequent in group B pts and with high IgIV posology. In these high risk pts we recommend to control RBC counts after IVIg therapy and one week later.

IMMUNOGENETICS AND IMMUNOREACTIVITY OF S-ANTIGEN PEPTIDES IN SYMPATHETIC OPHTHALMIA IN THE UK AND IRELAND

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Purpose: To assess possible HLA associations and predicted peptide immunodominance of retinal S-antigen (S-Ag) in sympathetic ophthalmia in the UK and Ireland. **Methods:** HLA typing by PCR-SSP was performed in 27 SO patients and 51 matched controls. Using HLA-DRB1*04 subtype associations with SO, computerised prediction was made of binding affinity of S-Ag 13-mer peptides from MHC-peptide chemiospatial relationships. Peptide immunodominance in DRB1*04+ SO patients was assessed by CD69 expression and pro-inflammatory cytokine (IL-2, TNF α , IFN γ) levels in CD4+ T cells after incubation with peptide. **Results:** In the UK and Ireland, HLA-C3 (pc<0.01), DRB1*04(0404) (pc<0.05) and DQA1*03 (pc<0.05) were significantly associated with SO. CD69 expression and CD69+IL2+/IFN γ + levels were highest in both high and low predicted affinity peptides in 7 DRB1*04+ patients. For DRB1*0404 and not -0401, increased CD69 expression was found for all 18 S-Ag peptides tested particularly peptide G (GELTSSEVATEVP) and peptide M (TNLASSTIIKEGI) homologues. **Conclusions:** As in Japan (0405), there is a DRB1*04-DQA1*03 association with SO, albeit weaker and with HLA-DRB1*0404 and not -0405 which differ by two amino acids in sequence. Peptide G and M homologues, known S-Ag epitopes, were immunodominant in DRB1*0404+ patients. Predicted peptide affinity and actual immunoreactivity supports both affinity maturation and altered avidity model of T cell selection.

A RANDOMIZED TRIAL OF THREE LONG-TERM CENTRAL VENOUS ACCESS CATHETERS UTILIZED FOR APHERESIS

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Background: Patients (pts) undergoing high dose therapy (HDT) with peripheral blood progenitor cell (PBPC) support require long-term vascular access. Traditionally, pts have had a catheter inserted exclusively for apheresis; however, the size and material of this catheter restricted it to short-term use only and thus was routinely removed on completion of the PBPC collections and a second catheter (for long-term use) was inserted immediately prior to HDT. Methods: To reduce the number of invasive procedures for pts we evaluated 3 new catheters, all intended for apheresis and further treatment including administration of chemotherapy, PBPC and blood transfusions, blood sampling etc. A total of 19 pts (M=5,F=14) were enrolled onto the study and pts were randomized to one of three catheters at the time of attending the transplant clinic. The catheters evaluated were the triple-lumen Pheres Flow® (n= 8), and the double-lumen Circle C® (n=5) and Soft Cell® catheters (n=6). Results: Pts had a variety of solid tumor and hematological malignancies including breast cancer (n=8), NHL (n=6), myeloma (n=3), germ cell tumor (n=1) and Ewings’ sarcoma (n=1). For the purpose of apheresis all catheters performed well with a mean blood flow of 69ml/min, (range = 20- 80ml/min). There was no report of high pressure in the return lumen as determined by the apheresis equipment monitoring system. Each of these catheters performed apheresis with few problems. The care and maintenance was similar to other, conventional apheresis catheters. Overall, sixteen (16) catheters were removed throughout the study period; ten (10) due to tunnel infection, four (4) due to thrombosis in one or all catheter lumens and the remaining two for other reasons (off-trial, fell out). The catheters remained in-situ for a median of 26 days (range 5-62 days). To date, no statistical difference in terms of performance or complications has been demonstrated. The trial continues to accrue patients.

EXCHANGE TRANSFUSION IN DOUBLE HETEROZYGOSIS HbS/BETA-THALASSEMIA. A CASE REPORT.

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Background: Sickle cell anemia and beta-thalassemia may occur together through gene combination HbS/beta-thalassemia (double heterozygosis). The patients show anemia, pain crises and chronic organ damage. Transfusion can take the form of top up or exchange transfusion and both can be applied therapeutically or prophylactically. **Case report:** We introduce a case of a young girl affected by double heterozygosis, that since she was 8 years old, she had repeated pain crises, with HbS level more than 80% of total hemoglobin. In order to avoid the high risk of severe vaso-occlusion crises, the patient underwent exchange transfusion. **Methods:** A protocol for automated, isovolemic exchange transfusion available on Dideco Vivacell and then on Fresenius AS104 was used. One theoretical erythrocyte volume was exchanged every three months (about 1 litre = 4 red cell units), employing leucocyte-depleted red blood cells, in order to decrease the HbS level to 30%. **Results:** After 58 months of treatment we had performed 20 exchange transfusion. The hemoglobin average values remained stable around 10-10,5 g/dl. The average HbS value was 60,6% ($\pm 7,8$) before the exchange and 29,7% ($\pm 4,7$) after. Pain crises disappeared. Body growth was regular. Alloimmunization and infection transmission did not occur. **Conclusions:** Automated red cell exchange was well tolerated, keeping the patient isovolemic. Red cells exchange reduces the risk of hyperviscosity and iron overload, in comparison with top up transfusions. In this case automated exchange transfusion was both safe and effective.

Analysis of the Thermal Stability of Human Albumin during a New Viral Inactivation Process using Differential Scanning Calorimetry (DSC)

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The technique of Differential Scanning Calorimetry (DSC) provides a rapid and sensitive means of probing protein structure and therefore can be employed to confirm protein stability during manufacture. In this study, we describe the use of DSC as a technique to monitor molecular changes in the structure of albumin following exposure to physiochemical conditions of pH, temperature and sodium caprylate concentration encountered during the course of a new viral inactivation process - Low pH Caprylate Incubation. The viral inactivation step makes use of sodium caprylate, which has traditionally been employed for product stabilisation during the pasteurisation process, to act as a viral inactivation agent at low pH incubation. The mechanism of action relies upon the fact that caprylate at pH 4.5, is in the acid form (non-ionised) which is able to partition itself across membranes of lipid coated viruses (eg. Pseudorabies virus, Sindbus virus, Hepatitis A, Herpes Simplex virus, HIV-1).

Albumin samples were diafiltered against HCl to achieve solutions of pH4.1, pH4.0 and pH3.9. Analysis of these samples by DSC demonstrated that as the pH was reduced there was an associated reduction in the thermal stability parameters. Caprylate was then added to the solutions and pH re-adjusted to pH 4.3 - 4.4. The DSC profiles shifted in a fashion corresponding to the pH shift, with the ΔH_d increasing due to the addition of caprylate. The samples were subsequently incubated for 10 hours at 32.5°C prior to being readjusted to pH 6.9. The results obtained indicated that during the low pH caprylate incubation the pH is a critical parameter that effects the onset of denaturation. In the case of albumin, the temperature of denaturation onset (T_i) decreases as a function of the drop in the pH. Furthermore, the enthalpy of denaturation (ΔH_d) also decreased as a function of the pH. However, in the final formulated samples there was no difference between control and samples that had been subjected to the Low pH Caprylate Incubation regimen.

The albumin molecule undergoes well recognised structural transitions that are dependent on the local pH conditions. The molecule becomes progressively more elongated as the pH is reduced below pH7.0, with distinct structural conformers noted at pH 4.3 (F-form) and pH 2.7 (E-form) (Peters, 1996). These structural transitions are however thought to be fully reversible upon pH readjustment to physiological conditions (Peters, 1996). This is consistent with the findings of the DSC analysis and indicates that it is possible to use this tool to assess the impact of individual processing steps on the conformation of therapeutic proteins.

Peters, T. (1996) All about Albumin: Biochemistry, Genetics and Medical Applications. Academic Press.