My abstracts on blood donor retention and seroprevalence of Hepatitis B in Blood Donors of Shimla, HP India.

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AC01

STRATEGIES FOR BLOOD TRANSFUSION IN CRITICAL BLEEDING

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Background: Annual survey of the critical incidents related to anesthesia by the Japanese Society of Anesthesiologists (JSA) demonstrated that critical bleeding in the perioperative period was the leading cause of intraoperative cardiac arrest and perioperative death in the first week postoperatively. About half of them were related to hemorrhagic shock due to trauma, rupture of the large blood vessels, and so on. The rest were related to intraoperative bleeding due to surgical manipulation. Although the possibility of massive and rapid bleeding was anticipated and some measures were to taken to deal with such critical bleeding, prognosis was still grave. The JSA analysed the data. Contributing factors include far greater rate and amount of bleeding than anticipated, delay in decision to start blood transfusion and to order additional blood products, hesitation to use ABO-compatible blood including O-type blood, prolonged blood transportation from the blood banks, and lack of man power. The JSA established the Guidelines for Critical Bleeding in collaboration with the Japanese Society of Blood transfusion and Cell Therapy in 2007.

Outline of the Guidelines: There are a few basic strategies as follows:

1) Physicians and nurses, medical engineers in the operating room and emergency room, staff in the transfusion department, and staff in the blood bank work as a team. Intense communication between them is mandatory.
2) The commander takes charge of the important decisions related to blood transfusion strategies.
3) Surgeons concentrate on hemostasis rather than to proceed the planned procedure. Damage control should be considered.
4) Anesthesiologists insert a few large bore intravenous lines, draws blood for CBC and coagulation studies, and order blood products according to the amount and speed of hemorrhage, vital signs, laboratory data, and the prospect of hemostasis.
5) ABO-compatible blood products should be used without hesitation.
6) Euvolemic status should be maintained to keep adequate perfusions pressures for major organs.
7) Hypothermia should be best avoided to worsen bleeding tendencies.
8) The institutional structure of blood transfusion should be understood by the staff concerning blood transfusion.
9) The institutional guidelines for critical and massive bleeding should be established according to the Guideline.
10) Simulation training involving all departments related to blood transfusion should be performed.

Future: Improvement in outcome in patients with critical bleeding has not been demonstrated after establishment of the guideline. We hope to see some improvements in prognosis of the patients. Guidelines for blood transfusion in critical obstetrical bleeding have been underway to be publicized.

AC02

HEPARIN-INDUCED THROMBOCYTOPENIA

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When encountering a patient developing thrombocytopenia in a critically ill situation, e.g. in the postoperative period, bleeding with a high risk of mortality is a major clinical concern and consideration is given to platelet transfusion even for prophylactic purpose. However, it has recently been recognized that platelet transfusion has the potential to cause thrombosis in some thrombocytopenic patients, particularly those with thrombotic thrombocytopenic purpura and heparin-induced thrombocytopenia (HIT). In recognition of this fact, the Japanese guideline for transfusion practice was revised several years ago to specify HIT as a contraindication of platelet transfusion. Heparin is administrated for the treatment and/or prophylaxis of thromboembolism in many critically ill patients. However, in some situations, this anticoagulant turns procoagulant through an immune-related mechanism. Heparin is usually given to patients who have thrombosis or undergo procedures such as cardiac surgery. In these patients, platelet activation or lysis often occurs, causing release of platelet factor 4 (PF4) from alpha-granules. Heparin has a high affinity for PF4, and when injected into the circulation rapidly results in complexes with PF4. The binding of heparin causes the conformational change of PF4 exposing neoantigens, and induces antibodies against the complexes of PF4 and heparin (anti-PF4/heparin antibodies). A subset of anti-PF4/heparin antibodies (HIT antibodies) can activate platelets through the engagement of Fc receptors by the immune complexes and release platelet-derived procoagulant microparticles, resulting in increased thrombin generation and more platelet activation leading to thrombocytopenia. The immune complexes also induce tissue factor expression on monocytes and endothelial cells (ECs) via EC damage and/or activation, resulting in more thrombin generation. As a result, many of the patients with HIT suffer from arterial and venous thrombosis due to their thrombin-induced hypercoagulable state, rather than the bleeding that would be expected from their thrombocytopenia. Previous reports indicate that about 50% of HIT patients suffer from thromboembolic events within 30days after the onset (if they are treated inappropriately) and HIT is independently associated with thrombosis with higher odds ratio as compared to other well-known risk factors, e.g., protein C (or S) deficiency, antithrombin deficiency, and lupus anticoagulant. If platelet concentrates are transfused to HIT patients in a hypercoagulable state, it is conceivable that the platelet transfusion can trigger the onset of new thromboembolism or exacerbate HIT-associated thromboembolism. Several case reports describe thrombotic events occurred soon after platelet transfusions in acute HIT patients. However, it is still uncertain whether platelet transfusion can be a risk factor for thrombosis in acute HIT patients, since this issue has not been investigated systematically. Thus, the recent guideline for HIT treatment in U.S. gives the additional comment as follows. In situations of diagnostic uncertainty or high bleeding risk, or if overt bleeding occurs, platelet transfusions in the setting of possible or probable HIT may be appropriate, particularly if heparin has been stopped for several hours. In conclusion, when (prophylactic) platelet transfusion is prescribed for the patients developing thrombocytopenia during or after heparin treatment, the underlying cause of thrombocytopenia should be carefully investigated considering the probability of HIT.

AC03

REGISTRY OF 919 PATIENTS WITH THROMBOTIC MICROANGIOPATHIES ACROSS JAPAN: DATABASE OF NARA MEDICAL UNIVERSITY DURING 1998–2008

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Background: Thrombotic microangiopathies (TMAs) are pathological conditions that are characterized by generalized microvascular occlusion by platelet thrombi, thrombocytopenia, and microangiopathic hemolytic anemia. Two typical phenotypes of TMAs are hemolytic-uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP). Severe deficiency of plasma ADAMTS13 activity (ADAMTS13:AC) is more specific for TTP but not for HUS. Since 1998, our laboratory has functioned as a nationwide referral center for TMAs by analyzing ADAMTS13.

Patients and methods: Of 1564 tested patients from 426 hospitals, 919 were positive for TMA. Levels of ADAMTS13:AC and the ADAMTS13 neutralizing autoantibody (ADAMTS13:INH) in these patients were determined by chromogenic act-ELISA and/or by classic von Willebrand factor multimer assay.

Results: TMA patients consisted of two groups, those with severe (less than 3% of normal control) and those with non-severe deficiency of ADAMTS13:AC. Additionally, both groups were divided into congenital (n = 65) and acquired (*n*=854) TMA. Of the congenital TMA patients, 41 had ADAMTS13:AC deficiency due to gene mutations, while the remaining 24 had disease of unknown etiology. The 854 patients with acquired TMA could be largely grouped into three categories: idiopathic TTP (n = 284), idiopathic HUS (n = 106), and secondary TMAs (n = 464). The secondary TMAs were observed in heterogeneous patient groups and were associated with drugs, connective tissue diseases, malignancies, transplantation, pregnancy, E. coli O157:H7 infection, and other factors. All of the patients with acquired severe ADAMTS13:AC deficiency were positive for ADAMTS13:INH

Conclusions: Although TMAs are highly heterogeneous pathological conditions, one third of TMA patients have severe deficiency of ADAMTS13:AC. Platelet transfusions to such patients are contraindicated. Rapid ADAMTS13:AC assays are therefore prerequisite in medical facilities where TMA patients are treated.

TTID

AC04

EMERGING INFECTIONS IN ASIA AND ITS POSSIBLE GLOBAL EFFECTS

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Challenges continue to arise in maintaining a safe and sufficient blood supply. The nature of these challenges has changed during the current decade. In the late 20th century the focus was on managing risks related to well recognised pathogens such as HIV and the hepatitis viruses (B and C). Continued vigilance is needed in these areas. A range of new concerns are however emerging. A number of factors are contributing to this. These include the impact of globalisation of commerce and the increased travels associated with this, the impact of climate change and in some instances advances in technology that enable us to intervene more effectively to address long standing blood safety concerns. Improvements in technologies can also provide an opportunity to address known risks more effectively e.g. the introduction of HBV DNA testing to identify donors with Occult Hepatitis B infection.

Emerging infections can impact on blood services in a number of ways. Firstly they might be readily transmissible by transfusion of blood components. Secondly, they can reduce significantly the availability of donors leading to potential or real shortages of blood components for transfusion and finally the infections can lead to an increased demand for blood components. In practice many agents will impact in a number of ways.

Asia is the most populous continent. Infections emerging in this environment can quickly lead to global safety concerns. Current concerns include Dengue Fever, arthropod borne infections such as Chikungunya virus, Hepatitis E virus and new influenza strains including the Novel A H1N1 09 strain. Occult hepatitis B and malaria also continue to present challenges for blood services in the region.

Dengue infection is caused by a flavivirus that is spread by mosquito, primarily Aedes aegypti. The infection is seasonal and is widespread in Asia, South America, Africa and increasingly in the Pacific. There is now good evidence that is transmissible by transfusion and the virus can be detected in the blood of asymptomatic blood donors. Nucleic acid based tests are currently being developed.

Chikungunya virus is an alpha virus transmitted by Aedes aegypti and albopictus mosquitos. Interest in the agent increased following large outbreaks in Reunion Island and northern Italy. The primary impact on blood services is a severe temporary shortage of donors.

Hepatitis E virus is predominantly spread by the faeco oral route. Recent outbreaks in the Hokkaido region of Japan linked to eating pork products have provided evidence that it can be transmitted by transfusion.

Pandemic influenza remains a real concern for public health services. Initially this related to the H5N1 avian influenza strain. The emergence of novel influenza strain H1N1 09 in 2009 and the resulting pandemic have inevitably raised questions for blood services.

Blood services will need to develop effective strategies to manage the risk associated with emerging agents. This might include donor exclusion criteria and testing of selected donations. Pathogen reduction systems also provide a highly effective mechanism to manage the impact of these agents.

Stem cells and transplantation

AC07
STEM CELL HARVESTING AND TRANSPLANTATION

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Allogeneic hematopoietic stem cell transplantation is an accepted treatment for a variety of malignant and non malignant diseases. Today, peripheral blood progenitor cells (PBPC) are increasingly used instead of bone marrow (BM) as stem cell source. Additionally, umbilical cord blood (UCB) has been established as an alternative source of donor cells. Many differences between the distinct stem cell sources do exist. PBPC contain a higher amount of CD34+ cells compared to BM and UCB leading to more rapid engraftment with the disadvantage of a higher incidence of chronic graft-versus-host disease. In contrast to adults, children seem to have a better outcome after BM than PBPC transplantation. Therfore, especially patients with non-malignant diseases and children should be transplated with BM. The disadvantage of the significantly lower CD34+ cells count in UCB products can be overcome by transplantation of two different units. The risk for allogeneic BM and PBPC donors is considered neligible. Letal and serious side effects due to the harvest procedure are rarely reported with an incidence of 1/10,000 and 7.25/10,000, respectively. Granulocyte-colony-stimulating factor (G-CSF) is widely administered to PBPC donors but questions have been raised about its safety in respect of development of hematologic malignancies. To evaluate this concern, more long-term safety data from larger prospective studies are needed.

In the therapy with adult stem cells, a genotypic HLA class I and class II identity between donor and recipient for the loci HLA-A, HLA-B, HLA-C, HLA-DR and HLA-DQ is optimal; for UCB, HLA matching seems to be less important due to the relative low number of mature T cells in cord blood. The influence of other HLA linked or not HLA linked loci in stem cell transplantation (HLA-DP, KIR, [3DOTS]) is under investigation.

Due to the fact that the AB0-System is inherited independently from the HLA system, in about 50% of allogeneic transplants blood groups of donor and recipient are different. In about 25% of transplants a major AB0 mismach (mm, recipient's AB0 agglutinins directed against donor's erythrocytes), in another 25% a minor AB0 mm (donor's AB0 agglutinins against recipient's red blood cells) and in about 5% a bidirectional AB0 mm (major+minor) is present. To avoid hemolytic complications during stem cell infusion especially if BM is used as stem cell source, incompatible red cells (in case of major AB0-mm) or plasma (in case of minor AB0-mm) have to be removed. Delayed severe immune hemolysis; however, due to donor-derived passenger lymphocytes may be observed in minor and/or bidirectional AB0 mismatched grafts, especially in cases with reduced-intensity conditioning. In order to avoid this complication, prophylactic exchanges of red blood cells can be performed prior to transplantation.

AC08
ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANTATION: AN ASIAN PERSPECTIVE

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Allogeneic HSC transplantation is now a standard treatment for many haematological conditions. The donors may be siblings, haplo-identical family members or unrelated donors. Because of the decreasing small family size in many Asian countries, there is an increasing demand for unrelated HSC donation, especially for paediatric patients. With better coordination, for example among the Chinese registries, matched unrelated donors are now more readily available. The relative HLA homogeneity is also helpful. Homozygous HLA alleles may provide suitable haplo-identical family donors.

There is a unique disease distribution among Asians. Some indications for transplant are relatively more common. They include beta thalassaemia major, disseminated NK/T-cell lymphoma and acute myeloid leukaemia with t(7;11). On the other hand, less common indications are Hodgkin lymphoma, low grade lymphoma, multiple myeloma, myelodysplasia and aplastic anaemia.

Some post-transplant issues are more important here. They include a unique spectrum of red cell antibody, thalassaemic syndromes, glucose-6-phosphate dehydrogenase (G6PD) deficiency, association of thyroid diseases and HLA A2-B46-DR9, hepatitis B infection (HBV), tuberculosis, HTLV-I infection, aspergillosis, and high sero-positivity rates of EBV and CMV infections. There are also differences in pharmacogenetics requiring special attention in drug usage.

G6PD deficiency may affect donors or recipients. We must be cautious when using co-trimoxazole or other oxidizing drugs in these patients. There is usually no problem with engraftment and the recipients may even have a higher enzyme level than the donor.

HBV infection is endemic in many parts of Asia. Reactivation of the infection is common following cytotoxic chemotherapy or intensive immunosuppression, and is associated with a high mortality. Hepatitis B surface antigen (HBsAg) positive patients must be identified before transplant. A high pre-transplant serum HBV DNA level is predictive of reactivation. HBV reactivation is largely preventable with the use of pre-emptive anti-HBV therapy, such as lamivudine. There may however be a problem of drug resistance if the lamivudine therapy is prolonged, especially beyond one year. Early use of other new anti-HBV drugs, which are associated with lower incidence of drug resistance may be advisable. The HBV status of the donors also needs to be considered. HBsAg positive donors should be avoided as far as possible. If this is unavoidable, intensive anti-HBV therapy for both donors and recipients is essential. Transplantation using HBsAb and HBcAb donors for HBsAg positive patients may result in clearance of HBsAg in the recipients, as the result of adoptive immune transfer.

AC09
WORLDWIDE NETWORK FOR BLOOD AND MARROW TRANSPLANTATION (WBMT)

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The first preparative meeting for the creation of Worldwide Network for Blood and Marrow Transplantation (WBMT) was held in Lyon in 2007, accompanying European Blood and Marrow Transplantation (EBMT) annual congress. A trigger of this movement was the creation and the steering of Asian Blood and Marrow Transplant Registry (Asian BMT Registry) whose creation was proposed in Bangkok in 2000 and initiated the first activity survey of the hematopoietic stem cell transplants in 15 member countries in 2006. Center of International Blood and Marrow Transplant Research (CIBMTR) and EBMT paid attention to this movement and these three international transplant registries initiated the preparation for making WBMT, involving East Mediterranean Blood and Marrow Transplant Registry (EMBMTR) and other international societies. WBMT was approved by World Health Organization (WHO) in 2008 as a model system of global network for organ, tissue transplantation and cell therapy. The aim of WBMT, which is described in the byelaw, is that "Promote excellence in stem cell transplantation (SCT), stem cell donation, cellular therapy (CT) and accreditation through collaboration of existing international societies using coordination, communication and advocacy. The purpose of this cooperation is to engage exclusively in charitable, scientific, and educational activities and endeavors including specifically, but not limited to, promoting and fostering, among the many scientific and clinical disciplines, the exchange and diffusion of information and ideas relating to SCT and CT and encouraging investigations on these matters. The focus of the Network is to collaboratively advance the field of SCT and CT while not pre-empting the activities of its member societies."The candidate organizations of WBMT are WMDA (World Marrow Donor Association),EBMT, CIBMTR, APBMT, EMBMTR (East Mediterranean Blood and Marrow Transplant Registry), ABMTRR (Australia-New Zealand Blood

an Marrow Transplant Registry), AABB (American Association of Blood Bank), ISCT (International Society for Cell Therapy), ASBMT (American Society for Blood and Marrow Transplantation), FACT (Foundation for the Accreditation of Cell Therapy), JACIE (Joint Accreditation Committee ISCT-EBMT), NETCORD, EUROCORD, ASHI (American Society for Histocompatibility and Immunogenetics), EFI (European Foundation for Immunogenetics), AHCTA (Alliance for Harmonisation of Cellular Therapy Accreditation). WBMT has so far, 5 standing committees; Committee for Transplant Center Issues, for Donor Issues, for Grant Processing Issues, for Accreditation Issues and for Dissemination/Education Issues. As the initial works by WBMT, two projects are now underway. One is to set global transplant center number (GTCN), which is a unique ID for each transplant center and is expected to contribute more accurate acquisition of transplant situation in the world. The other work is global activity survey of hematopoietic stem cell transplantation, to which CIBMTR, EBMT, APBMT, ABMTRR and EMBMTR have submitted data and as a result, the first global map which exhibited transplant rates in the four continental regions was drawn. It is conceivable that WBMT, as an umbrella for pre-existed international organizations, would be a powerful apparatus to develop HSCT further in global level.

Transfusion practice

AC10

HOSPITAL TRANSFUSION COMMITTEE – THE ESSENTIALS FOR SAFE AND PROPER TRANSFUSION MEDICINE

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Introduction: In Japan, the following requisites are necessary for the appropriate transfusion medicine practice. First, guarantee of sufficient amounts of high quality blood donation and the establishment of a safe blood collection system by the Japanese Red Cross (JRC). Second, NAT-based screening tests for infectious markers, good quality blood group typing methods, irradiation of blood for prevention of TA-GVHD, prestorage leukoreduction, as well as an accurate and timely supplying system of blood components by JRC. Finally, the establishment of the hospital transfusion committee (HTC) to actively promote the safe and appropriate transfusion practice based on The New Blood Law, The New Guideline for Transfusion Practice (TP) and The New Criteria for Indication of Blood Products, is essential.

Background: The New Blood Law, the Law to Assure the Stable Supply and Safety of Blood Products, was approved in Japan in 2003 to further improve the safety of blood products and their appropriate use and to assure their stable supply. It defines the principles of TP and determines the responsibility of the related parties to achieve domestic self-sufficiency in blood products. The New Guideline for TP and the New Criteria for Indication of Blood Products for Transfusion were revised in 2005. And the Hospital Management Fee for the Appropriate TP was introduced in 2006. The Essentials of the Guideline and the Criteria: The New Guideline for TP defines the essentials of the hospital management system for TP. The establishment of a transfusion service, fully-equipped for laboratory testing, and centralizing the management system of TP, is necessary, as well as the full-time medical doctor responsible for TP, specialized laboratory technicians, the transfusion manual containing the transfusion error checkpoints and a 24h system for transfusion testing and provision, are required.

The establishment of HTC actively taking part in the practical management system and control of TP is an essential condition. Leading doctors of each specialty requiring blood transfusion and some representatives of paramedical staff must join the HTC, where the important issues related to transfusion practice, such as the principles, guidelines and manuals of transfusion, are discussed. Regular inspection of the improvements achieved, preservation of the records of HTC discussions and the notification of the decisions taken to the whole staff of the hospital are also required.

The New Criteria for the Indication of Blood Products for Transfusion: The New Criteria for the Indication of Blood Products for Transfusion describes the principles for the appropriate use of blood products and summarizes the criteria for their indication. The trigger levels and the achievement levels for each blood component, and the timing, the dosage and the rate of transfusion for each blood product are described. Additionally, the methods of transfusion- related record database and of the evaluation of transfusion effectiveness are shown.

Discussion: Safe and appropriate transfusion practices should be achieved through active control by HTC. HTC should routinely check the appropriateness of TP based on the new criteria, and always keeping in mind the following question ''Is the hospital transfusion management system consolidated and working?''

AC11

BLOOD TRANSFUSIONS IN NEONATAL CARDIAC SURGERY AND EXTRACORPOREAL MEMBRANE OXYGENATOR (ECMO)

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Background: In high income countries, infants below the age of one year use less than 2% of the blood component supply. Approximately 20% of red blood cell (RBC) and most of plasma products are used for surgical procedures, of which cardiac surgery is the most common indication. Platelet transfusions (PT) are considered as a marker for the level of illness. Dependent on the number of PT received, mortality risk is 10–30 times higher as compared to other critically ill neonates. Receipt of more than 20 PT is not rare for newborns treated with ECMO. Currently 1–2% of newborns with treatment refractory pulmonary and/or cardiac failure are supported for 8±5 days by ECMO, requiring daily 0.5–1 units RBC and 0.5–2 units of PT. The survival rate is over 80% as compared to an estimated mortality rate of 80% should ECMO not have been applied. Long-term morbidity, due to intracranial hemorrhage (ICH) or chronic lung disease is however frequent.

ExtraCorporeal Circuits (ECC): All ECC cause haemolysis leading to increase of plasma-free haemoglobin (PfHb) and decrease of the haptoglobin scavenger. Free RBC constituents may increase the systemic and pulmonary vascular resistance, affect the coagulation profile, platelet dysfunction and renal tubular damage. The risk of an increased degree of hemolysis is higher in young children, extensive surgery and ventricular assist devices (VAD) and ECMO.

Besides hemolysis, a fall of approximately 40–50% of the platelet count is an inevitable consequence of ECC. In cardiac surgery this happens immediately, while after ECMO the nadir is reached after 3 days. Besides, there is dysfunction of platelets, which are activated and at the same time show impaired aggregation to agonist.

A severe complication of ECMO is ICH. Identified risk factors are a low pH, bradycardia, hypotension, un-maintained target values of the activated coagulation time (ACT), number of PT and a larger volume infused the first 8–24h after start of ECMO. All these identified factors may not be causal but indicate more sick patients.

Despite epsilon amino caproic acid (AECA) and activated factor VIIa may have life-saving effects in case reports and reduce bleeding and transfusion needs, a lower incidence of ICH has not been demonstrated and increased thrombosis has been reported.

Supportive Transfusion Therapy: Both cardiac surgery and ECMO are associated with massive transfusions and exposure to multiple donors. Questions on transfusion targets in relation to the shift of HbF to HbA, product selection with respect to storage time, use of autologous red cells, side effects and approaches for improvement exist, but are as yet hardly solved. Development of systems lowering the volume needed for priming of the ECC may reduce transfusion needs for cardiac surgery in children weighing more than 6 kg. One third of PT given to patients on ECMO are indicated for bleeding. Because transfused platelets acquire platelet dysfunction the value of prophylactic transfusions to prevent bleeding can be questioned.

AC12

TRANSFUSION ERRORS AND THEIR PREVENTION

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''A man's errors are his portals of discovery''
James Joyce

Error is an inherent component and by-product of every activity in which humans are involved in the process. Transfusion errors although only recently recognized and formally reported probably existed once blood transfusion became an essential component of medical treatment.

Errors can happen at any point along the transfusion chain. Records of transfusion errors in the New York State for the past 10 years (Linden)

showed that erroneous blood administration was observed in one out of 19,000 RBC units administered. Half of these events occurred outside the blood bank (administration to the wrong recipient, phlebotomy errors, testing of the wrong specimen, transcription errors, and issuance of the wrong unit) with 15% of cases involving multiple errors.

While transfusion transmitted infections have always occupied a prominent focus in blood safety, the prevention and management of errors is often not emphasized sufficiently; despite contributing significantly to patient mortality and morbidity. Most transfusion errors are benign but some of them can result in catastrophic consequences for the patient; particularly if it involves transfusion of ABO incompatible blood. Sazama and colleagues studied transfusion-related deaths reported to the US FDA for 10 years and identified 131 fatal ABO incompatible transfusions. The study also found that the most frequent error leading to a fatal outcome was administration to someone other than the intended recipient.

Because of the certainty of human errors, it is important that there should be a system or process in place to identify all errors occurring at any stage of the transfusion chain. Current Good Manufacturing Practices must be followed to ensure that institutions document and validate each Standard Operating Procedure (SOP) and process. Errors can then be identified, documented, analyzed and categorized so that one can gain more insight into root causes and future prevention.

Near misses are equally important as they indicate situations with the potential for adverse outcome. Systematic data collection and root cause analysis is perhaps the most significant process in both identifying errors but also changing and educating mindsets. The long established pharmacovigilance system worldwide has demonstrated this. The Haemovigilance system, which pioneered in France and later spearheaded by the UK (SHOT) system is another example of a systematic reporting, collection and analysis of data on a larger scale.

For comprehensive management of errors, a quality culture must exist that emphasizes the responsibility of everyone involved as well as a non punitive approach that encourages frank reporting and open discussions.

The consequences of transfusion errors are clear and this mindset has to be filtered out of blood establishments and into hospital environment where the actual transfusion occurs. Equal effort should be put into adequate systems and new technologies like Radio Frequency Identification Devices (RFID), laboratory computer systems and bar codes represent significant advances in the field. Hospital Transfusion Committees should also play a vital role in making sure that guidelines are implemented and adequate staff education and training is conducted.

Safe blood

AC13
PREVENTION OF BACTERIAL CONTAMINATION

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Blood centers and hospitals worldwide face a continuing challenge to improve the safety of blood components for transfusion. Important improvements have been achieved due to careful donor selection, and advances in collection, testing, storage and handling. However, haemovigilance system reports reveal that bacterial contamination remains an important residual risk

A wide range of bacteria have been reported in association with contamination of blood components from clinical case reports, detection by quality control testing and more recently from the introduction in some countries of routine surveillance programs for bacterial screening of platelets. Many of these are skin flora able to grow up during storage, especially room temperature storage of platelets. Other sources include donor bacteremia and contamination during collection, manufacturing or subsequent handling. Of these, many but not all appear to cause clinically apparent sepsis in transfusion recipients. Gram negative bacteria are associated with a substantial proportion of severe cases and fatalities. However, even seemingly innocuous bacteria such as common skin flora may be associated with very serious outcomes, especially at high levels of contamination.

Important elements in reduction of bacterial risk include careful donor selection based on relevant health history and examination. Improved skin disinfection measures have been shown to reduce presence of bacteria in platelets. Diversion of the initial volume of blood away from the collection bag has been introduced in many countries following studies showing reduction in bacterial contamination of both whole blood and various components. Appropriate environmental conditions for collection, processing and storage and handling are important.

In recent years, some centers have introduced routine bacterial screening for platelets. Most use a culture-based method. These generally have very high sensitivity, designed to detect small numbers of bacteria present in the initial inoculum, but also often have substantial rates of initial reactive results which are later not confirmed. Differences in method and timing of sampling, volume inoculated, aerobic with or without use of anerobic culture, quarantine period of components, and definitions of confirmed positive results, among other factors, can make comparisons between published reports somewhat difficult. False negatives do occur, and cases have been reported of clinically significant events following transfusion of platelets with negative screening results. However, where these systems have been introduced they are generally considered to have contributed to a reduction in bacterial risk. Some rapid tests for use immediately prior to transfusion are available and others are in development, including using flow cytometry and molecular methods. Pathogen reduction technologies applicable to platelets already exist. Preliminary results of a WHO-ISBT International Validation Study on Blood Bacteria Standards were presented in early 2009. These will greatly assist comparisons between laboratories and between methods, including evaluation of new technologies.

Many clinicians are not knowledgeable about the small but real potential for bacterial contamination of blood components, yet clinical awareness, including appropriate prescribing, component inspection prior to transfusion, careful patient monitoring, prompt recognition of signs of sepsis, and appropriate intervention can save lives. More remains to be done in this area of clinical transfusion practice.

AC14
TRANSFUSION-RELATED IMMUNE REACTIONS: PATHOGENESIS AND PREVENTION

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Blood transfusion introduces a plethora of foreign antigens to the patient and it may stimulate the immune system. This involves the development of antibodies against the donor (alloantibodies) and/or against the patient (autoantibodies).

Antibodies may develop against all types of blood cells. Red blood cell (RBC) antibodies occur mostly after RBC transfusion. While the immunogenicity of a single RBC unit may depend on its attributes, the patient's immunization is mostly determined by the transfusion history, but also by the patient's diagnosis.

In addition to alloimmunization, RBC transfusion may also result in the appearance of autoantibodies. This concomitant autoimmunization is mostly transient, it does rarely cause hemolysis, and there is no known association to underlying diseases. Concomitant autoimmunization does thus differ from autoimmunization in autoimmune hemolytic anemia that is often secondary to autoimmune or lymphoproliferative disorders.

Immunization due to blood transfusion is cell-specific. Apart from RBC antibodies, antibodies to platelet specific antigens, to human leukocyte antigens (HLA), or to granulocyte antigens can be induced that may cause post transfusion purpura (PTP), platelet refractoriness, or transfusion-associated lung insufficiency (TRALI).

Immunization to structural or conformational epitopes of plasma proteins is limited by proteolysis, excretion, or other types of antigen removal. IgA is an exception to this, and antibodies to IgA may cause anaphylactic reactions. It is not known, if this is related to the dimeric structure of IgA.

Blood transfusion may not only cause antibody development, but it may also have more complex effects as in hyperhemolysis or in transfusion-induced immune suppression. Hyperhemolysis may be seen in certain patients after dissimilar transfusion without antibodies being detectable. Due to the similarity with PTP, an immune mechanism is generally assumed. Here as in transfusion-induced immune suppression, the pathophysiology is unknown.

While it was sometimes claimed that transfusion of old units lead to an augmented mortality, it has to be mentioned that older units are more likely to be transfused to patients that are in worse condition.

An influence of blood products to the immune are still possible and may be due to cytokines or chemokines released by remaining lymphocytes, an altered membrane composition with the exposure of inner layer molecules, and microvesiculation of the stored cells. It is also possible that immunomodulation only occurs, if individually certain clinical conditions are met.

The prevention of unwanted immune effects of blood transfusion is based on three mainstays. First of all, the transfusion threshold needs to be addressed. Especially the less ill patients do profit from a restrictive transfusion strategy.

Secondly, not all patients will develop antibodies, but some will require extended matching.

Thirdly, patients with antibodies may require attention, though not all of them. Some may be transfused liberately, e.g. as in cases with most of the naturally occurring antibodies, if they are not reactive at 37°C. Autoantibodies have always to be disregarded, if blood transfusion is required. However, prior immunosuppression is advisable.

Finally, there is a hope that tolerance might become feasible in transfusion medicine. Protocols are in clinical use for patients with antibodies to IgA, factor VIII, or insect allergy. With the characterization of regulatory T and suppressor cells further applications might become conceivable.

AC15

HOW WE COULD OVERCOME TRANSFUSION ASSOCIATED GRAFT-VERSUS-HOST DISEASE (TA-GVHD) IN JAPAN

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TA-GVHD has been one of the most serious hazards of transfusion. In 1955, Shimoda reported 12 cases of post-operative erythroderma (POE). Of these patients developed a skin rash and high fever, six patients died, and five showed severe leukopenia. For one case of POE, Aoki et al diagnosed as GVHD by histological specimens in which lymphocytes attacked hematological stem cells in the bone marrow, and published it as the first case of TA-GVHD in an immunocompetent patient in 1984. This paper gave an extremely strong impact to Japanese scientists in transfusion medicine, because so many patients might have been suffered from this newly proposed pathological condition after transfusion. By a retrospective analysis on 63,257 cardiovascular patients operated between 1981 and 1986 in 137 hospitals in Japan, it was reported that a case of TA-GVHD would be developed per 659 cases of cardiovascular patients operated and transfused. These data pressed us to establish a nationwide system to gather and analyse serious hazards of transfusion including TA-GVHD, and we (Juji and Tadokoro) moved to the Central Blood Center of Japanese Red Cross from the Blood Transfusion Service at University of Tokyo Hospital, and started to establish Japanese Red Cross Hemovigilance System(medical information activity) in 1992. We employed totally more than 100 pharmaceutists as MR (medical representatives) in all the blood centers across the country, and educated them for 1 year on serious hazards of transfusion, and asked them to visit medical institutions, and to see and discuss with physicians and surgeons about cases with serious hazards of transfusion. Simultaneously, we established a laboratory to analyse patients' materials for identification the cause of the transfusion reactions. Then, we started our activities in the beginning of 1993. In 1993, we received 228 cases of serious transfusion reactions, and of 32 suspected cases, nine cases were confirmed to be TA-GVHD by the comparison of tandem repeat numbers in microsatellite marker loci of DNA obtained from patient's peripheral blood mononuclear cells and finger nails in the year. In 1997, when confirmed TA-GVHD cases reached 14, the Ministry of Health sent ''yellow cards''to all the medical institutes across the country, and recommended to use irradiated blood. However, there were four cases of TA-GVHD in 1999. So, it was finally decided to supply all the cellular blood components irradiated from blood centers in 2000. Fortunately, since then, we have no longer had any case of TA-GVHD, as long as blood supplied by Japanese Red Cross Blood Centers, were used. The irradiation dose was determined to be not less than 15 Gy, and not more than 50 Gy based on in vitro lymphocyte response tests to allogeneic cells (MLC). By our more than 9 years our experience, this irradiation dose was confirmed to be suitable and safe.

Immuno-haematology

AC16

UPDATE ON CURRENT ISSUES IN THE IMMUNE RESPONSE IN TRANSFUSION MEDICINE

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Transfusion of allogeneic cells may induce strong immune responses in the recipient. The strongest immune reponses are caused by non-familiar MHC class I and class II molecules inducing both cellular immune repsonses and anti-MHC antibodies. Also less severe immune reponses to non-MHC molecules occur in transfusion medicine like immune responses to minor transplantation antigens, red blood cell and platelet antigens and to various infectious agents.

In the lecture the basis for immunity with special regard to alloimmunity will be discussed emphasising direct and indirect allorecognition and immune reponses to foreign antigens on blood cells and infectious agents.

The following topics will be discussed:

1. Transfusion of leucocyte filtered and non-filtered red cells in immune competent and non-competent hosts.
2. Transfusion of leucocyte filtered and non-filtered platelets in immune competent and non-competent hosts.
3. Transfusion of plasma and plasma products.
4. Immunological aspects of transfusion of autologous and allogeneic hematological stems cells.
5. Pregnancy and immunisation towards antigens on blood cells.
6. Immune responses towards infectious agents transmitted with transfused blood.

AC17

NON-INVASIVE DETECTION OF FETAL RHD STATUS AND OTHER GENETIC CHARACTERISTICS BY CIRCULATING NUCLEIC ACIDS IN MATERNAL PLASMA

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The discovery of circulating cell-free fetal DNA in maternal plasma in 1997 has opened up new possibilities for non-invasive prenatal diagnosis. Circulating fetal DNA represents some 5–10% of the total DNA in maternal plasma. Fetal RhD status can be robustly determined from the plasma of RhD-negative pregnant women by a variety of PCR-based approaches. This approach is already in routine use in a number of centres. The detection of fetal-specific single nucleotide polymorphisms, on the other hand, is more challenging. Our group has explored single molecule analysis methods such as digital PCR for this application. We have shown that digital PCR is a promising method for the detection of such single nucleotide variations. Very recently, our group has further shown that massively parallel genomic sequencing, in which millions of plasma DNA molecules are analysed in a single run, is another very powerful method for the quantitative analysis of fetal DNA in maternal plasma (Chiu RWK et al. PNAS 2008; 105: 20458–63). We have shown that quantitative genetic characteristics, such as chromosome aneuploidies, can be detected non-invasively and robustly using this approach. It is likely that maternal plasma DNA analysis will play an increasingly important role for the future development of prenatal diagnosis.

AC18

LEUKOCYTE ANTIBODY SCREENING OF DONORS FOR THE PREVENTION OF TRALI

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Antibody-mediated (immune) transfusion-related acute lung injury (TRALI) is a frequent cause of transfusion-associated major morbidity and death in the western world. In contrast to other immune transfusion-reactions, the causative leukocyte antibodies are present in the transfused blood component and are mainly formed by female donors with a history of pregnancy. Alloimmunized male donors have been rarely implicated, especially when transfusion of leukocyte-depleted cellular blood components is the use. Leukocyte depletion of packed red cells has also eliminated reverse TRALI which is due to leukocyte antibodies of the recipient binding to neutrophils present in the transfused non-leukocyte reduced red cells. The leukocyte antibodies are directed against HLA and HNA (Human Neutrophil) antigens. Among them antibodies to HLA-A2, HLA class II and HNA-3a are the most frequently implicated in severe TRALI in which patients require artificial ventilation. Although antibodies to HLA class I antigens are frequently formed by the donors at risk, the incidence of severe TRALI due to these antibodies is comparatively low. This might be due to the fact that the likelihood for a transfused HLA class I antibody to bind to a cognate antigen on neutrophils is low as 90% of the HLA antigens in the blood are located on platelets or are present as soluble antigens in the plasma.

The provision of plasma from only male donors for transfusion in the UK has been shown to efficiently reduce the incidence of severe and fatal TRALI. In order not to lose too much blood components with high plasma volumes from potentially immunized donors, especially platelet concentrates, blood services began to look for leukocyte antibody screening programs. In contrast to screening for viral markers, only donors with a history of immunization have to be tested and retesting is only necessary after re-exposition to leukocyte alloantigens.

For the detection of HLA class I antibodies, many techniques including CDC, immunofluorescence, ELISA, and flow cytometry are in use. Most commercial assays have been developed for transplant needs and, therefore, they are very sensitive. Since HLA class I antibodies have rarely induced severe TRALI despite their high frequency, extremely sensitive tests will result in unnecessary deferral of blood donors. Adaption to the needs of donor testing for TRALI reduction is necessary. The same applies for the available commercial tests for HLA class II antibody screening. For the detection of HNA antibodies a combination of granulocyte immunofluorescence and agglutination assays together with a typed cell panel has been recommended by the ISBT working party on granulocyte immunobiology.

Leukocyte antibody screening has been successfully established in even large blood services and provides a useful tool in reducing the number of unnecessarily deferred donors.

Blood products/management

AC19
PROVISION OF BLOOD PRODUCTS FOR THE HIGHLY IMMUNIZED PATIENT

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Introduction: Rarely, in the pre-transfusion compatibility testing of patients requiring blood transfusion, a complex serological picture involving alloimmunization presents. Often this is found in patients with exposure to blood through transfusion or pregnancy. Patients who have received many transfusions are more likely to have higher rates of alloimmunization. Patients with autoantibodies with complex serology often need testing to insure no underlying antibodies to common antigens are present. There are several ways to define highly immunized; one way is the relative frequency of the desired blood type in the population. Alloimmunization to a high prevalence antigen or if multiple antibodies to common antigens are present, the frequency of that type may be quite low. The serological red cell antibody detection methods vary in sensitivity. Once the antibody identification is complete, the determination of clinical significance is vital. If an antibody is clinically significant, then the search for the blood commences. Various sources may be explored; autologous, family siblings, or allogeneic. Provision of these products in the volume needed is critical to meet patient needs.

The patient population exposed to blood products from various sources are commonly represented in the highly alloimmunized patient population. Diagnoses in this group often include Sickle Cell Anemia and Beta Thalessemia. Sometimes autoimmune hemolytic anemia may be represented if the autoantibody is to a rare antigen or there are many underlying alloantibodies.

The definition of highly immunized and the specificity of the contributing antibody specificities varies in countries. Globally, some blood types considered rare in some countries are not rare in others (example Fy(a-b-) in Asian countries compared to USA). In addition, a patient with a combination of antibodies to common antigens (for example, anti-e anti-s, anti-Fyb and anti-Jka) may define a rare type.

Antibody detection methods vary. It is obvious that a saline detection method will be less sensitive than a Gel method. Also, if the reagent red cells used in the detection phase not diverse enough, it may not detect all alloantibodies. Molecular methods are commonly employed in partial D and e cases. If products are not easily attainable, it may be helpful to determine the clinical significance. Many use the rule of requiring reactivity at 37C for clinical significance. Others may rely solely on the specificity and still others utilize the Monocyte Monolayer Assay.

Finding the blood may be a challenge. Autologous blood may be considered, especially in times of future surgical need or in pregnancy. Family members may be valuable. Allogeneic donors are generally found in Blood Centers who make it a priority to collect and store rare donor units. When the blood is not available domestically, international requiest may be required to meet the blood needs of the patient.

AC20
COST-EFFECTIVENESS IN HEMOTHERAPIES AND TRANSFUSION MEDICINE

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Incremental cost-effectiveness analyses have become standard methodologies in public health decision-making. When applying these important tools in evaluating various hemotherapies, several caveats should be taken into account.

First, the true cost of transfusion is still poorly understood. Although public health decision makers, hospital administrators and clinicians are aware that blood components often do account for the largest proportion of all therapeutic products purchased by hospitals, they usually overlook that the acquisition costs are only a fraction of the overall transfusion cost. A full cost assessment of transfusion must cover all related processes before, during and after the actual transfusion. This includes the number of tests performed in the blood bank, logistic processes, the management of transfusion reactions, informed consent procedures etc. Therefore, it is necessary to apply process cost analysis to determine the cost of transfusion and competing therapies prior to incremental cost-effectiveness analyses

The second challenge in determining the cost of transfusion is that acquisition costs for the same allogeneic blood product can vary widely not only between countries but also within the same country. Different numbers of volunteer workers in the blood collection system, the level of competition and the lack of transparency in terms of pricing and cross subsidization may play an important role.

A third issue is the future scarcity of allogeneic blood products. Increasing life expectancy combined with decreasing birth rates impact on the demand and supply of blood products. A recent analysis in Western Australia showed a significantly higher per capita blood utilization in the older patient population compared with the younger population. Patients 70-years of age and older received 179.6 RBC units per 1,000 population compared with 33.5 RBC units per 1,000 in the 40–69 age group and 10.7 in the 0–39 age group. The overall utilization of RBCs in the 70+ age segment already represents more than 45% of all RBCs transfused. With the long-term trend in population dynamics, the older patient segments will leverage the demand for allogeneic blood components unless transfusion practice undergoes substantial change. At the same time the over-aging population impacts on the supply because the old age segment is excluded from the donor population. Therefore, increasing supply challenges and higher cost are anticipated. As a consequence, present results of cost-effectiveness analyses might not be valid in the near future.

The fourth challenge relates to ΔE, the denominator of an incremental cost effectiveness ratio. Several analyses have been performed on the safety of blood transfusion, but very few on the effectiveness of transfusion per se. For instance it has been measured at what cost infective transfusion risks have been reduced by introducing tests for HCV, HBV, HIV, West Nile virus etc., but not at what cost intended outcomes of transfusion were achieved. The overall conclusion is that neither the cost nor the effectiveness of transfusion is well defined. Therefore incremental cost-effectiveness analyses in transfusion medicine, although strongly needed, are still of questionable value.

AC21
EXTENDING PLATELET STORAGE: SAFETY AND QUALITY CONSIDERATIONS

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Background: Although the natural lifespan of platelets is seven to 10 day, the permissible storage time of platelets intended for transfusion is 4–7 days, depending on the regulatory authority and whether bacterial risk mitigation is performed (see Wood, ISBT Academy Programme 2 and Satake, S10). Not only the risk of bacterial contamination, but also storage lesion should be considered.

Platelet storage lesion: Storage lesion is the accumulation of deleterious changes in structure, function, and viability from the time of blood withdrawal to the time of transfusion and/or apoptosis. Studies of platelets stored over a period of 7 days have linked storage lesion to platelet activation as measured by the generation of CD40L, CD62P and CD63. Other in vitro measures include gross morphology (swirling) and hypotonic shock response. Still another aspect of storage lesion is the generation of microparticles from platelets, leukocytes, and red cells. Platelet-derived microparticles (PDMPs) increase during storage, and, by expressing surface antigens characteristic of activated platelets, may participate in coagulation and thrombosis.

Extension of storage period of platelets: Cold Storage: Cold storage is desirable to prevent bacterial growth and thereby extend the storage period, however, glycosylation of beta-GlcNac residues with UDP-galactose failed to prevent rapid *in vivo* clearance of transfused platelets after cold storage via clustering the GPIb/V/IX and recognition by hepatic macrophages. Homeostasis-Enhancing Containment: Declining pH parallels decreased blood gas pO2 and increased pCO2 as well as reduced glucose and accumulation of lactic acid. Below pH 6.2, the in vivo viability of platelets is supposed to decrease, but at pH 7.6 and above in vivo platelet viability, as determined by recovery and survival, seems not to be affected. Room temperature storage makes platelets require even more oxygen than refrigerated red cells to maintain efficient aerobic metabolism. In case of oxygen insufficiency, platelets easily switch to anaerobic glycolysis, which exhausts glucose more rapidly while generating more lactic acid and causing the pH to fall. Three strategies to increase gas exchange and support aerobic metabolism exit: (i) enlarge the storage bag to increase its surface area; (ii) decrease the bag's thickness to increase gas permeability; and (iii) use more gas-permeable plastics and/or plasticizers in the bag's construction. A fourth strategy is to improve buffering by suspending a given dose of platelets in a larger volume of plasma or other additive solution. Polyolefin (PL2410 and PO-80) and polyvinylchloride (CLX, ELP and F730) bags with 1–1.3 L of bag size have been widely used, and storage solutions continue to be widely investigated.

Conclusion: Strategies to prevent bacterial contamination, including initial flow diversion, bacterial detection, and other measures, seem to enhance the safety profile of stored platelets, provided that further efforts are taken to mitigate storage lesion. Improved oxygen permeability of storage containers, and the quality and quantity of the solution in which platelets are stored, may enhance the therapeutic efficacy of platelets stored for 7 days or more.

Donor recruitment and donor care

AC22

COLLABORATION OF STAKEHOLDERS FOR PROMOTION OF VOLUNTARY NON-REMUNERATED BLOOD DONORS

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Blood transfusion is essential for current medical treatment, while the main purpose of usage may be different country by country. Blood programme to secure adequate supply of safe blood for patients in need is therefore an indispensable part of national heath care system. As blood is a live organ from human, blood needs to be collected from voluntary non-remunerated blood donors for blood safety and ethical reasons and also must be used appropriately.

In Japan paid donations prospered in the 1950s when a Japanese Red Cross blood bank started. ''Yellow blood''from anemic frequent paid donors that caused the high incidence of post-transfusion hepatitis (PTH) became a social problem. In 1964, a cabinet decision was made to establish a system to secure an adequate supply of stored blood through voluntary donations by the cooperation between government, local authorities and JRC blood centers. By the efforts of stakeholders, commercial blood banks were closed until 1972 and the incidence of PTH decreased. However, HIV infections through imported unheated coagulation factors manufactured from paid donors' blood in 1980s provoked social concern about the management of a blood program

The Law on Securing a Stable Supply of Safe Blood was enforced in July 2003 reflecting the HIV incidence. This law provides four basic principles of the blood program and defines the responsibility of stakeholders. In Japan, blood services are conducted solely by JRCBS. As for promotion of blood donation, Subcommittee for Blood Programme, the Pharmaceutical Affairs and Food Sanitation Council deliberates annually the national blood donation promotion plan compiling those submitted by prefectures governments based on the data of blood supply from the blood center. Amount of donations is then decided by the government. Corresponding blood collection plan by the JRC Blood Service is also deliberated and authorized by the government. Liaison Council for blood donation promotion movement is held annually by the government at each block to prescribe the annual plan to the representatives from prefecture governments, local authorities and blood centers. Almost all prefectures and half of the local authorities have the blood donation promotion council consisting of representatives from physicians, town groups, collaborating companies, and various voluntary groups to decide the annual activities for the promotion and recruitment. Information on the inventory volume of red cells in each blood center is daily shared with teh respective prefecture government, the blood service headquarters and the MHLW. When it declines to attention or alert level, actions are taken by the blood centers in collaboration with these stakeholders. By the collaboration in promotion of donation and promotion of appropriate use guided by several revisions of guidelines for transfusion medicine, stable supply of blood has been achieved so far. However, facing the rapid population change into highly aged society with local unbalance, we are challenging the structural change of blood donation focusing on promotion in and education of young generations, increase of registered active repeat donors, and expansion of cooperative companies/organizations in collaboration among stakeholders.

AC23

DONOR MANAGEMENT: SOCIAL MARKETING, MAINTENANCE AND TRUST

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Introduction: Social marketing was born as a discipline in the 1970's when Kotler and Zaltman realised that marketing principles could be used to sell ideas, attitudes and behaviours. In the last two decades, blood services have also began to apply social marketing concepts in the development of donor management programmes.

Social marketing and donor management: Marketing is about talking to the consumer and not about the product. Like commercial marketing, effective donor management programmes seek to discover how blood donation can become an integral part of a donor's life, rather than to persuade people to give a unit of their blood. In this context we examine the marketing mix, commonly know as the ''Four Ps''and the additional ''Ps''in social marketing, in relation to donor management:

Product - the idea and practice of voluntary blood donatio
Price - what consumers must do to obtain the social marketing product
Place - the way the product reaches the consumer
Promotion - how to create and sustain demand for the product
Publics - the external and internal groups involved in the programme
Partnership - other ogranisations in the community
Policy - changes needed to sustain a social marketing programme in the long run
Purse Strings - the money to create your programme
Example of a marketing mix strategy - The Singapore experience

A study was conducted amongst youths age 16–25, whom we identified as identity builders, and young adults 26–35, who are the career shapers to develop a strategy to influence behavioural change. The result of this study shows that both groups have a strong desire to define oneself and express one's individuality.

A marketing campaign using demand strategy to bring about behavioral change was designed to bring about a change in the current perception of blood donation to a desired perception of blood donation i.e. ''blood donation is character building''. The campaign focused on message relevance for donor motivation recruitment and retention in the first year - ''It takes all type''; message resonance to fuel growth in the 2nd year - ''Everyday Heroes''; and finally increasing momentum to a message permanence - ''Someone in my family is a blood donor''to inculcate blood donation as an integral part of life.

Conclusion: Despite challenges faced with the emergence of diseases and the economic downturn, we managed to ensure a sufficient blood stock level of 6 days for 326 days in 2008 - a 30% improvement from 2007. Evidence of support and trust for the national blood programme was reflected by the response rate of our sms recalls which successfully returned bloodstock levels of specific blood types to the comfortable level within 2–4 days of each recall.

To ensure sustainability, we shall continue to examine each element of the marketing mix as the programme develops. This will be done with the help of research to elucidate and shape the donor management programme towards making it an integral part of everyday life.

AC24

QUALITY ASSURANCE IN BLOOD BANKING: THE BASIS FOR SAFETY

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Blood bank institutions have a recognised obligation to ensure the optimal use of blood and blood products while protecting the health of the donor and the recipient.

There has been significant improvement in blood banking safety over the last 20 years. Much of this can be attributed to implementation of measures such as the use of donor selection criteria and advances in screening of

blood for infectious agents, however the implementation of quality assurance systems within the blood bank setting has played a key role. While many government and regulatory agencies require a quality assurance program to be established, careful planning can ensure an integrated and dynamic system that delivers benefits for the blood bank beyond compliance.

The main objective of a blood bank quality assurance system is to minimise risk to donor and product safety by ensuring that there are processes in place to control and monitor all critical activities, i.e all activities that have the potential to adversely affect safety or quality.

There are a number of strategies that can be implemented to achieve this level of control and monitoring, however one of the most important is to ensure that all critical processes are accurately specified in documented instructions, are based on principles of good practice and comply with relevant regulatory requirements. These instructions should be supported by effective training programs to ensure that all staff are competent to perform these critical activities. In addition, there should be documented programs for managing the safe and secure release of all materials, the use and control of equipment and reagents, and the manufacture and handling of all blood and blood components.

However, establishing documented processes and programs on their own will not guarantee donor safety or the delivery of a safe product. All processes and programs need to be validated or verified to confirm that they are delivering the required outcome, and any changes should be revalidated to ensure they have not affected the outcome.

While quality assurance is largely focussed on the prevention of safety and quality problems, an effective system includes processes to identify any problems and introduce corrective actions where necessary. This monitoring and feedback process generates data that, when analysed, can also be used to drive improvements in safety and quality.

Quality assurance programs within Blood Services address the whole manufacturing process, however to be fully effective, quality assurance should be applied to the full ''vein-to-vein''process from donor assessment to transfusion of the final component into the patient. Blood Services and Hospitals are increasingly adopting a collaborative approach in acknowledgement of the shared obligation to both donor and patient.

Quality assurance therefore provides a strong basis for donor and product/patient safety in the blood transfusion setting in addition to a powerful framework for encouraging improvement and meeting regulatory requirements.

Monday: Parallel Session S1: Management and Organisation

2A-S01-01

RISK AND CRISIS MANAGEMENT IN BLOOD PROGRAMME

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We live in a changing, unpredictable and unstable environment - the Asia Pacific region is no exception. Every blood centre may potentially face various risks, crisis, or even catastrophic disaster. Events of the past have shown that when a threat or disaster strikes, the level of preparedness makes the critical difference between success and failure in managing the situation. It is true that none of us can possibly be prepared for every potential eventuality, no matter how hard we work, but if we plan for the predictable, then it will be easier to deal with the unpredictable and limit its potential damage.

Risk management is a structured approach to managing uncertainty related to a threat. It includes: assessment and strategy development to mitigate and manage identified or potential risks. A crisis is a major, unpredictable event that threatens to harm an organization and its stakeholders. The practice of crisis management involves attempts to prevent technological failure as well as the development of formal communication systems to avoid or to manage crisis situations

Crises and disasters may be caused by external or internal hazards. External hazards are generated externally, and may be natural events (e.g. earthquakes, hurricanes, pandemics) or human events (e.g. terrorism, industrial accidents). During large-scale catastrophic external hazards, the plans and responses of the blood centre becomes a critical part of the overall national or regional emergency response plan, and requires effective communications with other emergency management professionals.

Internal hazards are events that occur internally in the organization. These may result in disruption to operations (e.g fires, hazardous spills, power outages, supply disruptions), disruption to blood supply (e.g. blood shortage, test kit recalls), or impact on the public image and integrity of the blood centre (e.g. contaminated blood, corporate scandal). Contingency plans developed to manage these hazards aim either to avoid it occurring or to manage its occurrence, and are proportionate to the risk of the event and its impact.

In managing risk and crisis, there are some key functions, activities and issues that blood centres should consider. Risk assessment and business operations planning are important components of an effective programme. Key issues to be considered include donor management, determination of true medical need, personnel management, communications, and logistics and utilities. A particularly critical component in managing crisis situations is the ability to manage effectively the communication with the public, in particular the media.

2A-S01-02

BLOOD SUPPLY MANAGEMENT IN AN INFLUENZA PANDEMIC

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A pandemic (derived from the Greek words ''all''and ''people'') occurs when there is emergence of an infectious disease spreading through the human population worldwide. Although the current 2009 influenza A/H1N1 pandemic comes most easily to mind, other pandemics would include the HIV pandemic as well as historical instances of smallpox, cholera and tuberculosis pandemics.

The impact of a severe influenza pandemic on the blood supply is likely to be significant. Blood donor availability is most likely to be affected as donors become ill, have less time to donate due to the need to care for others or work commitments, or are unwilling to donate due to fear of being infected. Restrictions on collection sessions and additional donor exclusion criteria also contribute to donor loss.

This will be accompanied by a changing need for blood components. Elective surgery and other non-urgent clinical interventions will often be deferred due to shifting healthcare priorities. However, demand for some blood components (including those with limited shelf life like platelets) may not change significantly and red cell demand may decrease by no more than 10–25% in most cases.

Disruptions may occur in blood supply operations as a result of employee illness and absenteeism. Contractors, suppliers, maintenance and support providers will also be affected, resulting in interruptions in transport, supply of consumables, and services. Supply chain problems are exacerbated by an increased demand for certain consumables such as face masks and gloves, which are in short supply during a pandemic.

In order to maintain a safe and adequate blood supply during a pandemic, blood services must plan and prepare so that appropriate actions can be taken as the situation unfolds. Varying measures will need to be taken at different stages of the pandemic to address issues of blood supply safety, donor loss, clinical demand, and operational disruptions. These include strategies to manage blood supply shortages, donor and staff safety, and recipient safety.

A clear command and control structure must be in place to manage pandemic responses. Contingency plans for the blood supply should be included as part of the national contingency plans, and must be consistent with whatever measures are taken at national level. A critical area that cannot be overlooked is maintaining effective and timely communications with all stakeholders involved.

2A-S01-03

DEVELOPING A DATA BASE OF BLOOD SAFETY INDICATORS IN PAKISTAN

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Background: In Pakistan, the blood transfusion services are fragmented and are predominantly hospital based. They lack financial resources, have no capacity building programs, lack a planned system of donor motivation, recruitment and retention. Quality assurance and accreditation systems are non-existent. There is no proper mechanism of documentation and records are not maintained accurately. The Ministry of Health, Government of Pakistan has decided, therefore, to launch a National Blood Transfusion Service (NBTS) project. The implementing organization is National AIDS Control Program (NACP) which in order to know about the current situation developed a questionnaire based on WHO blood safety indicators questionnaire.

Aims: To collect baseline Data-base on Blood Safety indicators in Pakistan

Methods: Questionnaire was send to blood transfusion focal points in all the four provinces, Federally Administered Tribal Areas (FATA), Federally Administered Northern Areas (FANA), Azad Jammu Kashmir (AJK) and all the Federal government hospitals. The questionnaire was also send to Non-Governmental Organizations (NGOs) working in the field of blood transfusion namely Hussani blood bank, Fatamid foundation, Pakistan Red Crescent Society (PRCS) and a private hospital, Shifa international hospital. No response was received from Agha Khan University and Armed force Institute for Transfusion (AFIT).

Data received was only from public sector institutes as there is no mechanism to collect data from private institutes in provinces. All data received was entered and analyzed in Microsoft Excel ver.2003.

Results: It was seen that in all the public sector blood banks and private hospitals, the voluntary donation ranges from nil in Balochistan to around 23% in AJK whereas in the NGO run blood banks, voluntary donation is higher than that of public institutions but it is also only 31% (Table1).

Table 1: Percentage Distribution of Types of Donation in different Provinces and Institutions

Type of donation %	Institutions and Provinces									
	Federal institutions	Punjab	Sindh	Balochistan	NWFP	AJK	FATA	FANA	NGOs	Shifa
Voluntary non-remunerated donations	3·4	11	12·5	0	0·9	23·5	23·3	0	31·3	0·5
Family / replacement donations	96·6	89	87·5	100	99·1	76·5	76·7	100	68·7	99·5
Paid donations	0	0	0	0	0	0	0	0	0	0
Other	0	0	0	0	0	0	0	0	0	0

Percentage Distribution of Types of Donation in di

Of all five WHO recommended transfusion transmissible diseases -HBsAg, HCV, HIV, malaria and syphilis, blood screening of malaria and syphilis is not done in all the institutions. Prevalence of Transfusion transmissible infections (TTIs) in public institutes and NGOs was comparable. Prevalence of Hepatitis C was the highest among all the TTIs and ranges from 1.1% in FANA to 5.8% in Punjab (Table 2).

Table 2: Percentage Distribution of Types of Donation in different Provinces and Institutions

TTIs %	Institutions and Provinces									
	*Federal institutions	Punjab	Sindh	Baluchistan	NWFP	AJK	FATA	FANA	NGOs	Shifa
HIV	0·02	0·0006	0·007	1·7	0·01	0	0·03	0	0·74	0·1
HBV	2·62	2·75	3·6	5·7	2·9	1	6·26	4·55	3·6	1·1
HCV	3·64	5·88	5·2	3	2·03	1·3	4·88	1·1	3·9	2·5
Syphilis	0·35	ND**	0·09	ND	0·18	0·12	0	ND	0·82	ND
Malaria	0·02	ND	0.014	ND	ND	0·005	0·06	ND	0·006	ND

*of the 6 institutes all five WHO recommended diseases screening is done in only 3 institutes.
**ND: not done

Percentage Distribution of Types of Donation in di

As regards the separation of whole blood into components, in the private hospitals all donations are processed into components, while good numbers of donations in federal institute (40%) as well as the NGOs (54–54%) are converted into components. However, in the provinces the situation is very alarming, ranging from nil in FANA and FATA to only 12% in sindh. No record is available from Balochistan.

A Transfusion committee was present only in the private hospital, where it was functioning poorly.

External Quality Assessment was seen only in the private hospital and two NGO blood banks namely Fatimid Foundation and Hussani Blood Bank.

Conclusion: There is a need to collect data on a regular basis and to develop database to use the diverse information easily and manage limited resources adequately.

2A-S01-04

PARTICIPATION OF THE EGYPTIAN NATIONAL BLOOD TRANSFUSION SERVICES IN AN EXTERNAL QUALITY ASSESSMENT SCHEME FOR BLOOD BORNE DISEASES

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Background: Serological Screening for transfusion transmitted infections (TTIs) in the Egyptian National Blood Transfusion Services (NBTS) has always been a challenge. Although testing strategies of donated blood for TTIs were developed and implemented, still the service lacked a complete system for quality assessment in the laboratories of blood banks.

Since 2002, the National Blood Transfusion Center (NBTC), the headquarters of the Egyptian NBTS, has worked to implement a comprehensive quality control program for TTIs screening to ensure quality and safety of blood.

Participation in an External Quality Assessment Scheme (EQAS) was a crucial step in this quality control program. However, limited financial resources of a developing country such as Egypt, together with proving the need of such a tool as a part of quality assessment in the lab, were the main challenges faced.

Aim: Achieving the approval of the Egyptian Ministry of Health (MoH) for the continuous funding of participation in a regular laboratory EQAS, on a nationwide basis, as a tool of monitoring and improving lab performance.

Methods: In 2007, the Microbiology Reference Laboratory (MRL) of the NBTC stepped forward with a proposal to the Egyptian MoH to elaborate the importance of participation in an EQAS. The proposal explained how this program would provide an insight to the performance of the participating labs, and how such participation would reveal areas of weakness and provide educational stimulus for improvement.

Meanwhile, the MRL studied a variety of EQASs coordinated by several international laboratories in terms of their reputation, regularity, number of annual rounds, cost and markers available in the scheme.

In 2008, the Egyptian NBTS reached an agreement with MCA laboratory, Netherlands, to secure a free trial participation in one round for three markers for two of NBTS laboratories; the Microbiology Screening Laboratory (MSL) and the MRL in the NBTC.

Furthermore, to provide additional evidence about the benefits of participation in EQASs, an agreement was settled between the Egyptian NBTS and MCA laboratory for the participation of the former in two EQA rounds annually coordinated by the later. The first round was in January 2009, with participation of 11 laboratories from the Egyptian NBTS. The second round would take place in June 2009.

Results: In the free trial, round (2008), results of both the NBTC MRL and MSL were satisfactory. This round included HBsAg, HIV Ag-Ab and Syphilis Ab.

In the second round (January 2009), all of the 11 participating laboratories (including two from NBTC and nine from regional blood centers) scored 6/6. This round included only HBsAg and HCV Ab.

Conclusion: Participation of the Egyptian NBTS in an EQAS proved to be a valuable tool to assess the performance of screening laboratories within the transfusion service. Participating labs were enthusiastic to assess their performance and encouraged by the satisfactory results. The results of trial participation in the EQAS assured reliable results of NBTS screening labs, and encouraged MoH to agree to fully fund the participation of more if not all screening labs of the Egyptian NBTS in scheduled EQAS.

Monday: Parallel Session S2: TTI

2A-S02-01

PREVALENCE OF HEV INFECTION AMONG JAPANESE BLOOD DONORS

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Hepatitis E virus (HEV) infection had been recognized to be extremely rare in industrialized countries. Recently, however, increasing reports of hepatitis E including transfusion-transmitted cases are reported in Japan and other industrialized countries.

So far, we have experienced four cases of transfusion-transmitted hepatitis E (TTHE). In 2004, we reported the first molecularly confirmed case of TTHEV, where infection route of the causative donor was not very clear. Then surveys of HEV prevalence in blood donors were undertaken and showed that higher prevalence of IgG anti-HEV in eastern Japan, and the positive rates in female donors were lower than that in male donors. There is a clear age-dependency in IgG anti-HEV prevalence in blood donors in Japan.

Meanwhile, we experienced the second case of TTHE. The causative donor had a barbecue party at a restaurant with his family and enjoyed yakiniku dinner including pig liver and/or intestines. Six of whom including the causative donor and his father were positive for IgM anti-HEV. The father died of fulminant hepatitis E after the barbecue party. HEV isolates from the donor also showed 99.9% homology with that from his father based on nearly entire HEV genome and was classified into genotype 4 that was indigenous to Hokkaido.

This case suggests that there are HEV carriers among blood donors in Japan and HEV infection does not necessarily lead to hepatitis symptoms. For this possibility, we decided to implement preliminary HEV NAT screening in Hokkaido in 2005. However, after the start of this preliminary NAT, we experienced two additional cases of TTHE in 2005 and 2006 because of the delay of the results of NAT. It took almost a week to obtain the results and some blood products, especially platelets concentrates, were already transfused before the NAT. In 2006, we implemented real-time HEV NAT screening.

Up to the end of 2008, the frequency of HEV RNA-positive donors is approximately 1/7700. Male positive donors were dominant. Also, genotype 3 was a dominant genotype. About half of the donors showed the elevation of their ALT level above 45 IU/l during follow-up period. In all of the HEV RNA-positive donors, ALT level came down below 45 IU/l within 50 days after their donation. The HEV RNA-positive donors were also followed-up for their HEV RNA. In all of them, HEV RNA became under the detectable level up to 62–76 days after the donation. The significant increase of the virus level after the donation was observed in 43% of the donors. Compared to hepatitis E patients, a) HEV NAT-positive donors were younger, b) genotype 3 is dominant in contrast to genotype 4 dominance in hepatitis patients. Sequence analyses showed that most of the representative strains from HEV NAT-positive blood donors exhibit over 93% sequence homology with the corresponding swine isolates suggesting that most of HEV are from pigs through food-borne routes.

2A-S02-02

EPIDEMIOLOGY OF HEV INFECTION AMONG BLOOD DONORS IN HOKKAIDO, JAPAN

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Background: Recent studies have revealed that indigenous hepatitis E virus (HEV) strains cause domestic hepatitis E in industrialized countries including Japan. Several cases of transfusion-transmitted hepatitis E have been reported there.

Aims: To clarify the characteristics of HEV infection among blood donors in Hokkaido, Japan, and to consider preventive measures for HEV transmission via blood transfusion.

Methods: A total of 1,098,989 serum or plasma samples from blood donors in Hokkaido from January 2005 to December 2008 were tested for the presence of HEV RNA by real-time reverse transcription (RT)-PCR using 20-pooled samples. Blood samples positive for HEV RNA were tested for the presence of IgM and IgG anti-HEV by ELISA, and measured for HEV viral load by real-time RT-PCR. HEV strains from the HEV positive donors were phylogenetically analyzed by direct sequencing of RT-PCR products of regions of HEV ORF1 and ORF2. Questionnaire was mailed to the HEV RNA positive donors to collect the data on their history of intake of animal meats within 2 months previous to the donation. The donors positive for HEV RNA were looked-back and followed-up before and after their positive donations.

Results: HEV RNA was detected in 142 (105 males and 37 females) donors and the overall prevalence of the HEV infected donors was 0.013% (0.015% in males and 0.009% in females) between 2005 and 2008 in Hokkaido. The yearly prevalence of HEV RNA-positives in male and female donors were 0.01% and 0.011% in 2005, 0.016% and 0.011% in 2006, 0.017% and 0.003% in 2007, and 0.02% and 0.009% in 2008, respectively, suggesting progressive expansion of HEV infection in male donors. No clear seasonality of the infection was observed during the period. Of the 142 donors, 109 (77%) donors had neither IgM nor IgG antibodies against HEV at their HEV RNA-positive donations. The strains detected in the donors were segregated into genotype 3 (132) and genotype 4 (6), which were assumed to be Japan-indigenous strains. Of the 103 donors responding to the questionnaire, 71 (69%) had a history of eating the animal viscera such as intestine and/or liver. Of the 39 donors followed-up at least twice a month after the donation, 21 (54%) showed transient elevations of ALT higher than 45 IU/L.

Conclusions: A total of 142 sporadic HEV infection were observed among blood donors during 2005 through 2008 in Hokkaido with male superiority in the prevalence, which were caused by Japan-indigenous HEV strains and appeared to be associated to ingestion of the animal viscera. HEV NAT screening may be more adequate to exclude the HEV-infected donors than HEV antibody screening.

2A-S02-03

LOOK-BACK STUDY ON RECIPIENTS WHO WERE TRANSFUSED HEPATITIS E VIRUS (HEV)-POSITIVE BLOOD

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Objective: Up to 2004, we observed at least two cases of transfusion-transmitted HEV infection. Since then, we have implemented NAT screening for HEV in addition to HBV/HCV/HIV-1 in Hokkaido area. The purpose of this study is to evaluate the factor(s) that may lead to transfusion-transmission of HEV by looking back the recipients who were transfused with HEV-positive blood.

Materials and methods: From 2002 to 2004, donor samples with high ALT (>=200 IU/mL) were tested for HEV-RNA. From 2005.1–2006.3, all donor samples were screened by HEV-NAT. However, a part of blood products were already transfused before the NAT results turned out. Since 2006.4, blood products have been issued after HEV-NAT screening. The recipients of HEV-positive blood products that were disclosed mostly by look-back

study with stored samples at previous donations were tested for HEV markers including antibody to HEV and HEV-RNA as well as liver functions.
Results: Look-back study disclosed 13 recipients who were transfused HEV-positive blood products. None of them was positive for HEV RNA or anti-HEV in pretransfusion samples. Of four recipients showing signs of HEV infection, three developed hepatitis E and one showed a transient elevation of ALT (peak: 61 IU/mL). The amount and genotypes of HEV in the four transfused blood products were 5.4 (G4), 5.5 (G3), 5.8 (G4) and 6.8 (G3) 10^n/bags, while four blood products that did not cause HEV infection in four recipients contained <4.4 (G3), <4.4 (G3), 4.3 (G4) and 5.5 (G3) 10^n/bags. Five of the 13 recipients died soon after transfusion and were not able to be evaluated for HEV transmission.
Conclusion: The higher amount of HEV (>5.4 log/bag) in blood products may be associated with the virus transmission. Also genotype 4 may be more virulent than genotype 3.

2A-S02-04
ESTABLISHMENT OF A KOREAN HBSAG LOW TITER PERFORMANCE PANEL FOR QUALITY CONTROL OF HBV DIAGNOSTIC KITS

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Background: Currently, International Standards or commercially available reference materials are used for the validation or quality assessment of domestic *in-vitro* diagnostic medical devices. However, due to their high cost and limited quantity a sustainable supply cannot be guaranteed. Also, these materials might not reflect the viral characteristics common in Korea. This study was conducted to establish a low titer performance panel to be used for quality control of HBV diagnostic kits.
Materials and methods: 371 plasma units with OD values less than 1.0 on EIA screening and 105 units with S/C ratio less than 10.0 on CIA were collected from Korean Red Cross blood centers. HBsAg testing with three EIA assays [GENEDIA HBsAg EIA 3.0 (Green Cross MS), BIO-RAD Monolisa HBsAg Ultra (BIO-RAD), and Murex HBsAg V.3 (Murex Biotec.)] and one CIA assay [Architect HBsAg (Abbott)] was performed on all units. Units with reactive results on CIA or units that were reactive on more than two assays were further subjected to HBV DNA quantification, HBV genotyping and subtyping. Units reactive on HBV DNA quantification were confirmed for HBsAg by HBsAg neutralization. The reactivity of a commercial low titer performance panel to various HBsAg assays was determined to be used as a selection criterion for candidate materials. Based on these results, 13 HBsAg positive units and two HBsAg negative units were selected as candidates. After addition of Bronidox as a preservative, the candidate materials were distributed into the final containers. Collaborative study with seven participating laboratories was conducted using two CIA assays [Architect HBsAg, Prism HBsAg (Abbott)], one ECA assay [Elecsys HBsAg (Roche Diagnostics)], one MEIA assay [AxSYM HBsAg V.2 (Abbott)], and three EIA assays [Behring Enzygnost HBsAg 5.0 (Dade Behring), BIO-RAD Monolisa HBsAg Ultra, Murex HBsAg V.3]
Results: Based on the results of the collaborative study, 11 HBsAg positive units and two HBsAg negative units were selected to constitute the low titer performance panel. The mean S/C ratio of HBsAg positive units was less than 10.0 and mean concentration of HBsAg of ten HBsAg positive units was less than 1.0 IU/mL. The panel members were of genotype C, subtype adr and ayr.
Conclusions: As a result of this study, a low titer HBsAg performance panel for quality control of HBV diagnostics kits has been established. This will enable supply of quality control materials at an affordable cost on a long-term basis.

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2A-S02-05
STATUS OF HEPATITIS VIRAL MARKERS CALCULATED FROM PRETRANSFUSION VIRAL MARKER TEST RESULTS OF PATIENTS AT ASAHIKAWA MEDICAL COLLEGE HOSPITAL

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Background: In October 2004, the Japanese government recommended that six viral markers be tested in patients scheduled for transfusion: hepatitis B surface antigen (HBsAg), antibody to HBsAg (HBsAb), antibody to HBV core antigen (HBcAb), antibody to hepatitis C virus (HCVAb), HCV core antigen (HCVcAg), and antibody to human immunodeficiency virus (HIVAb). At our hospital, we started testing these markers of pretransfusion patients in July 2005.
Aim: Japan is regarded as an endemic area of HBV and HCV. Therefore, it is considered that many Japanese are in a state of asymptomatic or latent HBV or HCV infection. At our hospital, a series of hepatitis marker tests (HBsAg, HBsAb, HBcAb, HCVAb, HCVcAg) was prepared. Physicians used this set menu to evaluate the status of hepatitis viral markers before transfusion. For this study, we calculated the status of hepatitis viral markers at our hospital from results of the pretransfusion viral marker tests conducted routinely before transfusion.
Materials and methods: Hepatitis viral markers of 3353 patients during July 2005 and December 2008 were evaluated. Data were collected from the database of our hospital information system. Measurement methods and positive values were the following: HBsAg (CLIA, > 0.5 IU/ml), HBsAb (CLIA, >10 mIU/ml), HBcAb (CLEIA, >70%INH), HCVAb (CLIA, >1.0 C.O.I.), HCVcAg (CLEIA, >50 fmol/l).
Results: The cases were those of 1721 men and 1632 women. Their average age was 59.9 years (0–96 yr). The positive rates of HBsAg, HBsAb, HBcAb, HCVAb, and HCVcAg are presented as a table. The rate of positive HBsAb with negative HBcAb was 8.9%, the rate of negative HBsAb with positive HBcAb was 9.9%, the rate of both positive was 20.3%, and the rate of both negative was 60.9%. Among 204 HCVAb positive cases, 118 cases were HCVcAg positive. The others were HCVcAg negative. No HCVcAg positive case was HCVAb negative. Among the 107 cases that were positive for both some HBV marker and some HCV marker, 88 cases were HBcAb positive.
Summary: We determined the status of hepatitis viral markers of a hospital based on results of pretransfusion viral tests. We assessed the status of apparent or latent hepatitis viral infection from a hospital level to a nationwide level if a pretransfusion viral marker test were strictly implemented for all patients scheduled for transfusion. Furthermore, these data provide background information for developing preventive measures against hepatitis viral infections, including transfusion-transmitted infections and hospital infections.

Table 1. Age related positive rate of viral marker

age	number of pts.	positive rate of viral marker				
		HBsAg	HBsAb	HBcAb	HCVAb	HCVcAg
0-9 yr	87	1·1%	6·9%	3·4%	0·0%	0·0%
10-19 yr	42	0·0%	2·4%	0·0%	2·4%	0·0%
20-29 yr	130	1·5%	12·3%	4·6%	1·6%	0·0%
30-39 yr	266	2·3%	14·7%	8·6%	1·5%	0·4%
40-49 yr	285	3·2%	20·0%	15·4%	3·5%	1·1%
50-59 yr	538	6·5%	30·1%	32·5%	4·3%	3·1%
60-69 yr	755	7·3%	36·7%	41·3%	8·5%	4·7%
70-79 yr	900	1·6%	32·2%	34·4%	9·1%	6·1%
80-89 yr	323	1·2%	38·4%	40·9%	7·4%	3·1%
over 90 yr	27	0·0%	25·9%	29·6%	0·0%	0·0%
total	3353	3·7%	29·2%	30·2%	6·3%	3·6%

Monday: Parallel Session S3: Blood Donation

2A-S03-01

GLOBAL CONSULTATION ON VOLUNTARY NON-REMUNERATED BLOOD DONATION

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More than 30 years after the first World Health Assembly resolution (WHA28.72) addressed the issue of safety and sufficiency of blood and blood products, many countries still lack consistent supplies of sufficient safe blood to meet the needs of their health care systems. Family/replacement and paid donation continue in many countries even though there is convincing evidence that systems based on 100% Voluntary Non-Remunerated Blood Donation (VNRBD) are fundamental to safe and sustainable blood supply.

A Global Consultation on 100% Voluntary Non-Remunerated Donation of Blood and Blood Components' was organized in Melbourne, Australia on 9–11 June 2009. Its objectives were to review current barriers in achieving a safe global blood supply based on 100% VNRBD and then to identify strategies and systems that will assist in meeting this goal. The Consultation brought together global and local partners to reconfirm their commitment to 100% VNRBD and to support countries in achieving this goal, which is founded on the policies articulated in World Health Assembly resolution WHA28.72 in 1975 urging Member States to promote the development of national blood transfusion services based on voluntary non-remunerated blood donation. This is also supported by resolution WHA58.13 in 2005 on establishment of World Blood Donor Day (WBDD) on 14 June. The consultation was timed to precede a global event marking WBDD 2009, being hosted by Australia.

Based on country experiences, key issues and challenges in achieving 100% VNRBD were identified as: role of government in achieving 100% VNRBD; establishment of a quality system and blood safety programme; building capability for education and training of professional donor recruiters; establishment of a programme to support VNRBD recruitment, retention and recognition; raising public awareness of blood supply as a public health and equity issue; and review of the ongoing importance of VNRBD for plasma for fractionation in addition to its established role in provision of labile blood components. Goals and strategies for 100% VNRBD, based on country success stories, were outlined as: creating an enabling environment for 100% voluntary blood donation; fostering a culture of voluntary blood donation; building and maintaining a sustainable and safe voluntary blood donor base; and providing quality donor service and care.

Global Initiatives and Regional Initiatives in the Asia Pacific to promote and support voluntary blood donation were presented, including WHO/IFRC training toolkit 'Developing a Voluntary Blood Donor Programme', WHO recommendations on blood donor counselling, World Blood Donor Day, international colloquium on VNRBD, Asia-Pacific Blood Network, 'Securing Safe Blood': regional initiative from the Japanese Red Cross/Thai Red Cross and Emerging Partnerships in the Pacific region.

The consultation resulted in the development of a 'Melbourne Declaration' and identification of priority actions and recommendations to different stakeholders for achieving 100% VNRBD. The 'Melbourne Declaration' is an advocacy document that affirms the unanimous support of participants for provision of a sufficient supply of safe blood obtained from 100% VNRBD and calls for action from governments to support its achievement by 2020. The Declaration also urges stakeholders to work collaboratively to support governments in achieving the goal.

2A-S03-02

CURRENT STATUS AND CHALLENGE OF DONOR RECRUITMENT

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The status and challenge of donor recruitment in blood establishments vary among Asia Pacific regions because of the diversity of culture, economics and health infrastructure. Some have not yet created a dedicated recruitment program due to limited resources. For those with established Voluntary Non-Remunerated Blood Donation (VNRBD) system, new challenges arise such as from the decline of young donors, evolutions of communication technology, new emergence of diseases and economic recessions.

Taiwan has succeeded in transforming the practice of blood supplies from paid donors to totally voluntary donors since 1974. The Taiwanese experience of various challenges during transition may be helpful to others facing different stages. Generally, the public reaction to blood donation evolves from fear to self-serving to altruism. In the first decade (1974–1983), the main challenge was about health issues and the most efforts focused on educating the public that donating blood was safe. In the second decade (1984–1993), the challenge was to meet the increasing demand of blood for transfusions by retaining existing donors while enlarging the donor population. In the third decade (1994-present), the efforts were concentrated on meeting higher expectations from donors and to improve the quality of donor services.

During three decades of VNRBD development in Taiwan, national blood donor participation rate reached 2% in 1985 and 5% in 1995. The donor population shifted from predominantly soldiers/students to community-based donors. The male/female ratio changed from 3.3:1 to 1.6:1. The major age group of donors (17–30-years-old) decreased from 78.14% to 44.59%. The first time donor dropped from 42.3% to 16.7%. In 2008, the blood donation was 1.8 million with a national blood donor participation rate as high as 7.86%. The majority is repeated donors (83.4%) and each donor gave an average of 1.64 donations per year.

Nevertheless, new challenges in the coming era are different than ever. The evolution of communication media is an attractive marketing tool to educate young people; however, news of negative blood donation experiences would spread quickly. Donor shortage is a potential risk if not enough young new donors are enrolling continuously. Low birth rates also contribute to the decrease of young donors. The percentage of trade population (16–24-years-old) who are donating, a measurement of young donor participation rate used by Asia Pacific Blood Network (APBN), is a good index to monitor the efficacy of young donor recruitment. New emergence of diseases leads to increasing travel deferrals and donor complaints. Economic recession results in lost blood campaigns in workplaces and diminishes the motivation to give blood due to non-employment. Furthermore, some donors are seeking payment compensation which hinders the VNRDB noble behavior.

In conclusion, donor recruitment requires collaboration and commitments at the national level. The key to success is customer (donor) focus. By periodically assessing the donor population with surveys, obtaining key metrics of the donor population, we can construct suitable strategies to target potential donors and to manage various challenges.

2A-S03-03

BLOOD DONATION BEHAVIOUR AMONG THE YOUNG POPULATION OF HONG KONG

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Background: Similar to many countries over the world, only 3% of the total population in Hong Kong donate blood (Hong Kong Red Cross Blood Transfusion Service 2003). The effects of an aging population will become a crisis as early as 2013 when many baby boomers reach the age of 60. Furthermore, 27% of the population will be over 65 years old in

2033(Census and Statistics Department 2003). On the other hand, Hong Kong's birth rate is one of the lowest in the world. Due to the steep decline in fertility, the number of students donating blood in schools dropped by 7.3% from 43,844 in the 2006/07 school year to 40,647 in the 2007/08 school year (Hong Kong Red Cross Blood Transfusion Service 2008).

Aims: It is of paramount importance for the Blood Transfusion Service (BTS) to expand its donor pool and recruit new blood donors. This study aims to (1) examine the characteristics and intention of young blood donors versus non-donors in Hong Kong; (2) explore the factors that may motivate or hinder Hong Kong young people's behaviour towards blood donation.

Method: This is a cross-sectional study using questionnaire to solicit information from young people, both blood donors and non-blood donors, on their personal characteristics, attitude towards blood donation; social, moral and descriptive norms, perceived control as well as past experience related to blood donation and the perception towards the BTS. The study was conducted at the blood donation venues and schools.

Young people aged 18–25 were the target population of this study. The donors were recruited from multiple blood donation centers in Hong Kong, and non-donors were recruited from schools/ institutes including those who accompanied their friends to the centers but did not donate blood.

Results: A total of 3,316 young people (2494 blood donors and 785 non-donors) were recruited from the five blood donation centers and mobile collection teams in Hong Kong in August and September 2008. Results showed that more non blood donors were underweighted (26%) than blood donors (16.9%). Blood donors demonstrated to have more knowledge on the usage of donated blood (87.2%) and were also aware of that blood is donated to save life (94.7%). About half of the young blood donors admitted that their donation were mainly for blood testing (53.1%), free physical check up (47.3%) and souvenirs (66.8%). Poor service of the BTS (OR=1.77) and the blood donation barriers (OR=1.76) such as fear of pain, fear of sight of blood, too thin, faint after donation, afraid of affecting own health, adverse reaction after donation were all identified as the factors that would hindered young people for donation. Many youngsters (non blood donors) also claimed that they did not know blood was needed to save life.

Conclusion: Recruitment strategies should focus on the development of health education programmes related to blood and blood donation for the youngsters. Enhancement of collection services will help to retain more people for donation.

2A-S03-04
KNOWLEDGE, ATTITUDES AND PRACTICES REGARDING HIV/AIDS AMONG BLOOD DONOR

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Background: There were 88,648 units of blood collected at NIHBT in 2008 of which 54,000 units were given from VNRBDs. HIV infection is now becoming a dangerous threat to blood safety. It is estimated that from 50 to 90 percent of people who have HIV do not know they are infected. A number of those people might be blood donors; however, some give blood just in order to get a free HIV test.

Aim: To assess and evaluate the existing level of knowledge about HIV/AIDS and the risk behavioral factors among blood donors to assist blood programmers in the development of an effective VNRBD program.

Methods: A cross-sectional study and in-depth interview was conducted to assess the knowledge, attitude and practice on HIV/AIDS prevention among 832 donors, who were randomly selected by multi-stages sampling at the collection sites in Hanoi. Structured questionnaires were used to collect data between April and October 2008.

Results: The average age of the donors was 22.8. A majority of the donors knew blood transfusion was one of the ways to transmit HIV (83.7%); however, 27.6% did not know about ''window period''. There were still 41% of participants who had a limited level of knowledge. In term of attitude toward AIDS prevention, 48.2% supposed that a person who is suspected to have HIV could still donate blood because they are able to either get free testing or help in saving life if the test is negative; 6.7% of donors were not willing to contact blood centers after donation if they realized that they had high risk behaviors. There were 17.3% of single participants having sexual activities; the percentage of condom use was 25% in extra-marital sex; 15.6% had blood related accidents with sharp instruments. Alarmingly, 17% of donors suspected that they might be HIV infected and 81.2% wanted to get HIV test result after blood donation.

According to the study, there were no significant differences of awareness level between first and repeat donors; level of knowledge was not improved through blood donations. The percentage of donors who really wanted to know their HIV test result is higher among (1) those who knew that blood transfusion is one of the ways to transmit HIV (1.86 times) and (2) those who suspected themselves to have HIV (2.63 times).

Conclusions: Donors' knowledge on HIV/AIDS is limited, not only among first time but also in repeat donors. It is true that, no one knows and understands about a blood donor better than themselves whether they have high risk behaviors or not. Findings from the study recommended that donor recruiters and blood programmers should design and conduct evidence-based campaigns in order to ensure safe blood transfusion. It is essential to carry out some urgent actions such as (1) providing more education activities on HIV/AIDS prevention when implementing blood donor programmes, (2) improving the quality of pre- and post-donation counseling for donors to encourage high risk donors to self-refer their donations and (3) establishing an effective call-back system within blood centers.

Monday: Plenary Session Cellular Therapies

2B-PL1

FROM HUMAN SOMATIC STEM CELLS TO HUMAN IPS CELLS - STATE OF THE ART AND FUTURE NEEDS

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Although the enormous amount of information on disease mechanisms have been delivered from basic biomedical science, the development of new treatments, diagnostics and prevention are not commensurately gained. One of the root cause underlining in the gap between the basic biomedical science and the clinical research is the heterogeneity of human. Thus the clinical researches apply the statistic methods. Uniformity of the therapeutic entities is another basis of the clinical studies. In the context of cell replacement therapy, major challenge is the development of the technology to prepare the uniform cell entity. Progress of the epigenetics indicate that the heterogeneity of human are generated both by the difference in the genetic sequences and the epigenetic modifications. Diversity of epigenetic modification in somatic cells is generated by the cell-differentiation associated and the environmental exposure associated mechanism. Indeed, genome wide DNA methylation analysis indicated that the cells derived from same tissue of two different individuals have different DNA methylation profiles. Adult tissue stem cells and progenitor cells are required for both proliferative homeostasis and insult-induced cell genesis, and are present in all tissues in the human body (Angew Chem Int Ed Engl. 47, 5718, 2008). The environmentally induced epigenetic modifications are likely to be introduced in these tissue stem cells and progenitor cells during proliferation and differentiation. DNA methylations are also introduced during in vitro culture of stem cells and somatic cells. The culture-induced DNA methylation and the heterogeneity of DNA methylations in donor derived cells are the major cause which prevents the uniformity of cell based medicine. Reprogramming technologies are expected to reset these epigenetic modifications. The chromosomal DNAs receive two different physiological reprogramming during development; the post fertilization reprogramming and the primordial germ cell reprogramming. Artificial reprogramming including the somatic cell nuclear transfer (SCNT) and the iPS cell method mimic the post fertilization reprogramming. In the course of study to establish human iPS cell technology, we identified that alkaline phosphatase and nanog positive iPS cell colonies are heterogeneous in ES cell related gene expression (Stem Cell Res. 1, 105, 2008). In addition, we identified that genome wide gene expression profiles of human iPS cells are quite different from human ES cells (Stem Cell Res. 1, 105, 2008). It is well know that the cloning efficiency of SCNT remains lows and variety of abnormal phenotypes including the placenta overgrowth, large fetus syndrome, immune dysfunctions and a shorter life span were observed in cloned animals. Epigenetic abnormalities are also observed in cloned animals. If we take these observations together, we can predict that the present artificial reprogramming technology can't reset all of the epigenetic modifications in the somatic cell sources. The presence of reprogramming recalcitrant genes would be a critical problem for standardization of the reprogrammed-cell based cell therapy. In this context, I would like to discuss the future direction of human iPS cell research for the application in the cell based medicine.

Monday: Parallel Session S4: TTI

2C-S04-01
NAT SCREENING AND PATHOGEN INACTIVATION FOR BLOOD PRODUCTS

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Successful implementation and use of nucleic acid testing (NAT) for HIV, HBV, HCV and further viruses as well as improved donor selection have led to a dramatic risk reduction for viral transmission via blood transfusion over the last years.

Today, other risks like bacterial contamination get into the focus of hemovigilance: Particularly platelet concentrates (PC) are vulnerable to bacterial growth due to their storage conditions. Patients receiving such products have a small potential risk of severe complications or even death. Modern hygiene regimes, e.g. improved disinfection of the donors' skin or preparation of blood products in fully closed systems as well as diversion, led to a significant reduction of bacterial contamination risk in the past. However, a small risk remains.

(Re)emerging pathogens in a global setting pose a threat to blood safety: Examples like SARS virus, Chikungunya or Dengue viruses, avian flu (influenza virus H5N1), influenza A virus H1N1 or West Nile virus (WNV) are only some more recent examples out of the vast numbers of (re)emerging pathogens, which are rapidly spread throughout major parts of the world and pose a potential threat to blood product safety.

Therefore, two possible ways of further reducing such risks for blood products are feasible: a.) testing and/or b.) pathogen inactivation (PI).

Testing for bacterial contamination is possible by different methods: Direct detection methods for bacteria have disadvantages regarding sample size and detection limit. Biochemical methods like oxygen consumption might not detect anaerobic germs. Automated culture methods are still the most sensitive technique, but they have their downsides as well. Incubation periods require time and an early sample taken, therefore the risk of ''negative to date''products and sample errors. Novel molecular genetic test methods for detection of bacterial nucleic acid are in different states of development.

(Re)emerging pathogens require constant vigilance and must be included into the NAT testing program when posing a threat to a safe blood supply. Four different principles of PI can be distinguished: Solvent/ detergent methods can only be used for PI of pooled plasma and are only able to inactivate enveloped viruses. Physical methods like UVC irradiation still have to prove efficacy and safety in clinical use. Photodynamic PI methods produce oxygen radicals, which might also modify cellular structures. Photochemical PI methods are in clinical use and can be adapted for PC and plasma PI.

All PI methods have to proof, that they effectively inhibit pathogens while maintaining full functionality of the blood products. Toxic or mutagenic compounds must not remain in the final product. The PI technology must fit into the existing work cycle of a blood bank. Finally, costs per product must be acceptable.

In summary, both testing and inactivation have their advantages and disadvantages, which have to be weighed up against costs and benefits of both procedures.

2C-S04-02
FROM NAT/PI TO NAS/PO: PREPARING FOR NUCLEIC ACID SURVEILLANCE AND PRODUCT OPTIMIZATION

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The transfusion-transmitted infection (TTI) risk of pathogens for which nucleic acid testing (NAT) has been implemented is now insubstantial compared to other hazards of transfusion. Minimizing the TTI risk of non-tested pathogens is a goal of pathogen inactivation (PI); in particular, the interdiction of emerging pathogens is a major selling point. Incidental to PI is the possibility of eliminating certain patient-oriented procedures, e.g., selectively screening for cytomegalovirus (CMV) or irradiating to prevent transfusion-associated graft-versus-host disease (TA-GVHD). Do NAT and PI introduce new hazards? Perhaps. The product-oriented elements of ''SQuIPP''- Safety, Quality, Identity, Purity, and Potency - are interdependent in positive and negative ways, and every intervention, including NAT and PI, requires time and money (see Wood, S9). Time has a special impact on the quality and availability of labile products such as granulocytes and platelets. Money spent on one intervention is unavailable for any other, and is not necessarily allocated to yield the best overall cost per quality adjusted life year (cost/QALY) or similar measure. Thus, current debate often focuses on NAT vs. PI. The ''right''answer likely depends on circumstances; a recent Chikungunya epidemic in La Réunion provoked rapid deployment of PI to ensure a sufficient, local platelet inventory, but the quality of PI-treated products is a matter of ongoing investigation.

For the future, we should dare to imagine, and dare to insist on, better performance for lower cost in all safety interventions. Information technology (IT) and automation have probably shown the greatest cost/performance improvements in recent years, especially, as recording and processing approach the molecular level. A rational next step is to acknowledge that DNA and RNA are themselves data storage media, which await better techniques for reading (and editing). The issue will not be how little we can test, but how well we can handle the vast data available in every donor sample. In a world of nucleic acid surveillance (NAS), ethics and privacy will be at the forefront of donor-oriented considerations.

As for products manufactured from donated blood, today's photochemistry may be the beginning of a new wave. Now, we disable nucleic acid replication. In the future, we might regulate apoptosis or modify antigens. Product optimization (PO), starting with rational donor selection criteria, will proceed with manufacturing steps best suited to a particular patient's circumstances, e.g., the platelets given for an acute hemorrhage may very well differ from those given solely for prophylaxis. What is least likely to harm should be replaced by what is most likely to help.

2C-S04-03
ESTIMATION OF INCIDENCE AND RESIDUAL RISKS OF TRANSFUSION TRANSMITTED INFECTION FOR HIV, HCV, HBV AND HTLV IN HONG KONG: 2006 - 2007

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Background: Incidence rates and residual risks of transfusion transmitted viral infections are important parameters in the understanding of current blood safety and the effectiveness of blood safety related measurements implemented.

Aims: Use mathematical models to estimate the incidence rates and residual risks of transfusion transmitted HIV, HCV, HBV, and HTLV infections in Hong Kong in 2006 and 2007.

Methods: In 2006 and 2007, all donations to the Hong Kong Red Cross Blood Transfusion Services (HKRCBTS) were tested for anti-HIV-1/2, anti-HCV, HBsAg, and anti-HTLV-I/II using the PRISM ChLIA (Abbott, Abbott Park, IL). Prior to implementation of HIV-1/HCV/HBV triplex ID-NAT using Procleix Ultrio assay (Chiron-Novartis, Emeryville, CA) in April 2007, donor samples were tested only for HIV-1 RNA and HCV RNA in MP16 format. For estimation of incidence rates of HIV and HCV, seroconversion and NAT yield among repeat donor population were used. For HBV, HBsAg seroconversion among repeat donor population was adjusted for its in vivo transient nature (multiplied by a factor of 2.77). For HTLV, since there was no seroconversion in repeat donors, the prevalence in first time donors was used for estimation. Residual risk for each virus was estimated using the model: Residual Risk = Incidence rate X Window Period (in year). Reported window periods (in days) were used: HIV-1 MP-NAT 9.0 (7.8–10.2), HCV MP-NAT 7.4 (6.1–8.7),

HBsAg 38.3 (33.0–43.7). The incidence rate among first time donors was assumed to be twice the rate of repeat donors. In HKRCBTS approximately 80% of collections were from repeat donors.

Results: Based upon our data and calculation, the estimated incidence rate (per 100,000 person-year) for all donations were: 4.36 for HIV, 0.87 for HCV, and 84.54 for HBV; while the estimated residual risks per donations were: 1 in 930,233 for HIV, 1 in 5.6 million for HCV, 1 in 11,273 for HBV, and less than 1 in 10 million for HTLV. The actual risk for HBV could be higher since HBV NAT data were excluded from calculation, and therefore, cases of occult hepatitis B infection (OBI) were not included in estimation.

Conclusions: Most of the NAT data used for estimation reported here were conducted in a MP16 format. Therefore, the current residual risk, after implementation of routine ID-NAT, should be lower, particularly for HBV where OBI cases would likely be detected. Nevertheless, the risk of HBV transmission remained to be much higher than HIV and HCV, hence, an NAT assay with improved HBV sensitivity is needed to further protect our blood supply.

2C-S04-04
STABILITY OF TRANSFUSION TRANSMITTED VIRUSES IN WHOLE BLOOD

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Purpose: In order to reduce the diagnostic window period, real time mini-pool nucleic acid amplification technology (mini-pool NAT) was implemented into blood donor screening at the German Red Cross in 1997. Currently, approximately 10,000 donations from Austria, Luxemburg, Baden-Württemberg, Hessia, Saxonia, Berlin, Brandenburg, Schleswig-Holstein and from the German Federal Armed Forces (Bundeswehr) are tested daily by NAT for HAV, HBV, HCV, HIV-1 and B19 at our blood donation service by three testing laboratories in Frankfurt/Main, Plauen, and Ulm. Blood samples arriving by midnight are pooled over night, extracted and amplified the next morning. The results are ready for release by early afternoon. Results of blood donor screening by NAT over a time period of more than ten years showed a residual risk for transfusion transmitted infections of 1:10.8 million, 1:4.3 million and 1:360,000 for HCV, HIV-1, and HBV, respectively. Risk analysis according to the hazard analysis of critical control point method (HACCP) can be divided into pre-analytic conditions (e. g. sample collection, kind of sample tubes, storage conditions, time period before centrifugation) and analytic conditions (extraction method, amplification and detection system). Especially during the Christmas and Easter holidays the time period before separation into plasma and red cells should be possible to extend to 72 h. The current study evaluated the influence of different pre-analytic conditions on the analytical sensitivity of the Baden-Württemberg - Hessen mini-pool NAT system.

Methods: Blood donor samples (576 in total) were pooled into 144 pools (pools of four) with a total volume of 15 ml each. Haematocrit was examined from all pools by Sysmex XT 1800i. In the next step the whole blood pools were spiked with six different concentrations of HBV, HCV and HIV-1 (each concentration was tested in 24 replicates) and adjusted to the plasma volume in each mini-pool. After spiking, the plasma from one ml of whole blood from each pool was frozen at -40°C. The residual volume of the mini-pools (14ml) was divided into two aliquots and stored at room temperature and 4°C–8°C for additional time points. Plasma was taken from each mini- pool after 24h, 48h, 72h, and 168h, respectively. All plasma samples were extracted by the automated Chemagen extraction system and investigated by the German Red Cross NAT system for HBV, HCV and HIV-1. All data were calculated by probit analysis using SPSS 12.1 software.

Results: The analytical sensitivity remained stable from day 0 to day 7 independent of storage conditions (room temperature or 4°C–8°C) for HBV, HCV, and HIV-1, respectively.

Conclusions: Whole blood storage up to seven days in EDTA sample tubes did not influence the analytical sensitivity of transfusion-transmitted relevant viruses listed in annex II list A 79/98/EU in combination with the extraction and amplification method of the German Red Cross Baden-Württemberg - Hessen. These data enable an extension of the pre-analytic time period before centrifugation up to 72h at room temperature without increasing the residual transfusion- transmitting infectious risk for blood components, and gives blood banks more flexibility to organize screening processes especially on public holidays.

Monday: Parallel Session S5: Immune Haematology

2C-S05-01

ADVANCES IN ALLOIMMUNE THROMBOCYTOPENIA LABORATORY INVESTIGATIONS

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Alloimmune thrombocytopenia is mainly encountered during pregnancy when the mother becomes immunized against the fetal platelets antigens she lacks. Therefore during pregnancy the maternal IgG cross the placenta and recognize their target on the fetal platelets. The resulting fetal alloimmune thrombocytopenia, which incidence has been evaluated to 1/1000 live births in Caucasians, is usually severe and can lead to bleeding. Intracranial hemorrhage (20–30% in retrospective studies) is the most severe complication leading to death (10–15%) or neurological sequelae (20%). Due to the recurrence for incompatible fetuses in the subsequent pregnancies, antenatal management has been developed.

The diagnosis of fetal or neonatal alloimmune thrombocytopenia is of utmost importance for the management of the affected infant and the subsequent pregnancies.

The diagnosis is straightforward when a maternal alloantibody is detected directed against the offending antigen present in the infant.

The molecular basis of the platelet alloantigens has been elucidated and single nucleotide polymorphism (SNP) in the gene encoding the relevant glycoprotein is present in the 23/24 defined antigens. Antigen determination has evolved during the recent years. A number of genotyping methods have been developed: PCR-RFLP, PCR-SSP, real time PCR and more recently microarrays. Although genotyping is widely used, unknown genetic variants may alter the results, and ethnic diversity is of importance. Genotype is not always phenotype. However phenotyping is somehow problematic because reference human sera have limited availability.

The detection of the alloantibodies could be challenging. Currently the most widely techniques used are the antigen-capture assays with mouse monoclonal antibodies. However false-negative results may occur by steric hindrance between the human antibody and the monoclonal antibody. Excessive washing procedure may result in the dissociation of low avidity alloantibodies. In addition modification of the epitope during storage should be taken into account. Alloantibodies are heterogeneous, anti HPA-1a undetectable with the MAIPA technique HPA-1a, « false negative MAIPA », are detectable with surface plasmon resonance technology. Some anti HPA-3a alloantibodies are detectable only using fresh platelets in flow cytometry assays. Detection of alloantibodies directed against low frequency antigens is somehow more complicated. Cross-match with maternal sera and paternal platelets is to be performed. However it depends on paternal platelets availability and ABO mismatch. New techniques are developed to overcome these problems such as B-lymphoblastoid cell lines, stably transfected CHO cells and recombinant mini-b3 integrin fragments. Due to variation in sensitivity of antibody detection among laboratories and the absence of monoclonal antibodies against human platelet antigens, only HPA-1a antibodies have been made up to now, international standard reagents for detection of human antibody against human platelet antigen have also been developed: Anti HPA-1a for quantification, anti HPA-5b, anti HPA-3a and minimum sensitivity for anti HPA-1a.

Difficulties in laboratory diagnosis for fetal and neonatal alloimmune thrombocytopenia should not delay therapy when there is bleeding tendency or severe thrombocytopenia. When the diagnosis is equivocal, retesting is recommended with new samples.

In conclusion, international workshop exercises are of importance for further improvement and quality assurance proficiency.

2C-S05-02

NEONATAL ALLOIMMUNE THROMBOCYTOPENIA (NAIT) CAUSED BY ANTIBODY SPECIFIC FOR A NEWLY IDENTIFIED ALLELE OF HUMAN PLATELET ANTIGEN (HPA)-7

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Background: Neonatal alloimmune thrombocytopenia (NAIT) is a neonatal disorder characterized by maternal alloimmunization against fetal platelet antigens inherited from the father. A healthy 30-year-old Japanese woman gave birth to her second child after an uneventful pregnancy. Nine hours after birth, the infant presented with severe petechiae, and a platelet count of 6 x 10(3)/μL. Two years later, she delivered her third child, also having NAIT, (who presented with petechiae and a platelet count of 3 x 10(3)/μL).

Study design and methods: To elucidate the maternal cause of the NAIT in the second infant, serological and genetic studies, including platelet genotyping and sequence-based analysis, were conducted. In addition, serological screening for the new platelet antigen was performed.

Results: Serum from the NAIT infant's mother contained antibodies against human platelet antigen (HPA) of the newborn child. A family study revealed that this antigen was inherited in an autosomal codominant fashion. Using 5-cell lineage flow cytometry, we localized the antigen to a platelet glycoprotein (GP). Subsequent platelet immuno-fluorescence inhibition experiments localized the antigen to the GP IIIa subunit of the GP IIb/IIIa complex. GP IIIa localization was confirmed in sequence-based typing studies, which identified a 1297C>T (407proline>serine substitution) mutation in the ninth exon of the GP IIIa gene. We provisionally designated this antigen HPA-7osa. This C>T mutation occurs at the same nucleotide position as the single nucleotide polymorphism of HPA-7b, which involves a C>G substitution in the GP IIIa gene. The third child with NAIT also has HPA-7osa antigen. Serological screening HPA-7osa in the Japanese population revealed a phenotypic frequency of approximately 0.0015.

Conclusion: We identified a new third allele of HPA-7, which was characterized by a 1297C>T mutation of the GP IIIa gene. This allele designated as HPA-7osa was found in 0.15% of the Japanese population. An antibody against this antigen could be the cause of severe NAIT.

2C-S05-03

THE HUMAN PLATELET ANTIGEN (HPA) SYSTEM OF 1 THROUGH 10 GENOTYPE AND ALLELE FREQUENCIES BY PCR-SSP IN DONORS IN CHINESE CANTONESE

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Background: Platelet-specific antibodies may be involved in refractoriness to platelet transfusion, disorders such as neonatal alloimmune thromobocytopenia and post-transfusion purpura. Genotyping for major human platelet antigen (HPA) systems is of considerable help in establishing the diagnoses of these diseases. It is also important for seeking compatible donors for platelet transfusions in immunized patients.

Aims: To investigate the HPA system of 1 through 10 genotype and allele frequencies by PCR-SSP in Chinese Cantonese.

Methods: A total of 200 samples from random Chinese Cantonese donors were involved in the study. The genotyping for HPA system 1 through 10 was carried out by using PCR-SSP technique.

Results: The genotype and the allele frequency of human platelet antigen system 1 through 5 in donors in Chinese Cantonese are as in table 1. The genotype and allele frequency of HPA system 6 through 10 are as in table 2. In the study, none of HPA-1b/1b, 2b/2b, 5b/5b homozygosis was detected which were found in other racial groups

Table 1 Genotype frequency and Allele frequency of human platelet antigen system 1 through 5 (n=200)

	Genotype		Allele	
	Observed number	Frequency (%)	Observed number	Frequency (%)
HPA-1a/1a	198	99·0	398	99·50
HPA-1a/1b	2	1·0		
HPA-1b/1b	0	0·0	2	0·50
HPA-2a/2a	185	92·5	385	96·25
HPA-2a/2b	15	7·50		
HPA-2b/2b	0	0	15	3·75
HPA-3a/3a	60	30·0	217	54·25
HPA-3a/3b	97	48·5		
HPA-3b/3b	43	21·5	183	45·75
HPA-4a/4a	198	99·0	398	99·50
HPA-4a/4b	2	1·0		
HPA-4b/4b	0	0·0	2	0·50
HPA-5a/5a	196	98·0	396	99·00
HPA-5a/5b	4	2·0		
HPA-5b/5b	0	0·0	4	1·00

Table 2 Genotype frequency and Allele frequency of human platelet antigen system 6 through 10 (n=200)

	Genotype		Allele	
	Observed number	Frequency (%)	Observed number	Frequency (%)
HPA-6a/6a	189	94·5	388	97·00
HPA-6a/6b	10	5·0		
HPA-6b/6b	1	0·5	12	3·00
HPA-7a/7a	200	100·0	400	100·00
HPA-7a/7b	0	0·0		
HPA-7b/7b	0	0·0	0	0·00
HPA-8a/8a	200	100·0	400	100·00
HPA-8a/8b	0	0·0		
HPA-8b/8b	0	0·0	0	0·00
HPA-9a/9a	200	100·0	400	100·00
HPA-9a/9b	0	0·0		
HPA-9b/9b	0	0·0	0	0·00
HPA-10a/10a	200	100·0	400	100·00
HPA-10a/10b	0	0·0		
HPA-10b/10b	0	0·0	0	0·00

Conclusions: The allele frequency of HPA-3 may play an important role in HPA alloimmune disorders in Chinese Cantonese. HPA-2, -6ï¼Œ-5 ï¼Œ-1 and -4 should be considered substantially following HPA-1 in the HPA alloimmune disorders. The rarity of allele frequency of HPA-7b, -8b, 9b and 10b may lead to a low incidence of neonatal alloimmune thrombocytopenia and posttransfusion purpura in Chinese Cantonese.

2C-S05-04

PRESENCE OF PLATELET-ASSOCIATED ANTI-GPVI AUTOANTIBODIES AND RESTORATION OF GPVI EXPRESSION IN PATIENTS WITH GPVI DEFICIENCY

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Background: Glycoprotein (GP) VI deficiency is a rare platelet disorder with a mild bleeding tendency. However, its pathophysiology remains unclear.

Objectives: We characterized a novel GPVI-deficient patient with immune thrombocytopenic purpura and examined for the presence of anti-GPVI autoantibodies in this and another patient with GPVI deficiency.

Materials and results: A 12-year-old Japanese girl (case 1) with moderate thrombocytopenia and mild bleeding showed selectively impaired collagen-induced platelet aggregation. Flow cytometric analysis indicated that the patient possessed a defect in the expression of GPVI/FcRg. An eluate of her platelet-associated IgG contained anti-GPIIb-IIIa autoantibodies. Moreover, using GPVI/FcRg-transfected cells, we unexpectedly identified anti-GPVI antibodies against the soluble ectodomain of GPVI in the eluate despite the patient's GPVI deficiency. In contrast, anti-GPVI antibodies were not detectable in her plasma. In another case of GPVI deficiency (case 2) without detectable plasma anti-GPVI antibodies, we again detected platelet-associated anti-GPVI antibodies. In a two-year follow-up of case 1, the platelet count increased to within the normal range and the bleeding tendency improved. Interestingly, GPVI was again expressed on her platelets associated with a decrease in the relative amount of anti-GPVI antibodies.

Conclusion: This is the first demonstration of platelet-associated anti-GPVI antibodies in GPVI deficient subjects, in one case, with spontaneous restoration of GPVI expression. These results strongly suggest an autoimmune mechanism in GPVI deficiency.

Monday: Parallel Session S6: Cellular Therapies

2C-S06-01

UNRELATED HEMATOPOIETIC STEM CELL TRANSPLANTATION AND BANK

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Allogeneic hematopoietic stem cell transplantation (HSCT) from a human leukocyte antigen (HLA)-matched unrelated (UR) donor has been established as one mode of curative therapy for hematologic malignancies and other hematologic or immunologic disorders, when an HLA-identical sibling donor is unavailable. Unrelated voluntary donors are found through marrow donor registries in the country or the international donor exchange program.

After extensive research on genetic factors such as HLA, there is now accumulating evidence of the presence of HLA alleles that drastically affects the UR-HSCT outcome. Selection of HLA compatible donor reduces the risk of acute graft-versus-host disease (GVHD) and chronic GVHD, and improves the survival after UR-HSCT. Also, the induction of the graft-versus-leukemia (GVL) effect to reduce relapse of leukemia is considered one of the advantages of allogeneic HSCT. There have been several large-scale analyses of UR-HSCT. The Japan Marrow Donor Program (JMDP) demonstrated the effect of matching of HLA class I alleles (HLA-A, -B and -C) on the development of severe acute GVHD and the importance of HLA-A, -C and -B allele-matching for better survival in T cell-replete UR-HSCT. Some non-JMDP registries also reported the importance of HLA class II matching for GVHD and survival. Updated analysis of NMDP in US indicated that HLA-A allele level mismatching, HLA-B serological mismatching and DRB1 mismatching are significant risk factors for severe acute GVHD, and that disparity in HLA class I (HLA-A, -B or -C) and/or HLA-DRB1 increased the mortality. Furthermore, the role of HLA-DPB1 matching has been elucidated for acute GVHD and for leukemia relapse in some registries. However, the reports mentioned above have produced considerable conflicting results.

The association of ethnicity with the incidence of GVHD and the other clinical outcomes after HLA identical sibling bone marrow transplantation is well documented. However, there have been no international analysis for the association of ethnicity in UR-HSCT, and only the analysis based on each registries or institutions with different genetic background existed. The large scale international unrelated HSCT data base provides a unique opportunity to compare the incidence of acute GVHD after HLA matched UR-HSCT according to donor-recipient ethnicity, and results obtained from this analysis becomes basic data for further international analysis of HLA mismatch unrelated HSCT and for international donor exchange of unrelated donor.

Recently unrelated cord blood transplantation (UR-CBT) not only in children but also in adult has become one of standard for transplantation, and barrier of HLA in UR-CBT need to be explored.

2C-S06-02

CORD BLOOD TRANSPLANTATION: EXPERIENCE IN IMSUT

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Disease relapse should be solved in all kinds of hematopoietic stem cell transplantations. When we use cord blood graft, the engraftment failure and delayed recovery of graft function are other important problems awaiting solution. The hematopoietic engraftment was provided by graft and recipient factors. In cord blood transplantation, cell dose, viability of cell and human leukocyte antigen compatibility are recognized as graft factors. Underlying disease, infection, alloimmunization and damage of bone marrow microenvironment are important as recipient factors. Especially, inadequate conditioning regimens have been associated with failure to maintain donor hematopoiesis. The main aims of conditioning regimen are to eradicate malignant disease effectively and to obtain sufficient immune suppression for engraftment.

Since the 27-year old lady with treatment-resistant acute myelogenous leukemia (AML) transformed from myelodysplastic syndrome (MDS) received cord blood graft as the first adult recipient in Japan on August 1998, cord blood transplantation was performed for almost 200 adult patients in our institute. We are using total body irradiation (TBI) containing conditioning and cyclosporine plus methotrexate (CsA+MTX) as graft-versus-host disease (GVHD) prophylaxis in most of cases. In late '80s, we have developed recombinant granulocyte-colony stimulating factor (G-CSF)- combined conditioning regimen for myeloid malignancies to increase the susceptibility of S-phase specific cytotoxicity of cytrabine (Ara-C). For cord blood recipient, as same as for unrelated bone marrow recipient, we continue to use the conditioning regimen consist of 12 Gy TBI, G-CSF combined with Ara-C and cyclophosphamide (CY). We are using this regimen to obtain sufficient immunesupresion and effective cytotoxicity.

We studied the clinical outcomes of 102 adults (> 16 years old) with myeloid malignancies (AML 79, MDS 15 and chronic myelogenous leukemia eight) who received single unit of cord blood graft between August 1998 and December 2008. Overall rate of advanced disease was 62%. All patients received 12 Gy of TBI plus G-CSF-combined Ara-C plus CY regimen and most of patients received CsA + MTX method. Median numbers of leukocytes and CD34+ progenitor cells before freezing of cord blood grafts were 2.4x107/kg and 0.9x105/kg, respectively. Median follow-up of survivors was 64 months. Overall myeloid and platelet engraftment rates were 92% and 82%. The 2-year cumulative incidence of transplant-related mortality was 9%. The 3-year cumulative incidences of relapse in patients with standard and advanced stage were 8% and 22%.

Five-year probability of overall survival was 68% and those of disease-free survival in standard and advanced stage patients were 80% and 58%, respectively.

These data suggest that G-CSF combined myeloablative regimen might be promising for better engraftment and for lower relapse rate in patients with myeloid malignancies. The results of multicenter prospective studies in Japan are expected soon.

2C-S06-03

EFFECTS OF PLATELET SUPERNATANT AND LYSATE ON EXPANSION AND DIFFERENTIATION OF UMBILICAL CORD BLOOD CD133+ STEM CELLS TO MEGAKARYOCYTE PROGENITOR CELLS

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Background: In this study, we surveyed the effects of platelet growth factors including the platelet supernatant and platelet lysate on the expansion and differentiation of cord blood CD133+ stem cells into megakaryocytic progenitor cells.

Aims: The aim of this study was to examine the effects of PLATELET SUPERNATANT (P.S) and PLATELET LYSATE (P.L) on the expansion of umbilical cord blood CD133+ stem cells and differentiation to megakaryocyte progenitor cells.

Methods: Umbilical cord blood CD133+ stem cells were isolated using the MidiMacs separation system. CD133+ cells were placed in a serum free medium supplemented with IL-1, IL-6, IL-3, TPO and stem cell factor (SCF) for 7 days at 37°c and 5% co2 as a control cells. To investigate the effect of platelet supernatant and platelet lysate, the CD133+ cells were cultured at the presence of different concentration of the growth factors and cytokine cocktails. The concentration of cytokines of the platelet supernatant and platelet lysate were performed by ELISA. The expression of CD41 and CD61 antigens as a megakaryocyte progenitor cell markers were evaluated by

flow cytometry. A comparative study of means was performed using the analysis of variance (ANOVA) statistical test. The results were considered significantly different when $P < 0.05$.

Results: The results showed that platelet supernatant (50mg/ml) but not platelet lysate (50mg/ml) is able to suppress the expansion of CD133+ cells, significantly ($P < 0.05$). The flow cytometry assay showed that neither platelet supernatant nor platelet lysate can increase the expression of CD41 (18.05±4.19 and 16.75±4.05) and CD61 (19.18±4.3 and 17.91±4.29) in comparison with that of the control cells ($P < 0.05$). The concentration of growth factors were 240, 450, 0.8, 2.6 pg/ml and 18.1 ng/ml,1.8 ng/ml,10.2 pg/ml,31.0 pg/ml for PDGF-AB, TGF-B, FGF and EGF, respectively, in platelet supernatant and platelet lysate.

Conclusion: Taken together, platelet growth factors suppressed the expansion of UCB CD133+ cells and increased the differentiation of UCB CD133+ cells into megakaryocytic progenitor cells in a dose and time dependent manner. More studies are necessary to reveal the mechanisms of platelet growth factors effect on expansion of CD133+ stem cells, and differentiation to megakaryocyte progenitor cells.

2C-S06-04

SIGNIFICANT REDUCTION OF HPC QUALITY AFTER OVERNIGHT STORAGE OF PBSC PRODUCTS AT ROOM TEMPERATURE

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Background: Although cord blood has been recommended to be stored and delivered at room temperature (RT) within 24h after collection, there is no standard guideline for the optimal conditions for storage and transport of freshly harvested peripheral blood stem cells (PBSC) in Korea. Considering the number of leukocyte including CD34+ cells in mobilized PBSC is more than 10–20 times higher than cord blood, it needs to have validated and objective guideline for the optimal storage temperature and length.

Aims: In this study, we aimed to identify the influence of storage temperatures and various cellular parameters of PBSC harvests, and the optimal storage conditions before cryopreservation for higher CD34 recovery and eventually for better hematopoietic stem cell (HPC) transplant outcomes.

Methods: We included 231 PBSC harvests from 63 pediatric oncology patients for autologous hematopoietic stem cell transplantation which collected from 2007 June to 2009 January at Samsung Medical Center. The products were divided into two groups according to the pre-cryopreservation storage condition and compared the cellular parameters such as CD34+ count, viability and CFU-GM count.

Results: When we compared the 69 PBSC products which stored over night (O/N) at RT before cryopreservation to 162 processed on the day of collection, post-thawing viability (6–97%, median 73% vs. 24–99%, median 87%), CFU-GM count (0.0–2,156.0/ul, median 22.8/ul vs. 0.0–7,007.0/ul, median 99.2/ul) and CFU-GM/CD34+ ratio (0–13.2, median 0.015 vs. 0–25.5, median 0.122) were significantly decreased ($P < 0.0001$, Mann-Whitney test). Among O/N stored PBSC harvest at RT, 61% (42/69) had less than 0.005 of post-thawing CFU-GM/CD34+ ratio compared to 17% (27/162) of PBSC processed on the day of collection. Also higher the initial leukocyte count and poorer the post-thawing viability, lower the CFU-GM/CD34+ ratio ($P < 0.0001$, Kruskal-Wallis test).

Summary and conclusions: Storage of PBSC harvests during O/N at RT has significantly affected the quality of HPCs measured by CFU-GM assay after thawing. Although the tolerable duration for storage at RT or the optimal temperature which can guarantee the quality of HPCs needs to be further evaluated, based on this result, we strongly recommend precluding the O/N storage of PBSC harvests at RT.

Monday: Parallel Session S7: Clinical Transfusion

2C-S07-01

EVIDENCE BASED TRANSFUSION: A PARADIGM SHIFT IN GETTING SUPPORT OF EVIDENCE NEEDED?

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Background: Evidence-based medicine describes the process of incorporating critical appraisal of the literature, individual clinical expertise, patient values, clinical circumstances and societies' expectations into medical decision making. This formal approach is been increasingly utilized to formulate individual transfusion decisions and in setting national transfusion policies.
Aims: To describe the process of evidence-based decision making and its application to transfusion medicine through the analysis of the test case.
Methods: Critical appraisal of the literature and evidence based medicine techniques are described and applied to the issue of the duration of red cell storage and the incidence of complications after transfusion, as raised by the report of Koch et al (NEJM 358:12, 1229–39). This report compared the outcomes of patients transfused with blood after < 14 or >14 days of storage during and after cardiac surgery.
Results: The methods and logic of critical appraisal of the literature and evidence based medicine are reviewed. Critical appraisal of the paper by Koch et al reveals a case-controlled, retrospective observational study with Level III-IV evidence to support the use of fresher blood in cardiac surgery patients. Issues are raised concerning the equivalence of the prestudy prognosis of the test and control groups and the use of non-risk adjusted statistics in comparing data. After risk adjustment, the association of an older blood transfusion with a poorly defined composite index of adverse outcomes is of marginal significance (O.R. 1.16, 95% C.I. 1.01–1.33). Reports of systematic review of the literature reveals 19 publications: 14 studies are retrospective observational studies and many suggest an association of older blood with various adverse outcomes. Five prospective studies fail to show an adverse relationship, however these are small and under-powered. Society values emphasize the availability of blood products, which at this time requires inventory management practices that prevent the transfusion of blood < 14 days storage on a routine basis for all patients.
Conclusions: Evidence-based decision making provides a formal process to evaluate the information supporting individual clinical and national policy decisions in transfusion medicine. Application to the specific problem of whether to limit the use of older blood suggests that the level of evidence does not yet justify changing clinical practice. Adequately powered randomized control trials are necessary to determine the relative safety of older blood in transfusion practice.

2C-S07-02

MINIMIZING HUMAN ERRORS TO ENHANCE THE SAFETY OF BLOOD TRANSFUSIONS: A STUDY ON SAFETY OF CLINICAL TRANSFUSION PRACTICES IN NATIONAL HOSPITAL OF SRI LANKA (NHSL)

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Introduction: Blood and blood component transfusion therapy is a major aspect of the modern medical practice. Despite all its potential benefits it is associated with risk of serious adverse reactions, some of which are due to the human errors. Therefore quality assurance and adherence to accepted guidelines during all aspects of clinical transfusion practices is essential to ensure patient safety.
Aims: To evaluate the current clinical transfusion practices including transport of blood units to wards, storage in wards, cross-checking of blood before transfusion, documentation, transfusion process and monitoring in National Hospital of Sri Lanka (NHSL) using the existing guidelines published by National Blood Transfusion Service (NBTS) of Sri Lanka and identify the shortcomings to make recommendations to promote safe and appropriate use of blood and blood components.
Methodology: A prospective direct observational study of the clinical transfusion practices was done in NHSL. Study involved collection of data on clinical transfusion practices by direct follow-up and observation of randomly selected blood/blood component units by trained research assistants from their dispatch from blood bank to the end of transfusion. Study was conducted over a period of 1 month and all the data was computerized and analyzed using SPSS 15.0 for Windows statistical software package.
Results: Randomly selected 200 transfusion events (Red Cell Concentrates = 104, platelet concentrates = 30, fresh frozen plasma = 68, cryoprecipitate =134) were observed. Several types of transport containers including Regiform boxes (89%) and cooling boxes (8%) were used to carry the blood components from the blood bank to wards. Transport containers were not used in six events. On four occasions ice packs which were in direct contact with the blood packs were used inside the containers.
Time taken for the blood components to reach the ward after the dispatch from the blood bank was calculated. Delay of transporting to the ward was more than 30 min in 23% (n = 46).
After reaching the ward 26% of the products were kept outside the container till the commencement of the transfusion. Process of cross-checking of blood pack was correct only in 42%. Expiry date was checked only in 16% and none were checked for haemolysis or clots.
Positive patient identification was satisfactory only in 16%. Satisfactory information regarding transfusion reactions and symptoms were given only to 30% of the patients. Written instructions on monitoring were given only in 54%. Standard blood transfusion sets were always used and 18G cannula was used in 61% of transfusions. In 50% of the patients the same cannula was used to inject antibiotics (38%) and other drugs (12%). None of the transfusions were monitored with the use of a monitoring chart.
Conclusion: There is lack of safe transfusion practices and adherence to guidelines especially in the process of transport, patient identification, explanation of the transfusion process to the patient and monitoring. Formal training programmes to increase the awareness of the importance of safe transfusion practices at wards need to be implemented for the medical and nursing staff.

2C-S07-03

TRANSFUSION PRACTICES IN PAEDIATRIC CARDIOTHORACIC SURGERIES

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Background and aims: Blood requirement in complex paediatric cardiothoracic surgeries do not correlate well with the blood usage in adult procedures. Hence this study aims at:
1. Studying the present transfusion practices in different types of paediatric cardiothoracic surgeries
2. To study the crossmatch to transfusion ratio for each type of surgical procedure
Materials and methods: A total of 571 patients underwent paediatric cardiothoracic surgeries between Jan 2006 to May 2009. Patient details like age, gender, weight, Haematological parameters like haemoglobin(Hb), Platelet count, Prothrombin time (PT), INR, number of cross matches and transfusions, type of surgery were obtained form the Hospital information system. All surgical procedures except Blalock-Taussig shunt (BT shunt), were done using a Cardiopulmonary Bypass which was primed with packed cells and an electrolyte solution. None of the patients received pre-operative or intra- operative anti- fibrinolytic agents. Heparinisation and reversal with protamine were done according to the standard protocol. Patients who underwent the same type of surgery were grouped together.

The mean age, Hb, platelet count, PT, INR, crossmatches, transfusions, crossmatch to transfusion ratio were derived for each group.
Results: Table- I shows the different type of surgeries and the mean haematological parameters. Table - II shows the transfusion requirements and the cross match: transfusion ration for each surgical procedure.
The crossmatch to transfusion ratio was < 2 in most of the surgical procedures. The mean transfusions were more than the number of crossmatches in Coarctation of aorta repair and the repair of Patent Ductus Arteriosus.

Table I: Surgeries and the mean heamato

Type of surgery	mean age	Nos.	weight (mean)	Hb (mean)	PLT Count (mean)	PT (mean)	INR (mean)
ASD Repair	5·2	26	13·6	16·8	1·4	17·1	1·4
BT Shunt	4·8	15	14	17·9	1·4	18·9	1·6
TOF Repair	7	143	13·7	19·1	1·6	20·7	1·7
PDA Repair	5·4	89	17·7	18	1·6	19·6	1·7
COA Repair	6·8	29	16·7	17·3	1·7	18·9	1·5
VSD Repair	8·2	166	20·2	18	1·5	17·7	1·4

Table II: Transfusion requirements

Type of surgery	Cross match	red cells	platelets	FFP
ASD Repair	6	3	2	1
BT SHUNT	4	3	3	1
TOF Repair	6	4	1	1
PDA Repair	4	5	2	1
COA Repair	4	6	3	1
VSD Repair	4	4	2	1

Conclusions: Paediatric cardiothoracic surgeries require blood components because of the use of blood to prime the CBP in addition to the intra and post operative requirements. The Cross match to transfusion ratio was less tha two in most surgical procedures.

2C-S07-04

IMPLEMENTATION OF THE APPROPRIATE USAGE OF ALBUMIN IN JAPAN

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Background: Human albumin solution has been much more used in Japan compared with the international average level. It remains a problem to achieve self-sufficiency in all blood products including plasma derivatives such as albumin solutions.
Aims: To investigate the usefulness about implementation promoting appropriate usage of albumin.
Methods: A questionnaire of transfusion medicine including questions about appropriate transfusion therapy was sent to 2046 hospitals. Similar questionnaire study was perfomed every year form 2005 to 2008. Various implementation was studied in terms of the usefulness to promote appropriate use of albumin. Change in the amount of albumin used in Japan was based on the data of Blood Products Research Organization. A trend was analyzed in recent years.
Results: Replies were obtained from 1032 hospitals. In the early 1980s, the amount of albumin used in Japan accounted for about 30% of all over the world supply of it. The amount of albumin in Japan was gradually decreased in the early 2000s, and constantly declined from 2006 to 2008. Compared to 2006, there was a 10% decrease in albumin solution use in 2008. It's possible that transfusion management fee was useful, which was introduced by the national medical insurance system in 2006. A unified management system for albumin and doctors responsible for transfusion with full-time involvement in transfusion was also effective to promote appropriate use of albumin.
Conclusion: These results suggests that it's important to establish transfusion management system including plasma derivatives, and to design the policy promoting appropriate usage of albumin.

2C-S07-05

ANALYSIS OF THE TRANSFUSION-RELATED HAZARDS IN JAPAN

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Background and aim: In Japan, Red Cross Society (JRC) has collected the information on adverse reactions and infectious diseases of blood transfusion since 1993. The reporting system provided by medical institutions nationwide is based on voluntary. It is not clear how cover transfusion-related hazards by the current system. The National Survey on the present state of transfusion-related hazards in Japan was performed by the Japanese Society of Transfusion and Cell Therapy (JSTC).
Methods: The targets of the Survey were 2046 hospitals in 2008. Replies were received from 1032 hospitals. A questionnaire regarding transfusion medicine was sent containing identical questions to hospitals for each of the years from 2004 to 2008.
Results: Unified reporting systems to Hospital Transfusion Committee or transfusion-related department in each hospital for adverse reactions gradually spread and increased to 87.6% in 2008 compared to 81.7% in 2004. Although adverse reactions were widely reported in paper form reporting by Internet did not appear to be customary use as only 129 hospitals (13.0%) mentioned doing so in 2008. Transfusion was performed using 1,322,396 bags of red cell concentrate, 456,555 bags of fresh frozen plasma and 433,116 bags of platelets concentrate at 1032 hospitals in 2008, and was equivalent to approximately 40% or more in Japan. Serious hazards stemming from transfusion were investigated in 2008, and 23 cases demonstrated transfusion-related acute lung injury (TRALI). Severe allergic reactions were detected in 64 cases and transfusion-transmitted bacterial infections in two cases. No graft versus host disease (GVHD) was diagnosed. Hemolytic transfusion reactions excluding ABO-incompatible transfusions were 4 cases. ABO-incompatible transfusions were 10 cases, and the blood product used red cell concentrates in fivecases, fresh frozen plasma in four and platelets concentrates in one and nobody died cause of

transfusion. There were eight deaths reported among hazards stemming from transfusion, in five of whom the cause of death is thought to TRALI and one is thought to hemolytic transfusion reaction.
Conclusion: JRC is the only organization to supply blood products in Japan, and the confidential party like JSTC is necessary to collect the reliable information. In addition, survey of adverse reactions during transfusion is important in order to establish appropriate transfusion practices, including measures against the hazards of transfusion.

Monday: Parallel Session S8: Blood Products

2C-S08-01

MANAGING FRACTIONATED PRODUCTS IN DEVELOPING COUNTRIES

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Background: In the low and medium Human Development Index (HDI) countries where 82% of the world population lives, only 39% of the global blood supply is collected and used. It is highly likely that access to fractionated product is even lower. However patients like haemophilia are dependent on clotting factor replacement for management of their condition. Efforts are being made in many countries to provide these products. In the developing countries that has attained higher standard of living and where the healthcare system is better organised, the need to ensure transfusion dependent patients like haemophilia have access to safer blood products becomes a priority.

The three sources of fractionated products are: 1. Setting up a fractionation plant and fractionating plasma that is collected

2. Collecting plasma for contract fractionation

3. Sourcing fractionated products commercially

Setting up a fractionation plant requires huge resources that are beyond the reach of developing countries and the amount of plasma available for fractionation is too low to do so. Contract fractionation is a reasonable alternative. Commercial products are another way of making these products available for patients. All these have their advantages and disadvantages.

Many developing countries do not have fractionated products like Factor VIII, Factor IX, and Immunoglobulin. Haemophilia patients in these countries are managed by using cryoprecipitate or fresh frozen plasma (FFP). Where the safety and quality of these products are not assured, these patients are vulnerable to infections and inadequate management. In a poorly funded health system, the fate of these patients is rather bleak.

In developing countries with more advanced economies in what is termed as newly industrialised countries, patients can have access to commercial blood products. However, the cost of these products is prohibitive.

There are also occasions when the global plasma supply is threatened by external factors or there is a shortage in supply. In this situation developing countries face acute shortage that leaves patients without appropriate treatment.

In moderately well developed blood transfusion programme where blood components therapy is practised, generally there is an excess of FFP. This leads to wastage and high cost of components production which makes it uneconomical to continue component therapy. These FFP could be fractionated into blood products that are required. While setting up a fractionation plant is costly and not feasible, contract fractionation is a workable alternative. It may also be more cost effective. Examples can be seen in Iran and Malaysia. Contract fractionation may not provide the entire need, but it may be able to partially supply the total requirement and also supplement during periods of global shortages.

Conclusion: Although recombinant products are available in high HDI countries there are not within reach as far as low and medium HDI countries are concerned. These countries would have to try to provide safer treatment that is more affordable. Striving for self sufficiency in these circumstances is important against uncertain supply from commercial sectors that depends on plasma collected from developed or high HDI countries.

2C-S08-02

EVALUATION OF VESICLE FORMATION IN STORED BLOOD AFTER PRION AND LEUKOCYTE FILTRATION

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Background: Cell-derived vesicles arising in stored blood components may affect transfusion safety and efficacy. For this reason, processing and storage technologies should be evaluated for their impact on vesicle formation. Flow cytometry is a useful tool not only for the investigation of cell-sized vesicles, but also, microvesicles such as platelet-derived microparticles (PDMP), which are not detected by conventional hematology analyzers or light microscopy.

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

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

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

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

2C-S08-03

QUALITY CONTROL OF LEUKOCYTE-REDUCED BLOOD PRODUCTS IN JAPAN

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Introduction: The Japanese Red Cross (JRC) began a process to reduce the leukocyte counts in platelet concentrates derived from apheresis donations (PC) in Oct. 2004, then in fresh frozen plasmas derived from apheresis donations (FFP) in 2006, and lastly in blood products derived from whole blood donations (WB) in 2007. For quality control, the count of leukocytes in the blood products is determined by sampling inspections at a Quality Control Laboratory of the JRC Central Blood Institute. Twenty-eight blood centers of the JRC currently prepare blood products. For leukocyte counting, these centers perform fixation of the samples and send them to the Laboratory of the JRC Central Blood Institute. The JRC Central Blood Institute determines the counts by flow cytometry. According to regulation, the leukocyte number in 95% of the samples of the blood products should be lower than 1x10^6/bag.

Methods: Samples are fixed by Pallfix (Pall, Tokyo, Japan) at room temperature at the blood centers, and then sent to the JRC Central Blood Institute, where they are stored at 2–8 C until use. The leukocyte count is determined at the JRC Central Blood Institute by flow cytometry - FACS Calibur (BD Biosciences, SanJose, USA) or Cytomics FC500/BC (Beckman

Coulter, Tokyo Japan). The LeucoCOUNT Kit (BD Biosciences) is used for the sample preparation and staining for flow cytometry. The data from 2007 to 2008 were used for this analysis.
Results: The results revealed that the leukocyte counts in 57 samples out of 39,900 PCs (0.14%), 23 samples out of 85,243 FFPs (0.03%), and 183 samples out of 70,800 WBs (0.26%) were over 1x10^6/bag. Almost all products (over 99% of the PCs and FFPs, and 96% of WB products) showed a leukocyte count of under 5x10^5/bag; especially 77.3% of the PCs showed leukocyte counts of under 5x10^4/bag. Differences were found in the distribution of the leukocyte counts in FFPs according to the apheresis machine used: 55.4% of the FFPs prepared using an apheresis machine of one brand showed leukocyte counts of under 5x10^4/bag; on the other hand, only 15.2% of FFPs prepared using an apheresis machine of another brand showed leukocyte counts of under 5x10^4/bag. The counts in the WB products were widely distributed; 38.3% showed counts of 1–5x10^5/bag, 21.0% showed count of 1 x10^5–5x10^4/bag, and 37.2% showed counts of under 5x10^4/bag.
Conclusions: The leukocyte count in all blood products were within limits and conformed to regulation (95% of the products showed leukocyte counts of under 1x10^6/bag). However, in the products, the leukocyte counts varied widely depending on the blood collection method and the apheresis machine used.

2C-S08-04
PROBLEMS AND CHALLENGES OF PLATELETAPHERESIS IN YOGYAKARTA BLOOD TRANSFUSION SERVICE, INDONESIA

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Background: In Indonesia, plateletapheresis is a new technology in the blood transfusion service. In November 2006, the first procedures to collect single donor apheresis platelets applying MCS3p (Haemonetics®) were started. Until March 2009, 126 procedures were performed and the collected components were distributed to patients. However, we face some problems and on the other hand also some challenges.
Problems: One of the main problems in Indonesia is the costs of one unit apheresis platelets. The price is 20 times of that one unit of whole blood derived platelet concentrates (PCs), US$280 vs. US$14. In patient care, the platelet dose for an adult is equivalent to 5–6 PCs. Therefore, the price of one unit apheresis platelets is equivalent to four times of PCs. In Indonesia, the health insurance is not covering this product. Consequently, not all patients can afford apheresis platelets. Therefore, we can't keep apheresis platelets in stock, e.g. for emergency requirement. Plateletapheresis procedures are only performed on clinicians' request. A second problem is the availability of only one type of apheresis machine (MCS3p). The procedure time to take one unit of platelets by this machine is longer than that of newer types. As a consequence, reductions of donor comfort. Another problem in our 24/7 hour service is the availability of only four apheresis nurses. All are trained, however the skill and knowledge is not equivalent. Not always apheresis procedures can be performed and in some circumstances, difficult conditions/problems during a procedure can appear.
Challenges: At present, health insurance only covers whole blood derived blood components. Compared PCs, transfusion of apheresis derived (single donor) platelets show less transfusion reaction/adverse events, and reduction of transfusion transmittable infections. Therefore, it's important to persuade health insurance companies and government how beneficial apheresis platelets can be. By covering this product in their system, the product can be available for more patients. Because of higher cost, we can't deliver apheresis platelets without the clinician's request. The advantage of this technology is known and it is realized that this product is preferred above whole blood derived PCs. However, in our two years experience, the usage of this product still limited to patients from the pediatric and internal medicine ward. In periods of shortage of whole blood donor, e.g. the Dengue season, apheresis platelets are needed to cover the shortage of PCs especially in patients with Dengue Hemorrhagic Fever. Thus, increase of the knowledge of the clinicians regarding plateletapheresis is needed.
Unfortunately, in Indonesia adequate apheresis training is lacking. Since the first procedure of plateletapheresis, our technicians learn troubleshooting from the mistakes made performing a procedure: learning by doing. To increase service for donors and patients, improving of operator skills and knowledge is a must by introduction of routine (re)trainings.

2C-S08-05
GRANULOCYTE TRANSFUSION: DETERMINANTS OF LEUKOCYTE YIELD AND CLINICAL EFFICACY -EXPERIENCE IN A SINGLE TERTIARY ADULT HAEMATOLOGY UNIT

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Background: There was increasing interest in granulocyte transfusion(GT) since GCSF became available in 1990s. Its efficacy among adult neutropenic patients remained uncertain.
Aims: Describe and assess safety, feasibility and efficacy of granulocyte procurement, and GT among adult patients with severe neutropenic sepsis from March 2008 to May 2009 in a single tertiary haematology unit.
Methods: Retrospective analysis of clinical and laboratory parameters of granulocyte donors and recipients.
Results: Donors:48 granulocyte donations, with median leukocyte yield (LY) of 65.18(31.30–131.72)X10(9), were collected from 39 healthy donors(23 males, 16 females)using hydroxyethyl starch(HES) and intermittent flow centrifugation(IFC) aphaeresis, after 8 mg dexamethasone(three had 4 mg) and 300 mcg GCSF. 7donors donated twice and one donated thrice. Due to urgency of collection, four donations were collected 2–3h after premedication and produced good median LY of 56.18(45.68–62.90)X10(9). These were not significantly different from donations obtained >12h after premedication(median LY 65.48(31.30–131.72)X10(9); P=0.246). Donors' median age and weight were 29(20–51)years and 63(46–85)kg respectively. Median 431(272–716)ml of granulocyte were procured after processing median 6.85(4.40–10.42)l of blood volume(BV), which was 0.91–2.30(median1.58)times of total BV. Median predonation WBC, neutrophil count(ANC), platelets and Hb were 38.14(29.06–49.24)X10(9)/L, 36.46(18.82–46.77)X10(9)/L, 288(168–393)X10(9) and 14.9(12.30–16.90)g/dL respectively. Postdonation, platelet count dropped by median 55.5(37.8–82.3)% to median 127(52–263)X10(9)/L; Hb dropped by 15.2(4.0–33.0)% to 13.0(9.2–15.4)g/dL which were clinically acceptable. LY did not correlate with donors' weight, BV processed, predonation WBC/ANC, gender or age. No serious adverse donor reactions occurred.
Patients: 12 patients, aged19–61(median 32)years, received median 3(2–9)ABO-Rh-matched GT over median of 6(3–14)days without serious adverse reactions. They had AML(n = 5), ALL(n = 6)or severe AA(n = 1)-which were newly-diagnosed(n = 4), relapsed/refractory(n = 3) or in CR(n = 5). They received induction chemotherapy(3), salvage chemotherapy(2), consolidation chemotherapy(3), allogeneic BMT(n = 2), autoBMT(n = 1) or ATG(n = 1). All had severe neutropenia(SN)(median nadir WBC(WBCnadir) 0.03(0.01–0.15)X10(9)/L)and severe infections: 10 bacterial(nine bacteraemic, one other culture-proven)and nine fungal(four proven, three probable, two possible)and one severe viral pneumonitis. They received GT at median of 21(6–61)days after onset of SN. Median WBC rise(WBCrise)was 0.83(0–3.76)X10(9)/L 5–18(median11)h after GT. WBCrise did not correlate with LY, LY/kg, LY/m2, age, WBCnadir, SN duration, weight, body surface area, bacterial or fungal infections, but was significantly(P = 0.025)higher among females than males(1.43 vs 0.54X10(9)/L). Four (33.3%)patients survived after responding to GT and resolution of infections; the rest died of infections. Mortality did not correlate with patient's median WBCrise, median LY/kg, median LY/m2, fungal infections, GT duration, SN duration, duration from SN onset to GT or gender. On univariate analysis, there was tendency for mortality to be increased by age(26vs 44 years,P = 0.148), culture-proven bacterial infections(P = 0.091), ICU admission(OR21,P = 0.067) and ventilation requirement(OR21,P = 0.067), and it was significantly increased among

patients requiring inotropes (P = 0.018). On multivariate analysis, only inotropic requirement was significantly associated with mortality (P = 0.000).

Conclusion: Healthy donors can safely donate granulocytes, even repeatedly in some. With GCSF and dexamethasone, good LY was obtained using HES and IFC, irrespective of donors' weight, gender and processed BV. Four donors produced good LY despite collection at short duration (2–3 h) after premedication: this is worth further evaluation and may improve logistics of granulocyte collection. GT may benefit post-therapy haematologic patients with prolonged SN, especially young patients, before they develop bacteraemia or deteriorate haemodynamically. Large prospective RCT investigating the role of GT in prevention of severe infections among such patients are needed.

Monday: Parallel Session S9: Management and Organisation

2C-S09-01

COST EFFECTIVENESS OF BLOOD PROGRAMMES - ASIA PACIFIC BLOOD NETWORK PERSPECTIVE

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Globally, cost management of blood programs is seen as a critical strategic issue. This needs to be balanced with other strategic priorities, including sustainability of the blood system and provision of sufficient, safe and appropriate products and services for patient care. Asia Pacific blood services seek improvement in cost efficiencies through both local initiatives, and through partnering with other countries via international networks such as the Asia Pacific Blood Network (APBN).

Through the APBN, blood services participate in two key areas that have the potential to yield improved cost efficiencies. These are:

a. Comparison of Practice
b. Knowledge Exchange

This paper outlines the way in which the APBN members have utilised these opportunities to identify and understand internal and external cost drivers and through this, help address challenges. Cost data is less useful in effecting change than measures of operational efficiency, as well as utilisation and wastage rates. Some common tools and approaches will be discussed, although it is recognized that for each country with its unique set of stakeholders, geography, and socio-political, legal, regulatory and economic parameters, the decisions made may well be quite different but appropriate for the local context.

2C-S09-02

WHAT DOES IT REALLY COST TO TRANSFUSE A UNIT OF RED CELLS? THE AUSTRALIAN COST OF TRANSFUSION STUDY

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Health costs are escalating worldwide, driven by, among other reasons, changes in population demographics (for example, an ageing population) and community expectations, and changes driven by advances in clinical practice and new technologies. In transfusion, major investments have been made in product safety improvements, generally with less focus until recent years on improving clinical practice. The total costs of transfusion are generally not well captured or documented in the literature, and therefore we have had limited visibility and understanding of the true complexity, risks and costs of clinical transfusion practice.

The Australian cost of transfusion study is a collaborative study designed to capture, as far as possible, all the hospital costs of transfusing a unit of red cells. The two hospitals represent different institutional settings: Flinders Medical Centre (FMC) is a major tertiary referral centre in Adelaide with a broad range of activities: trauma service, cardiac and other major surgery, solid organ transplantation, obstetrics and pediatrics, operating over multiple sites. Peter MacCallum Cancer Centre (PMCC) is a single-site quaternary adult hematology/oncology referral centre providing comprehensive cancer care services in Melbourne.

Using data from January-December 2006, detailed clinical and laboratory process maps were constructed and validated for every step required to transfuse a single unit of red cells. De-identified aggregate transfusion episode data were extracted from clinical and laboratory databases at both hospitals. Non-red cell components, exchange transfusions, and transfusions in pediatric and trauma patients were excluded. Personnel and financial data associated with red cell transfusion, such as direct and indirect costs (including salaries, on-costs and other activities such as education; costs relating to clinical and laboratory equipment, reagents and consumables: costs attributable to quality programmes such as auditing and transfusion committee activities) and generic overhead costs, were extracted from administrative databases and assigned for each of the process steps. The data were incorporated into costing software modules developed by 'Medizinische Gesellschaft für Blutmanagement' based on 'ARIS Business Architect 7.0' and 'Business Optimizer 7.0' (IDS Scheer AG, Germany). The process maps and costings from the study will be discussed in the presentation.

The complexities of the activities described in this study demonstrate the importance of understanding all the processes, in order to create a safe and workable system. This includes a multidisciplinary team with relevant education and training, working with the appropriate equipment and other resources required, to manage every stage of the process, and operating within a comprehensive institutional quality management system. Better information regarding costs and complexities will inform assessments of transfusion practice, and alternatives to transfusion, including directing where we should invest energies and funds to improve the transfusion process and minimize risks and costs in a changing healthcare environment.

Disclosure: the study was funded by an unrestricted grant from Amgen Australia.

2C-S09-03

THE EFFICACY OF ALT MEASUREMENT BEFORE 400ML WHOLE BLOOD DONATION AT MOBILE BUSSES

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Aims: At Kanagawa Red Cross Blood Center, we collect 180,000 units of whole blood each year. Unfortunately, approximately 4% (7,049/184,989; 3.81% in 2008) must be discarded due to alanine aminotransferase (ALT) exceeding 61 IU/L. We did a trial to determine the efficacy of ALT measurement just before donation at our mobile busses at which over 70% of our whole blood is collected.

Materials and methods: From June 1 to June 22, two mobile busses were equipped with dry-chem ALT measuring machines (Reflotron, Roche). We exclude blood from donors whose ALT value exceeds 61 IU/L and use the remaining blood for measuring hemoglobin. The percentage of donations with abnormal ALT measured by Hitachi Autoanalizer at the Tokyo Blood Center was also calculated. The rates of abnormal ALT were determined by Reflotron plus Hitachi and by Hitachi alone. Measurement by Reflotron was limited to 1. first time donors, 2. an ALT value exceeding 50 IU/L in a previous donation, and 3. abnormal liver function at a periodic health checkup.

Result: Abnormal ALT was found in 32 (2.41%) of 1,326 units of whole blood collected. 19 donations were immediately stopped because of an abnormal Reflotron ALT value. The donations found later by Hitachi test resulted in a discard rate of 3.79% (51/1,345). Chi-square test showed a significant reduction in the discard rate ($P < 0.05$).

Discussion: Although the number of samples was too small to prove the efficacy of ALT measurement just before donation, we succeeded in reducing the discard rate by 1%. At this rate, each year we could save 2,000 blood bags, laboratory costs, and some of the expenses of our 50 mobile busses. Further investigation with a larger number of donations will be necessary to confirm the results of this preliminary trial.

2C-S09-04

UNDERSTANDING TRANSFUSION OUTCOMES THROUGH CLINICAL REGISTRIES: VALIDATION OF A LINKAGE TECHNIQUE

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Background: There is evidence of substantial variation in transfusion practice, but little understanding of how this variation affects patient outcomes. While information regarding blood products transfused is required to be retained, these data have not been correlated with clinical outcomes. However, several registries gather clinical outcomes data without transfusion information. An opportunity exists to use these two sources to explore the effect of transfusion on clinical outcomes.

Methods: Collection of transfusion data by record review or expansion of registry datasets has practical drawbacks. These data are captured by transfusion laboratory information systems (LIS). However, the validity of LIS data has not been confirmed.

Prospective validation of LIS data against individual patient records was undertaken at two major clinical centres in Victoria, Australia. Data regarding all transfusion episodes were compared over seven 24 hour periods at each centre. All clinical areas, all fresh blood product types, and each day of the week were included.

Results: Data regarding 596 units were captured; 218 centre 1 (37%), 378 centre 2 (63%), comprising 399 red cells, 95 platelets, 72 plasma and 30 cryoprecipitate units. 221 (37%) units were issued to inpatient wards including 99 (45%) to haematology/oncology; 109 (18%) to intensive care units; 95 (16%) to outpatient units including 63 (66%) to haematology/oncology day wards; 45 (8%) to operating theatres; and 27 (5%) to emergency departments. The location of transfusion was unrecorded for 99 (17%) units.

All products recorded as issued by the LIS were transfused to the expected patient. The time from issue of the blood product to the time of initiation of the transfusion could only be calculated for 482 (81%) of the blood products. The time of transfusion (including initiation) was not recorded for 61 (10%), and was recorded to have occurred before the product was issued in another 53 (9%) blood products.

Where there was a documented time of transfusion after time of issue, the median time to initiation of transfusion for each product type was for red cells 18 minutes (95% CI 16–20; IQR 10–33), platelets 20 minutes (95% CI 16–24; IQR 11–38), FFP 60 minutes (95% CI 17–96; IQR 13–163) and cryoprecipitate 44 minutes (95% CI 3–179; IQR 7–194). There was no significant difference in the median time to transfusion between the two hospitals for each of the four blood product types. Transfusion commenced within the recommended timeframe in 73% of cases for red cells, 69% platelets, 50% plasma and 68% cryoprecipitate.

Conclusions: Across a range of blood product types and destinations, at two different institutions, comparison of LIS data with clinical records demonstrated concordance. The difference between LIS timing data and patient clinical records reflects the expected time to transport, check and prepare transfusion but does not affect the validity of linkage for most research purposes. Linkage of clinical registries with LIS data can therefore provide robust information regarding individual patient transfusion. This enables electronic analysis of joint data sets, either periodically or continuously, to determine the impact of transfusion on clinical outcomes in particular patient populations.

Tuesday: Parallel Session S10: TTI

3A-S10-01
BACTERIAL CONTAMINATION: EFFECT AND LIMIT OF THE INITIAL FLOW DIVERSION

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Bacterial contamination in blood components is the major residual risk in modern transfusion medicine. The initial flow diversion is one of the techniques to cope with this risk. In this paper, the progress of implementation of the initial flow diversion into Japanese blood program is described.

Although the method had already been implemented and evaluated in routine blood collection process in some countries, we started with experiments using animal model in 2002. After the experiments of blood collection from bacteria-coated cervical vein of seven dogs, it was found that the diversion of 27 mL was enough to deplete bacteria in the blood even when dog cervical area was applied with the solution with the highest concentration of bacteria.

We next performed a small scale clinical trial in 2004 and evaluated the effect of the method in the setting of whole blood collection from blood donors. Whole blood was collected with or without initial flow diversion (approximately 3,000 for each arm) and the blood samples from collected blood were subjected to culture study. Although the difference in the contamination frequency between the two arms was statistically insignificant, the results suggested the beneficial effect of the method in routine settings, and the data served as a theoretical basis for the implementation of the method in Japanese Red Cross blood centers.

The initial flow diversion was implemented in October, 2006 for all blood collection in JRC. Diversion volume was 25 mL. To evaluate the effectiveness of the method and the actual frequency of bacterial contamination in platelet concentrates (PCs), JRC had started a culture study before the implementation of the method using a culture protocol designed such that it yielded the least possibility of false negative results. After its implementation, the number of contaminated PCs decreased to 11 (0.05%) out of 21,783 from 36 (0.17%) out of 21,786 PCs before implementation. A reduction rate of bacterial contamination was 71% for all bacterial species. The number of contamination possibly derived from the donors' skin flora except for P. acnes decreased from 8 to 1, while the contamination possibly derived from the bacteria from the donors' circulating blood or transient skin flora only decreased from 5 to 3.

It is theoretically difficult to expect a decrease in the frequency of contamination by bacteria originated from donor's circulating blood by the introduction of the initial flow diversion. Moreover, in facilities performing universal culture as a release test, the diversion of initial flow may ironically increase the frequency of false negative culture study because it decreases but does not deplete the bacterial input in PC allowing a PC having a trace of bacteria accompanied by an inoculum having no bacteria. The effect of initial flow diversion has to be reevaluated comparing the risk of false negative culture study with its capability of decreasing bacterial input in PCs.

3A-S10-02
BACTERIAL CONTAMINATION OF AUTOLOGOUS BLOOD DONATION IN JAPAN

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Background: Incidence of bacterial contamination in donated allogenic blood for transfusion available from the blood centers of Japan Red Cross is well documented. However, incidence of bacterial contamination in autologous blood donated from patients themselves remains to be studied.

Aims: To confirm the safety of autologous blood transfusion in Japan, incidence of bacterial contamination in autologous blood of patient's own was studied by multi-center trials prospectively.

Methods: Bacterial contamination of autologous blood of the patients was studied by cultivating the blood taken from the original blood bag aseptically between day 2 to day 5 after donation, in culture bottles. The aerobic bottles and anaerobic bottles for bacterial culture were purchased from Biomerieux or Becton-Dickinson. This study was performed prospectively in collaboration with 15 hospitals in Japan from October 2005 to March 2007.

Results: Three cases among 3,939 cases examined were positive for bacterial contamination. Incidence of bacterial contamination was 0.08%. The bacteria cultivated and identified were Propionibacterium acnes, Staphylococcus epidermidis, and Staphylococcus haemolyticus. All of the three positive cases were detected in summer.

Conclusions: Incidence of bacterial contamination of autologous blood was higher than that of allogenic blood for transfusion reported previously in Japan. Contaminated bacteria were thought to be originated from normal flora of the skin known as low toxic bacteria. Clinical effects of transfusion of contaminated autologous blood with low toxic bacteria still remain to be studied. Conclusively, sterilization procedure of the skin has to be done carefully and effectively before blood donation.

3A-S10-03
ANTI-HEPATITIS C VIRUS SCREENING OF DONATED BLOOD AND HYGIENIC IMPROVEMENTS HAVE DECREASED VERTICAL TRANSMISSION

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Background: As hepatitis C virus (HCV) is a blood-borne pathogen, donor screening for anti-HCV has effectively mitigated its transfusion transmission. We conducted a post hoc analysis to clarify the specific impact of donor screening on reproductive age females and their offspring.

Methods: Anti-HCV screening in Japan started in late 1989. In a cohort studied between May 1990 and November 2004, a total of 22,664 serum samples from pregnant women were screened for anti-HCV. Reactive samples were further tested for HCV-RNA. Data analysis was performed by applying Fisher's exact test and multiple regression using software packages Dr. SPSS II version 11.0.1J and SPSS version 16.0J for Windows. Values of $P < 0.05$ were considered statistically significant, and odds ratios with 95% confidence intervals were calculated for each parameter. For causality, linear structural regression models were calculated with the maximum likelihood method, using AMOS 16.0 (SPSS 16.0J).

Results: Of the 22,664 pregnant women studied, 103 (0.5%) were anti-HCV reactive,of whom 55 (54%) were also positive for HCV-RNA. From the data analyzed according to birth year, the positivity of anti-HCV (falling from 1.8% to 0.3%) and HCV-RNA (falling from 1.1% to 0.2%) has decreased over time. For 103 anti-HCV-reactive pregnant women, relevant risk factors were surveyed, with only 31 (30%) having received blood transfusion. Of the remaining 72 women, 14 had some iatrogenic risk factors, three had reported intravenous drug abuse or tattooing, 55 did not identify any risk factor. From the entire cohort, 532 (2.3%) had a confirmed or possible history of allogeneic transfusion; actual dates of blood transfusion were obtained from the medical records of 251 women. The incidence of transfusion history (from 6.2% to 1.5%) and positivity of anti-HCV (from 18.5% to 2.5%) among women who had received transfusion have decreased. Among 251 women with known transfusion history, 155 had been transfused before the introduction of anti-HCV screening, of whom 23 (14.8%) were reactive for anti-HCV. Conversely, only three (3.1%) of the 96 women transfused after screening were positive ($P < 0.01$). Structural

equation modeling indicates that anti-HCV is more strongly associated with blood transfusion than birth year.
Discussion: Anti-HCV screening of donated blood has markedly decreased HCV-infection of pregnant women with a transfusion history, however, 70% of anti-HCV-reactive women were deemed to be infected via the routes other than transfusion. According to the literature, iatrogenic exposure may be a factor in the patients with unknown transmitted route. Hygienic improvements such as the introduction of single-use syringes and needles for medical injections and single-use acupuncture needles, have reduced the incidence of HCV infections among the general population, including pregnant women. The frequency of HCV perinatal transmission from infected mother to infant ranges between 5 and 10%. Anti-HCV screening of donated blood and hygienic improvements have decreased the likelihood of HCV transmission, which in turn has reduced the number of next-generation HCV infection by vertical transmission from transfusion-infected women.

3A-S10-04
HUMAN T LYMPHOTROPIC VIRUS IN CANADIAN BLOOD DONORS

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Background: HTLV-I/II is a rare virus in Canada which is generally a life-long but frequently asymptomatic infection. HTLV-I is relatively common (prevalence > 1%) in the Caribbean basin, central Africa and southern Japan, and in non-endemic countries is generally associated with immigration from one of these regions, sexual contact, intravenous drug use and blood transfusion, but risk factors have not been described in Canadian blood donors previously.
Aim: We aimed to describe HTLV infection rates in Canadian blood donors and to assess their risk factors.
Methods: All blood donations have been screened for HTLV since 1990. Initially only HTLV-I was screened for, and in 1998 the assay was upgraded to include both HTLV-I/II. Beginning in 2005, all donors confirmed positive for HTLV were invited to participate in a telephone interview about risk factors. For each donor who participated, four control donors were selected matched for age, sex, place of donation and donation status (first-time or repeat).
Results: The HTLV rate decreased from 9.4 per 100,000 donations in 1990 when testing was implemented to 1.02 per 100,000 in 1994, and has remained at a similar low level since then (0.90 in 2008). In the last five years (2004 to 2008) most have been first-time donors (86%). Confirmatory testing shows that 68% (38) of donors are typed as HTLV-I and 27% (15) as HTLV-II. Most HTLV-I positive donations (76%) were in the province of Ontario in the central part of Canada, and most HTLV-II positive donations (66%) were in the western provinces of British Columbia and Alberta. In the case-control study, 18 of 36 (50%) HTLV positive donors participated (of whom 15 were HTLV-I positive), and 72 of 115 (62.6%) of controls invited participated. In logistic regression analysis, having been born in the Caribbean or Asia (OR = 29.38) and a history of blood transfusion (OR = 6.37) were predictors of HTLV positive status (P <0 .05). Ten of 18 HTLV positive donors were from the Caribbean or Asia as follows (three from the Caribbean, two from the Middle East, two from India, two from Taiwan and one from the Philippines.
Summary/Conclusions: HTLV-I/II rates have decreased since testing was implemented due to a large untested population initially, but rates are lower in recent years with most infections detected in previously untested first time donors. There is regional variation by HTLV type, and the main risk factors in donors are birth in the Caribbean or Asia, and history of blood transfusion. Of donors born in Asia or the Caribbean many were from regions not usually described as high risk areas.

3A-S10-05
A NON-INVASIVE NEAR INFRARED SYSTEM FOR DETECTING BACTERIAL CONTAMINATION OF PLATELET COMPONENTS

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Background: Bacterial contamination of platelet components (PCs) is a major hazard of transfusion that can lead to serious morbidity and mortality. In some countries bacterial culture systems and/or pre-transfusion surrogate test methods have been introduced. We are investigating near infrared (NIR) spectroscopy for rapid, non-invasive screening of bacterially contaminated PCs and normal controls.
Methods: Three ABO-identical PCs were pooled and divided equally into three conventional platelet bags. One unadulterated bag served as a control, while Staphylococcus epidermidis and Bacillus cereus were spiked at low concentration into each of the other bags. The major component of our test system consisted of a portable FQA-NIR Gun (Shizuoka Shibuya Seiki, Hamamatsu, Japan) operating in the near infrared (NIR) region of 700 nm to 1100 nm. A specially designed sample holder was assembled to allow transmittance mode NIR measurements of PCs during storage. NIR spectra were non-invasively acquired at six hours intervals until the total storage time reached 72 hours. Difference spectra were calculated by subtracting the second derivative spectra of individual PCs measured at intervals during storage from the second derivative spectra of the same PC measured at the starting point (hour 0).
Results: Applying the discriminant partial least squares (DPLS) technique to the difference spectra described above, satisfactory results could be obtained with no false negatives. For S. epidermidis, complete classification could be made by 60 hours after spiking. On the other hands, due to its fast metabolism and growth rate, the complete classification of *Bacillus cereus* could be made by 42 hours after spiking. Regression coefficient analyses of chemical changes during storage, such as glucose, lactose, and lactic acid concentration as well as pH changes caused by bacterial metabolism account for the changes in NIR spectra that resulted in success classification of the contaminating organisms.
Conclusions: This NIR-based system is promising as an additional way to evaluate the safety of PCs prior to transfusion. Further work to improve the system and to test it on other common bacterial species must be conducted.

Tuesday: Parallel Session S11: Platelets

3A-S11-01
WASHED/REPLACED PLATELET CONCENTRATES

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To prevent transfusion-related adverse reaction (AR) caused by platelet concentrate (PC), premedication using anti-histamines or steroids is performed in clinical settings in Japan. When premedication fails to prevent AR, washed/replaced-PC (W/R-PC) as plasma reduced-PC is administered because plasma reduction is believed to be effective. However, some skepticism lingers on because it is true that a patient who developed AR in the previous transfusion of plasma-PC does not always develop AR again in the next transfusion of plasma-PC. Therefore, the previous paper is reviewed herein, with explanations of the results of surveys related to the usage of W/R-PC in Japan and of our experience in dealing with W/R-PC, with discussion of plasma reduction's effectiveness for AR prevention.

Two earlier papers have reported that, irrespective of the AR type, the incidence of AR using W/R-PC was significantly lower than that using plasma-PC. No unexpected event was observed. According to the survey, hospital staff were satisfied with the preventive effects of W/R-PC and CCI values obtained after transfusion of W/R-PC. One problem for W/R-PC preparation was that no platelet additive solution (PAS) with satisfactory platelet preservative ability was available. We developed a new PAS, named M-sol, which can suspend platelets to less than 5% plasma concentration without degrading their ex vivo function for at least 7 days. Since March 2005, we have given technical assistance for preparing W/R-PC using M-sol when the physician in charge judged that plasma removal was necessary to prevent AR caused by plasma-PC transfusion.

To date, 276 bags of plasma-PC have been transfused to 12 patients who experienced AR; 117 ARs were observed. In contrast, 156 bags of W/R-PC were transfused to the same 12 patients and only one AR (mild urticaria) was observed in onepatient during 15 transfusions. A significant difference was found in the incidence of reaction ($P < 0.0001$). On a per-transfusion basis, the incidence of AR was reduced dramatically: from 42% (117/276) to 0.64% (1/156) ($p<0.0001$). The 1h CCI and 24h CCI of W/R-PC were, 2.29 ± 0.84–104 /μl and 1.54 ± 0.81–$104/\mu$l, respectively. These data leave no room for doubt about the preventive effect of plasma reduction on AR.

How much plasma should be removed? Approximately 90–95% of plasma can be removed using our procedure. The residual plasma volume is therefore no more than 20 ml. In contrast, recent apheresis devices make it possible to obtain so-called hyper-concentrated PC automatically. The reduced plasma can be replaced with PAS to make the absolute volume of the residual plasma 80–90 ml (plasma concentration 30–40%). The question is whether PC with 80–90 ml of residual plasma is effective for the prevention of AR or not. Several papers have addressed this issue: the incidence of AR is reduced only by half. Additionally, we experienced a patient who developed AR by PC with 80–90 ml residual plasma but who did not by W/R-PC. We conclude that residual plasma volume of less than 20 ml is preferred for a satisfactory preventive effect.

3A-S11-02
EVALUATION OF THE FUNCTION OF DONOR PLATELETS USING A PLATELET FUNCTION ANALYZER PFA-100

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Background: As the use of single-donor platelets increases, the platelet quality of individual donors has become more important. There has been little works on platelet function in blood donors at the time of donation.

Aim: The aim of this study was to determine the frequency of impaired platelet function among healthy platelet donors.

Method: Forty seven platelet donors who gave informed consent were enrolled. Platelet function was measured by a recently introduced platelet function analyzer PFA-100 (Dade Behring, Germany) using the collagen/ epinepherine (CEPI) and collagen/ ADP (CADP) cartridges. Plasma levels of vWF:Ag and vWF:RCo were measured in donors with abnormal closure times. Each donor completed a questionnaire on the intake of any food or drugs that has been known to affect platelet activation.

Results: Seventeen percent (8/47) of donors had prolonged CEPI closure time only which indicated an aspirin-like effect. Thirteen percent (6/47) showed a severe defect with prolonged CEPI closure times between 200–300s. One donor had maximal CEPI closure time of greater than 300s. Seven among thirteen donors with prolonged closure times had reduced vWF:Ag or vWF:RCo levels. Three out of six donors who showed aspirin-like effect had reduced level of vWF:Rco.

Conclusion: In this study, we found that the platelet dysfunction detected by PFA-100 was unexpectedly high among healthy platelet donors. The clinical implication of these dysfunctional platelets are not known. A large scaled study will be needed to determine exact clinical consequences of infusing platelets with prolonged closure times. However, this study suggests a possibility of the need for platelet function screening before donation to increase clinical efficacy of platelet transfusion.

3A-S11-03
COMPARISON OF FUNCTIONAL CHARACTERISTICS OF APHERESIS DERIVED PLATELET CONCENTRATES DURING STORAGE IN FOUR DIFFERENT PLATELET ADDITIVE SOLUTIONS

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Background: The use of platelet additive solutions (PASs) for the storage of platelets (PLTs) can conserve plasma for fractionation and thereby reduce plasma-related allergic and febrile transfusion reactions. Furthermore, new generation PASs have enabled the improvement of PLT storage conditions to increase PLT shelf life while maintaining viability and haemostatic function.

Aims: The purpose of the present investigation was to compare the in vitro quality of PLTs during 5 days of storage with different additives. Expression of activation markers and release of cytokines and chemokines by stored platelet concentrates (PCs) were particularly evaluated.

Methods: PCs were collected with a Haemonetics MCS+ blood cell separator four times from each of three healthy donors. PCs obtained were automatically resuspended in a PAS (Intersol, SSP+, Composol, or M-sol) with 35% residual plasma volume and then placed in gas-permeable polyolefin PO-80 containers. PCs were stored at 22°C on a flatbed shaker (50 cycles/min) for up to 5 days. Samples were taken from the PC container on days 0, 1, 3, and 5, and were tested for PLT swirling, number and mean PLT volume, residual white blood cell (WBC) counts, PLT-derived microparticles, surface CD62p expression, PLT aggregation, pH at 22°C, pO2, pCO2, soluble CD62p (sCD62p) and CD40 ligands (sCD40L), RANTES, glucose, and lactate concentrations, and hypotonic shock response (HSR).

Results: Twelve runs with MCS+ were performed, collecting 2.61 ± 0.23 x1011 PLTs in 226.7 ± 12.2 ml with 35.7 ± 2.5% residual plasma volume. Residual WBC count of PCs was below 1 x 106 per bag. PLT count remained nearly constant and swirling of PLTs was also well preserved during 5-day storage in all PASs. The pH of PC in M-sol increased from 7.62 on day 0 to 7.93 on day 5. The pH in Composol and SSP+ increased modestly between day 0 and day 5. In contrast, the pH in Intersol decreased modestly during the entire storage period, but remained above 7.0. The aggregation response of PLTs to ADP and collagen mixtures decreased remarkably between days 1 and 3 for all PASs, with differences among the PASs not

statistically significant. The HSR of PLTs was preserved during 5-day storage in all PASs. PLT activation as detected by surface CD62p expression was different among PASs. CD62p values of PLTs in Intersol were approximately 20% on day 1 and increased further to 30% on day 5. However, CD62p expression in other PASs was below 10% during the 5-day storage period. Secretion of RANTES, sCD62p and sCD40L increased gradually during 5-day storage periods in all PASs and was greater in Intersol than other PASs.

Conclusions: With the exception of PC in Intersol, the in vitro characteristics of PCs were stable over at least a 5-day storage period and comparable among PASs.

3A-S11-04

MEASUREMENT OF PLATELET MICROPARTICLES IN DONATED WHOLE BLOOD

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Backgrounds: Microparticles (MPs, 0.05–1.5 μm) are derived from platelets, leukocytes, and erythrocytes. MPs in blood product may have potential to cause unexpected consequences when they are transfused into patients. However, assay for MPs in blood products have not been implemented in the blood preparation step. Blood is considered to be most abundant in Platelet MPs (PMP) that has pro-coagulant activity. Therefore, assay for PMP in blood product may be useful to evaluate its quality.

Aims: In Japan, donated whole blood (WB) is leukoreduced before storage and divided into red cell concentrate and plasma. In order to evaluate the quality of WB that is transported from a distant collection sites, we measured PMP and platelet surface markers (CD42b, CD62P) in WB before (pre-WB) and after (WB-LR) leukoreduction.

Methods: We divided pre-WB into three groups based on the transportation time from collection site to our facility as follows: 1) Short: Within 2h of transportation. 2) Middle: More than 5h, and within 8h. 3) Long: More than 8h. Number of PLT in pre-WB and WB-LR were counted by automated cell counter K-4500 (Sysmex). FITC-conjugated CD61, PE-conjugated CD62P and APC-conjugated CD42b were purchased from Becton Dickinson (B.D.) for detection of PMP (%) and surface expressions of platelet P-selectin and GPIb. Multi-color flow cytometric analysis was performed using BD-LSR (B.D.). For statical analysis, Kruskal Wallis H-test and Mann-Whitney U-test with Bonferroni correction were used.

Results: As shown in the Table, there was no difference in number of PLT and percent removal of PLT among three groups. PMP (2.3±3.4%) and CD62P (26.2±13.3%) in pre-WB Long group were significantly higher than those in other groups. No difference in CD42b of pre-WB was observed among three groups. PLT were detected by flow cytometry (FCM) in two bags of Short WB-LR group. PLT was not detected in bags of other WB-LR group.

transportation period	Short (n=8)		Middle (n=23)		Long (n=23)	
	pre-WB	WB-LR	pre-WB	WB-LR	pre-WB	WB-LR
PLT ($\times 10^4/\mu L$)	17·3 ± 2·8	0·5 ± 0·2	18·0 ± 3·6	0·4 ± 0·2	16·8 ± 4·3	0·3 ± 0·3
% removal of PLT	—	96·8 ± 1·8	—	98·0 ± 1·4	—	98·0 ± 2·5
PMP (%)	0·9 ± 0·2	N.D.*	1·3 ± 0·6	N.D.	2·3 ± 3·4	N.D.
CD62P (%)	12·8 ± 6·2	N.D.*	16·7 ± 6·4	N.D.	26·2 ± 13·3	N.D.
CD42b (MFI)	376·7 ± 47·0	N.D.*	362·0 ± 64·5	N.D.	317·1 ± 81·2	N.D.

mean ± SD
N.D. = not detected
* PLT were detected by FCM in 2 bags

Summary: Although 2 bags in Short group were shown to contain activated PLT, no PLT was detected in most of the bags in WB-LR. Therefore we concluded that WB-LR and the derived products from WB-LR may not cause adverse reactions associated with PMP. The reason that activated PLT was observed in two bags remained to be addressed.

3A-S11-05

EVALUATION OF PLATELETS CONCENTRATION AS A LOCAL HEMOSTATIC FOLLOWING DENTAL EXTRACTION IN LIVER DISEASE PATIENTS

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Background: patients with chronic liver disorders have a several causes of bleeding secondary to hypoprothrombinaemia and thrombocytopenia. in case of dental extraction, he may suffer from bleeding, so local haemostatic methods may be a good methods.

Aim of the work: A study was done to evaluate the effectiveness of platelet concentrate as a local haemostatic following dental extraction for liver disease patients.

Method: the study consisted of 30 patients suffering from chronic hepatitis of both sexes. The age ranged from 30 to 60 years. The patients were divided into two equal groups; the control group and the study group. The control group Surgical, regenerated oxidized cellulose, was used as local haemostatic after dental extraction. The study group: In this group blood banked PC, platelet concentrate, together with calcium chloride and bovine thrombin were used as local haemostatic following dental extraction.

Results: Bleeding was managed and coagulation achieved within 15 seconds after the placement of Surgical on top of the extraction sockets and suturing with silk for the control group Bleeding was managed and coagulation achieved within 15 seconds following the injection of PC into the bleeding extraction sockets for the study group During the 3 months of postoperative clinical follow up, it was evident that the use of the local haemostatic's used in this study helped in the process of healing of oral wounds. The results were comparable in both groups as regards to the small sized sockets (those of anterior teeth and premolars), however; better haemostatic and healing results were achieved with PC when applied to the sockets of the posterior teeth (molars have a greater surface area) especially during the first 3 weeks postoperatively The post-extraction complications including pain, infection and delayed healing were overcome in this study through the action obtained by the occluding effect of the local haemostatic, the strict infection control measures, the prophylactic use of antibiotics and lastly, but not least by the healing promotion effect of the Pc Blood banked platelet concentrate proved to be an effective local haemostatic for operative bleeding following dental extraction in liver disease patients who are suffering from altered dotting functions Dental treatment of medically compromised patients necessitates: proper history taking, accuracy of dental and medical examinations, medical consultations, evaluation of pathological laboratory investigations and proper treatment planning.

Conclusion: Patients with chronic liver disease need more attention from: the medical sector, the civil society, and the governmental sector in an attempt to lessen their pain, health problems and the complications they might encounter The search for alternative materials is encouraged to replace bovine thrombin (needed to activate platelet concentrate) in an attempt to minimize the possibility of complications arising from its frequent use in the same individual We encourage dentists' and oral surgeons to get acquainted with the blood banked products which might have a great impact on the field of oral surgery.

Tuesday: Parallel Session S12: Cellular Therapies

3A-S12-01

CELLULAR THERAPY IN HEMATOLOGICAL DISEASES

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It has become well accepted, that the immune system plays an important role in the success and/or failure of allogeneic stem cell transplantation. Modern transplantation approaches therefore try to harness the graft-versus-leukemia effects to eradicate tumor cells, while at the same time trying to reduce the risk for graft-versus-host diseases and viral infections. In children with high risk leukaemia, who are devoid of any HLA-identical stem cell donor, the transplantation of megadosis (>10x10E6/kgBW) and fully T-cell depleted stem cell grafts has allowed to provide haploidentical stem cell transplantations from either parent. However, the incidence of graft rejection and delayed immune reconstitution has led to the further development of stem cell grafts that are depleted of CD3-T-lymphocytes and B-lymphocytes, while at the same time retaining other immune effector cells, such as monocytes and NK cells. These cells are expected to facilitate engraftment, speed up immune reconstitution and provide also anti tumour activity in vivo. Here we present the preliminary results of our pilot study in children and adolescents who were grafted from their haploidentical parents or from unrelated donors using CD3/CD19-depleted peripheral stem cell grafts. Since the recurrence of viral infections (such as e.g. CMV and EBV), as well as aspergillosis, are a major cause of post transplant mortality, the management of such complications is crucial for the success of haploidentical transplantations. Here, new technologies allow the adoptive transfer of highly purified, antigen specific donor lymphocytes. Hence, stem cell transplantation has developed to a sophisticated immune therapeutic regime that takes advantage of the different immune effectors cells provided with the graft to eradicate leukaemia and fight viral infections. The use of mesenchymal stroma/stem cells (MSC's) will further allow to reduce the intensity of the conditioning regimen.

3A-S12-02

DONOR TGF-BETA-INDUCED FOXP3+CD4+CD25+ REGULATORY T CELLS EXPANDED BY TOLERGENIC DENDRITIC CELLS CAN PREVENT ACUTE GRAFT-VERSUS-HOST DISEASE IN ALLOGENEIC BMT

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Regulatory T cells (Treg) were a subpopulation of CD4+ lymphocytes that were identified as potent suppressors in immune system. Tregs expressed the Foxp3 transcription factor and CD25, which were divided into naturally occurring Treg (nTreg) and Treg induced from Foxp3-CD4+CD25-T cells (iTreg). As discussed, there were two main challenges for clinic application for nTreg: the low frequency and contamination because of no reliable cell-surface markers of expanding nTreg. Therefore, converting non-Treg cells into functionally suppressive Foxp3-expressing iTreg would alleviate concerns over these challenges. Initial studies have shown that tolergenic dendritic cells (rDC) could expand nTregs in vitro. But how rDCs expanded iTregs in vitro and the suppression of expanded iTregs in vitro and in vivo are no clear.

In our study, first, naïve CD4+CD25-T cells were purified from splenocytes (C57BL/6). After 5 days stimulated by anti-CD3/CD28 in the presence of TGF-β1 and mIL-2, the percent of Foxp3+CD4+CD25+T cells increased from 0.38% to 82.45%. Functionally these cells were suppressive towards CD4+CD25-T cells proliferation stimulated by anti-CD3/CD28 in the suppression assay. Therefore, these functionally suppressive Foxp3-expressing cells were termed ''iTreg''. Then, rDCs derived from bone marrow (BM) of DBA/2 mice were induced by GM-CSF with IL-10 and TGF-β1. Compared with mature or immature DCs, rDCs had lower constitutive expression of surface MHC molecules and co-stimulatory molecules (CD86/80). In vitro, iTregs could be expanded effectively by allo-rDC, which increased 2.5 fold in 5 days co-incubation. These rDC-expranded iTregs (iTreg-rDCs) continued to express high levers of Foxp3 (78.6%). When suppressive activity was tested, these iTreg-rDCs were shown stronger inhibitory effect than iTregs induced only by TGF-β1. In order to determine whether iTreg-rDCs were able to regulate T cell allo-reactivity in vivo, the acute graft-versus-host disease (aGVHD) mouse models were used. In this aGVHD model, DBA/2 mice (hosts) were given total-body lethal irradiation and injected with 3–107 BM and 1–107 splenocytes from C57BL/6 mice (donors) after 24h. In addition, 1–107 donor-iTregs and 1–107 donor-iTregs expanded by host rDC were transferred respectively, along with the same counts of BM and SC as the aGVHD model, and named ''iTreg group''and ''iTreg-rDC group''. It was found that the severity of GVHD in the aGVHD mice was more intense than those transferred additional iTregs or iTreg-rDCs, including weight loss, hair loss, hunched back, swollen face and diarrhea. Meanwhile, half of the mice in the ''iTreg group''died by day 20 and 100% died within day 37 after. With the iTreg-rDCs treatment, 75% mice survived by day 60 while all of the aGVHD mice died within 14 days.

These results indicated that in vitro Foxp3 + CD4 + CD25 + iTreg could be generated by stimulation of Foxp3-CD4 + CD25-T cells in the presence of TGF-β1. After expanded by allo-rDC, iTreg continued to express regulatory phenotypes and had more effective suppression. In vivo, allogeneic donor-derived iTregs expanded by host rDC could inhibit aGVHD significantly. It is suggested that the expansion of iTreg converted from CD4+CD25-T cells via allo-tolergenic DC show the therapeutic potential of preventing allograft rejection.

3A-S12-03

ADHESION RECEPTOR USAGE OF HUMAN MESENCHYMAL STROMAL CELLS: IMPLICATIONS FOR EFFICIENT MSC GRAFTS

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Background: Intravenously applied Mesenchymal Stromal Cells (MSCs) are being increasingly used in preclinical and clinical applications. Thus, MSCs have been found to suppress immune reactions such as Graft versus Host Disease, to integrate into tumor microvasculature, or to improve outcome in patients with severe sepsis. Previously we have shown that this adherent growing cell type surprisingly can roll on endothelial cells, arrest on endothelial cells and extravasate into tissues (Blood 108:3938, 2006). Yet the repertoire of active adhesion receptors on MSCs has not been fully understood.

Aims: We aimed to better characterize the receptors which MSCs can employ in order to roll and arrest on endothelial cells, to polarize, as well as to transmigrate across endothelial cells.

Methods: Human MSCs were isolated from normal healthy donors and characterized using differentiation assays, RT-PCR, flow cytometry. Rolling, adhesion and transmigration were determined under shear stress in parallel plate flow chambers that were precoated with endothelial cells or recombinant adhesion receptor ligands.

Results: MSCs did not or at low levels express the selectin-type rolling receptors, P-selectin glycoprotein ligand (PSGL)-1, L-selectin and CD24, whereas CD44 which encodes an E-selectin ligand was highly expressed. Beta1 integrins which were expressed in MSCs included Very Late Antigens (VLA)-4 and -5, whereas the beta 2 integrins LFA-1 and Mac1 were present only in low amounts or absent. Chemokine receptors of both the CCR and the CXCR families were expressed intracellularly and to a lower degree on the cell surface. In shear stress assays using laminar flow chambers, MSCs

showed comparable frequencies of interaction and arrest on activated endothelial cells as human CD34+ progenitor cells or peripheral blood lymphocytes. Use of function-blocking antibodies as well as experiments on precoated recombinant single ligands showed that E- but also P-selectin are main mediators of MSC early interaction with endothelium. Arrest of MSCs was efficiently induced by co-coated chemokines, and was mainly dependent on beta-1 but not beta2 integrins. Once arrested, MSCs could transmigrate through ECs. Arrest of MSCs and subsequent transmigration were blocked after pretreatment of MSCs with the G alpha I inhibitor pertussis toxin and the Rac GTPase specific inhibitor NSC23766, thus confirming involvement of chemokine induced G protein coupled receptor signalling. We next analyzed responses to different immobilized chemokines to strengthen adhesion. Here we found that stimulation through six different individual chemokines including stimulators of chemokine receptors CCR3 and 5 (normally recruiting neutrophils, monocytes), CCR6 and 7 (recruiting mainly B- and T-lymphocytes into lymph nodes), CXCR4 (used by many cell types) and CXCR5 (the B cell lymph node entry receptor) efficiently increased integrin-mediated MSC adhesion

Summary/conclusions: MSCs show a characteristic profile of adhesion receptor usage when binding endothelial cells under shear stress, which is similar to hematopoietic cells. MSCs however lack or show little response to L-selectin and beta 2 integrins, and display an expectedly pleiotropic chemokine receptor usage. These data provide a basis for MSC graft engineering towards modified engraftment into tissues.

3A-S12-04

FINITE PROLIFERATION CAPACITY OF HUMAN CD1D-RESTRICTED NATURAL KILLER T CELLS DUE TO ACTIVATION-INDUCED APOPTOSIS

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Introduction: CD1d-restricted natural killer T cells (NKT cells) possess a wide range of effectors and regulatory activities that are related to their ability to secrete both T helper 1 (Th1) cell- and Th2 cell-type cytokines. Increasingly evidence shown NKT was going to be a kind of important therapeutic cell, especially in preventing GVHD following allogeneic bone marrow transplantation (BMT) which we have reported previously. The conflict, between scanty natural amount in peripheral blood and great amount needed for each clinical case, calls for efficient NKT proliferation for future use. However, NKT seemed fail to become a long-term clone familiar as other lymphocyte but turn to apoptosis in a short term shown by our data.

Aim: To present the phenomena and analyze the possible mechanism of the apoptosis during NKT induction and proliferation, which should be considered in future NKT cell development.

Methods: NKT cells were induced from PBMNC and proliferating in the present of alpha-GalCer and IL-2 for 3–4 weeks in vitro. The annexin-v expression and various phenotypes of the NKT were analyzed through flow cytometry. During the whole culture term, the cells were taken pictures every 2–3 days and harvested to analyze caspase-3 activity by western-blotting and Kinase activity assays every day.

Results: NKT clone arise after 7 days culture and mainly expanded during the later 2 weeks, but turn to apoptosis in a large scale from then, indicated by the flow cytometry assay on the cell surface markers and picture records. Shown by western-blotting, the activated Caspase-3 was stimulated to be present 24h later after each time alpha-GalCer was fed at the first week; however the highest Caspase-3 kinase activity was met during the second week. Moreover, the sensitivity and the reaction time point of NKT to alpha-GalCer stimulation on proliferation and caspase-3 kinase activity was various in different individual.

Conclusion: Since human NKT would be a potential therapeutic cell and be developed from blood buffy coat, the strategy that how to get them efficient should be taken into account first. According to the data, human NKT cells could be induced and proliferate excellently during the first 3 weeks, although NKT could not be expanded to a larger scale might because of the activation-induced apoptosis stimulated by alpha-GalCer.

3A-S12-05

SANITARY CONTROL IN THE CELL PROCESSING CENTRE AT AICHI MEDICAL UNIVERSITY HOSPITAL

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Background: The facility where cells for cellular therapy are processed should fulfill the good manufacture practice (GMP) standard according to the government published guideline and is called a ''cell processing centre''(CPC). The CPC at Aichi Medical University Hospital was established to perform cellular therapies in February 2006. After the cytotoxic T lymphocyte (CTL) therapy gained governmental approval as an advanced therapy in December 2006, we started to utilize the CPC for cell processing in August, 2007, and have so far performed CTL therapy for nineteen patients with advanced cancers. The CPC is 117 m2 in size and comprises eleven areas including gowning rooms, cell processing rooms, and degowning rooms. There are two types of clean cell processing rooms, rated class 10,000 and 100,000 after FDA Standard 209E. One of the most important issues in the CPC is sanitary control; personnel are required to follow not only standard operational procedures, but also strict standard precautions, including cleaning and gowning techniques. In addition, clinical samples, CTL lots, and environmental samples from the CPC are screened for pathogen contamination, and we report here on sanitary control in our CPC.

Methods: 1. Bacteria and fungi: Thirty-six tumour samples and 89 CTL lots were subject to bacterial and fungal screening tests. Tumour samples were examined on the day of manipulation and CTL lots two days before shipping. Environmental samples obtained by wiping (27 samples) or air-sampling (15 samples) from all the rooms in the CPC have been screened for both pathogens every week for about two years. Bacteria are examined using liquid culture for both aerobic and anaerobic kinds (BACTEC Plus).
2. Mycoplasmas: Eighty-nine CTL lots were screened for mycoplasmas by measuring enzyme activity using detection-kits (MycoAlert) on the day of shipping as well as endotoxins.
Endotoxins: Those lots were also subject to endotoxin screening using detection-kits (Endosafe-PTS).

Results: 1. Bacteria and fungi: Three samples (8.3%) out of 36 revealed positive for bacteria. In two cases, tumour tissues were resected again; in one patient, however, CTL therapy was not performed due to other reason. All CTL lots proved negative for both bacteria and fungi. For environmental samples, colony numbers exceeding 5 under class 10,000 and 25 under class 100,000 are considered unsatisfactory. Over the two years, colony numbers did not exceed the upper limit. Although numbers over 25 were detected several times in the gowning area (not a clean room), the number had fallen below this level by the following week, after some improvement of gowning technique.
2 & 3. Mycoplasmas and endotoxins: All CTL lots tested negative for both mycobacteria and endotoxins, and were administered to patients without any problems.

Conclusion: We have provided CTL therapy 89 times to nineteen patients with no contamination of pathogens or endotoxins. This is primarily due to having followed the standard operational procedures and precautions which we established. In addition to compliance with these standards, weekly monitoring of environmental samples from the CPC is considered helpful in keeping the facility sterile.

Tuesday: Plenary Session TTI

3B-PL3

OCCULT HEPATITIS B INFECTION: INCIDENCE, DETECTION, AND CLINICAL IMPLICATIONS

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It has been reported that chronic hepatitis B (CHB) carriers can lose the hepatitis B surface antigen (HBsAg) at an annual rate of 0.1–1%. In a long-term follow-up study of 298 CHB patients with HBsAg seroclearance, serum HBV DNA were detectable in 13.4%, 6.1%, and 3.7% of patients within 1 year and 5–10 and >10 years after HBsAg seroclearance, respectively. In all 82.1% patients had persistently normal alanine aminotransferase levels. Seven (2.4%) patients developed hepatocellular carcinoma (HCC) after HBsAg seroclearance. In 29 subjects with liver biopsies, intrahepatic total HBV DNA and covalently closed circular (cccDNA) were detected in 100% and 79.3% of patients, respectively.1

Patients with known CHB who have lost the HBsAg represent one end of the spectrum of occult hepatitis B (OHB) infection. CHB patients with HBsAg seroclearance are seldom included in studies of OHB, but these patients continue to harbor the hepatitis B virus and may continue to develop HCC.

Nearly all the other studies of OHB have been performed by detecting HBV DNA in the sera of HBsAg-negative blood donors. It has not been decided whether individual donation nucleic acid testing (NAT) may replace HBsAg testing.2 The incidence of OHB varies from 1:8209 in South Africa 3, to 1:36,137 in Poland4.

A recent study of OHB in Hong Kong, an area where 8% of the population have CHB, consisted of two cohorts. The first cohort of 3,044 donors from June 2005 to May 2006, were first tested negative for HBsAg and then individually with NAT. HBV DNA levels were then detected by the Artus HBV test (lower limit of detection 3.8 IU/mL). The prevalence of OHB was 0.14%. The second cohort consisted of 9,990 donors prospectively recruited from January 2006 to June 2008. This cohort was tested by NAT first. Donors who were positive by NAT, but HBsAg negative were then tested for HBV DNA using the Artus HBV test. The incidence of OHB in this cohort was 0.11%, very similar to the first cohort which had HBsAg screening first. As HBsAg screening followed by NAT is cheaper, this may be a more cost-effective method for transfusion services.

The OHB incidence of 0.11–0.14% in Hong Kong is much higher than those reported from other studies. This is presumably related to the high incidence of CHB in Hong Kong. The risk of transfusion-acquired acute hepatitis B in the recipients of OHB blood is low. It is of greater importance to investigate the possible clinical implications of OHB in affected subjects. Brechot et al have reviewed some of the possible complications.Five to six of the subjects with OHB in the Hong Kong cohorts had liver biopsies. All had near normal liver histology, with low or undetectable intrahepatic HBV DNA, signifying probable excellent long-term prognosis. However HBV DNA has been found inside HCC tissues in subjects negative for HBsAg.1,5 Recently acute fulminant reactivation of hepatitis B has been reported in OHB subjects following treatment with chemotherapeutic agents, the incidence being as high as 25% in OHA subjects receiving anticancer therapy with rituximab.6

3B-PL4

EPIDEMIOLOGY, CLINICAL FEATURES AND PREVENTION OF HTLV-I INFECTION

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Human T-cell leukemia virus type I (HTLV-I) is the main cause of Adult T-cell leukemia/lymphoma (ATLL) and HTLV-I associated myelopathy/Tropical spastic paraparesis (HAM/TSP). From the long-term epidemiological observation, the HTLV-I carriers are clustered in limited groups in the world, i.e., Southwestern Japanese, native Oceanians, Amerindians in Andes and Northeast British Colombia, North Iranians as well as Central Africans and their descendants in the Caribbean basin and South America. The annual risk of ATLL in endemic areas of Japan is approximately 1/1,000 over 40 years and the whole life risk is estimated at 2~5%, which are not very different in Central and South America. HAM/TSP is the second most common diseases caused by HTLV-I and its immunological feature showed high titers of anti-HTLV-I antibodies in serum and cerebrospinal fluid. The previous immunogenetic study showed that most ATLL patients have specific HLA haplotypes found only in a minority subpopulation in the HTLV-I endemic areas, while HAM/TSP patients share common HLA haplotypes appearing in the majority of Japanese. Such a different genetic background might determine the geographical epidemic pattern of ATLL and HAM/TSP in the world.

The final goal of epidemiology is the establishment of preventive measures and the promotion of better human health. The following strategies for primary prevention of HTLV-I related diseases include: 1) elimination or reduction of exposure to HTLV-I; 2) promotion of the effects of protective factors for progression of HTLV-I related diseases. The familial clustering of HTLV-I carriers indicated a couple of natural transmission routes, mother-to-child through breast milk and husband-to-wife through semen under natural life styles. To establish a desirable preventive measure against mother-to-child transmission of HTLV-I through breast milk, several prospective studies were conducted to evaluate the risk of HTLV-I infection among children born to HTLV-I carrier mothers in highly HTLV-I-endemic areas in South and West Kyushu, Japan.

The pregnant women were informed the results of anti-HTLV-I antibody test before 30 pregnant weeks and positives were advised to stop breast feeding to prevent mother-to-child transmission of HTLV-I. If they cannot accept it, alternative advice to shorten breast feeding and stop within 3~5 months was recommended. After following up until more than 24 months of age, the seroconversion rate among bottle-feeders (2~4%) and short-term breast-feeders (3~4%) showed much lower than those of long-term breast feeders (around 20%). The choice of being short-term breast fed for control of HTLV-I infection will provide a new gleam of hope for HTLV-I carrier mothers in the world.

Recent cancer registry in Nagasaki, West Kyushu, showed actually decreasing trends in age-adjusted incidence rate of ATLL in both sexes because age distribution of HTLV-I carriers in the endemic areas is relatively aging and HTLV-I carriers in younger generation is drastically decreasing. For the future prevention of ATL, risk reduction of maternal transmission of HTLV-I by stop or short-term breastfeeding would be effective. Actually infection rate among children delivered from HTLV-I carrier mothers was drastically decreasing from more than 10% to less than 3% after starting eradication programme.

Tuesday: Parallel Session S13: TTI

3C-S13-01

RECENT FINDINGS AND DEVELOPMENTS OF DENGUE AND CHIKUNGUNYA EPIDEMIOLOGY AND THEIR POTENTIAL IMPACT ON DONORS AND PATIENTS

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Dengue and chikungunya, transmitted by the Aedes mosquitoes, have caught world-wide attention due to increase in the frequency of major epidemics in recent years. Though transmitted by the same vectors, they are caused by different viruses (DENV and CHIKV) that belong to the genus Flavivirus and Alphavirus respectively.

There has been a worldwide resurgence of dengue in the last few decades. Today, it is estimated that it affects at least 50–100 million people every year. With more than 100 countries, between the 10°C isothermal lines, being endemic, 2.5 billion people are at risk of infection. Facilitated by rapid urbanization and increased travel, the disease continues to make its geographical spread, and the number of cases continues to rise across the world.

Chikungunya outbreaks have been reported in Asia and Africa. Major epidemics appear and disappear cyclically, usually with an inter-epidemic period of 7–20 years. However, its recent unprecedented explosive outbreaks since 2005, revealed an altered epidemiology. Millions of cases have been reported as the disease sweeps across the islands in the Indian Ocean, followed by countries in South Asia and South East Asia. An outbreak in Italy in 2007 demonstrated the threat of the tropical disease to temperate countries. These two Aedes borne diseases have also impacted travelers significantly, with numerous reports of imported cases in countries with no transmission.

In both diseases, viruses have been detected prior to the onset of symptoms, and asymptomatic cases have also been reported. As a result, under- and delayed diagnoses have led to dengue transmission through organ transplant and blood transfusion. The potential impact of the epidemiology of the two diseases on the safety of blood and organ donation will be discussed.

3C-S13-02

DENGUE VIRUS AS A POTENTIAL THREAT TO THE SAFETY OF THE BLOOD SUPPLY IN BRAZIL

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Background: Dengue virus (DENV) infection is on the rise in Brazil since 1986 with a record number of 764,040 cases reported for the first 9 months of 2008. These numbers represent an increase of more than 200,000 case compared to 2007. Potential transmission of DENV by blood transfusion is of concern although so far there are no published reports in this country and only two possible transfusion-associated cases in other Dengue-endemic areas.

Aim: To investigate the incidence of DENV viremic donations and assess the risk for infection.

Methods: Minipools comprised of 35 donors were prepared and potential DENV virus particles concentrated by ultracentrifugation. Automated extraction of viral nucleic acid was performed by the easyMag platform. DENV was detected by (1) serotype-specific real-time PCR, (2) a one-step RT-PCR followed by serotype-specific ''nested''PCR and (3) flavivirus universal RT-PCR. A total of 4,348 donations were tested by minipooling and over 700 donations were NAT-screened individually without pooling. In addition to NAT-testing, 531 donations were screened for IgG and IgM antibodies to DENV to determine the prevalence of infection in the blood donor population.

Results: A total of 4,348 donations from six different regions in which DENV incidence of infection extend over from 0.35 to 1,154 per 100,000 were tested. The overall prevalence rate for DENV viremic donations was 0.06% however individual sites with higher incidence of disease (600–800 cases per 100,000 people) displayed much higher prevalence rates; 0.2% for Fortaleza region or one out of 494 donations and 0.34% for Rio de Janeiro or one out of 296 donations. No other mosquito-transmitted flaviviruses (West Nile Virus, Yellow Fever Virus and St Louis Virus) were found in Brazilian blood donors. The NAT-positive DENV donations were found in three pools, none of the 700 individually tested donations were PCR positive which indicates sufficient sensitivity to detect DENV using the mini-pool strategy.

Evidence of previous Dengue infection based on seroprevalence was very high among blood donors in Fortaleza (86.4%) especially when compared to that of Sao Paolo (5%). This is not surprising as incidence of infection in Fortaleza was more than 1000 x the one in Sao Paolo. In addition 2.7% of blood donors in Fortaleza showed evidence of recent (IgM+) but not current (PCR negative) infection. The relevance of protective immunity is feasible. Studies of heterologous DENV infection in primates and mice more often indicate either full or partial protection rather than enhancement of infection. Homologous and heterologous immunity against the four DENV serotypes in blood recipients living in endemic areas may mute the effect of transfusion-associated Dengue and explain the lack of recognition of clinically overt cases of disease.

Conclusion: The minipool screening strategy described in this study exhibits sufficient sensitivity and is feasible in low-resource countries heavily affected by the current Dengue epidemic. The risk for DENV transmission through blood is quantifiable and proportionally related to the incidence of infection which may have implications for limited, regional versus universal NAT screening of blood donations in even in countries where Dengue is endemic.

3C-S13-03

HBV NAT TESTING IN CHINESE BLOOD DONORS WITH POSITIVE AND NEGATIVE ALT

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Background: Routine blood donor screening in China consists of two rounds of testing for HBsAg, anti-HCV, anti-HIV1/2, ALT and syphilis. Some blood centers also use rapid testing for ALT and or HBsAg in donor pre-screening as an added measure to prevent transfusion transmitted vital hepatitis. Nucleic acid testing for pathogens is currently not a part of donor testing in China.

Aim: To evaluate the current residual risk of HBV transmission in the Chinese donor population and to evaluate the value of ALT testing in preventing HBV infection in China.

Methods: Two groups of donors were included in this study: donors who have negative results on all donor screening tests (''negative group'') and donors who have abnormal results for ALT, but negative results for all other tests (''ALT group''). From Jan. 2008 to May 2009, 3,997 ''negative group''samples and 3,132 ''ALT group''samples were collected from four Chinese blood centers participating in the REDS II International Programme sponsored by the NHLBI in the United States: Kunming, Luoyang, Urumchi and Liuzhou. Samples were shipped to the NAT Lab of Beijing Red Cross Blood Center and were individually tested for HBV DNA by the

Procleix(R) Ultrio(TM) Assay system. All HBV NAT reactive samples were tested by HBsAg EIA to confirm their HBsAg negative status.
Results: Of 3,997 ''negative group''samples tested, four were HBV NAT reactive (0.100%, 1 per 999); of 3,132 ''ALT group''samples, three were HBV NAT reactive (0.096%, 1 per 1044). (Table 1).

Table1 HBV NAT Results in Negative group and ALT group

	ALT group		Negative group	
	samples tested	HBV NAT Reactive	samples tested	HBV NAT Reactive
Kunming	1228	0	1000	0
Luoyang	1000	2	997	0
Urumchi	594	1	1000	3
Liuzhou	310	0	1000	1
Total	3132	3	3997	4

Conclusions: Our preliminary results suggest that the current risk of HBV transmission in the Chinese donor population is around 1/1,000. ALT testing seems to have no significant value in preventing transfusion transmitted HBV infection in China. We plan to confirm these preliminary results with additional donor samples and additional testing on NAT-reactive, HBsAg-negative samples.

3C-S13-04
INCIDENCE OF OCCULT AND WINDOW PERIOD HEPATITIS B INFECTION AND SIGNIFICANCE OF HEPATITIS B CORE ANTIBODY TESTING AMONG INDIAN BLOOD DONORS

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Background: Regardless of efforts to ensure the safety of the blood supply, hepatitis B residual risk remains the highest among transfusion transmitted infections in India. To strengthen our blood safety program, Indraprastha Apollo Hospitals introduced Individual Donor Nucleic Acid Testing (ID-NAT) for Human Immunodeficiency Virus-1 (HIV-1), Hepatitis C Virus (HCV) and Hepatitis B Virus (HBV) using Procleix® Ultrio® Assay in April 2006.
Aims: To evaluate the impact of ID-NAT on blood safety and the significance of anti-HBc screening on blood supply.
Material and methods: 59,515 blood donations collected at Department of Transfusion Medicine, Indraprastha Apollo Hospital, New Delhi from 1st April 2006 till 30th April, 2009 were tested by Individual Donor Nucleic Acid Testing (ID-NAT) using the Procleix Ultrio Assay (Chiron Corporation, Emeryville, CA) on the semi-automated Procleix eSAS platform according to manufacturer's instructions. All samples were tested individually. The Procleix Ultrio Assay is a multiplex test which provides simultaneous detection of HIV-1 RNA, HCV RNA and HBV DNA in human plasma using Transcription Mediated Amplification Technology (TMA). All the positive results were subjected to Discriminatory Testing on the same Procleix eSAS system platform to find out the incidence of HBV. Samples were also tested for HBsAg and anti-HBc by ELISA technique (Bio-Rad's Monolisa) on automated ELISA processor (EVOLIS Walk away system).
Results: Out of the 59,515 donations tested, 6390 (10.73%) were positive for anti HBc and 604 (1.01%) for HBsAg. A total of 563 (0.95%) were positive for HBV DNA. Of the NAT-positive samples, 10 were negative for HBsAg for a HBV NAT yield rate of 0.02%. When compared with other HBV serologic markers, four of these 10 were positive for anti-HBc and therefore considered as occult Hepatitis B infection, while the remaining six were considered as window period (WP) HBV infections since they were anti-HBc negative. Due to the large number of migrant blood donors, follow-up testing on these donors were not possible.
Conclusions: Our data suggest that the exclusion of anti-HBc positive donors leads to an unnecessary loss of 10% of our donor pool. Although a small proportion of these donors might be infectious, implementation of the sensitive ID-NAT should be able to effectively eliminate those potentially infectious donations. Our Hepatitis B NAT screening program has resulted in considerable progress towards prevention of transfusion transmitted HBV infections. With the implementation of routine ID-NAT, the HBsAg and anti-HBc test algorithms is currently under debate. Our data indicates benefit of HBV NAT in detecting both window period and occult HBV infections.

3C-S13-05
LIVER DISEASE, ANTIVIRAL TREATMENT AND BEHAVIORAL RISK FACTORS IN A RETROSPECTIVE COHORT OF HCV SEROPOSITIVES DETECTED AT THE TIME OF BLOOD DONATION

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Background: We have previously reported three-fold increased mortality in a large retrospective cohort of HCV seropositives detected at the time of blood donation (Guiltinan Am J Epidemiol 2008). Higher mortality from liver disease, trauma, substance abuse and cardiovascular disease was associated with HCV.
Aim: These findings induced us to study the prevalence of liver disease, cardiovascular disease, trauma and health behaviors among living cohort members, We also determined history of antiviral treatment for HCV infection.
Methods: The cohort included 10,259 HCV+ subjects who donated blood from 1991 through 2002, and 10,259 age-, sex-, zip code- and donation year-matched HCV- blood donors. A health history questionnaire was mailed to a sample of 2000 HCV+ and 2000 HCV- living members of the cohort. Chi-squared tests and odds ratios (OR) with 95% confidence intervals (CI) were calculated.
Results: Questionnaires were returned by 312 (16%) HCV+ and 446 (22%) HCV- subjects. Both groups had similar age, sex and race/ethnicity, but HCV+ had lower income and educational attainment. HCV+ subjects reported chronic hepatitis (21%), cirrhosis (8%), other liver disease (4%) and hepatocellular carcinoma (0.6%). Among HCV+, ALT testing was done in 211 (68%). In the 89 (42%) with abnormal ALT, 68 had liver biopsies and 54 (79%) were abnormal; among the 101 (48%) with normal ALT, 53 had liver biopsies and 15 (28%) were abnormal. Interferon treatment was started in 112 HCV+ and was successful in 62 (55%), ineffective in 18 (16%) and stopped for side effects or other reason in 31 (28%). Myocardial infarction (4% vs. 2%) and stroke (3% vs. 2%) were slightly but not significantly increased in HCV+. Cigarette smoking ($P < 0.0001$) and family history of CAD ($P < 0.01$) were more frequent among HCV+ but high cholesterol ($P = 0.03$) and hypertension ($P < 0.01$) were more frequent among HCV-. Psychiatric disorders (OR=2.74), substance abuse (OR=5.52) and drug overdose (OR=5.94), as well as motor vehicle (OR=2.98) and workplace accidents (OR=2.65), were all significantly ($p<0.05$) associated with HCV+ status.
Conclusions: Former blood donors living with HCV infection had substantial rates of chronic hepatitis and cirrhosis. Medical management of HCV, and the implementation and success of interferon treatment were similar to reports from previous case series. We had insufficient power to detect possible small increases in cardiovascular disease and stroke; previously reported HCV associations with these conditions may be confounded by lower socioeconomic status, higher cigarette smoking and more frequent family history of CAD. Lifestyle issues associated with past or current drug abuse may account for substantial psychiatric and trauma co-morbidity.

Tuesday: Parallel Session S14: Red Cell Molecular Biology

3C-S14-01
MOLECULAR BIOLOGY OF BLOOD GROUPS

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For about half a century following their discovery in 1900, blood groups were studied almost exclusively by serological methods. From around 1950 biochemical analyses were introduced then, in 1986, the molecular era of blood group analysis began with the cloning of the glycophorin A (MN) gene. Cloning of the ABO (1990) and Rh (1990 and 1992) genes soon followed. A total of 308 blood group antigens are now recognised, with 270 of them belonging to one of the 30 blood groups systems. Each system represents either a single gene or a cluster of two or three closely-linked homologous genes. All of the 35 genes representing blood group systems have been identified and cloned, with the exception of the gene for the P1 polymorphism.

Most blood group polymorphisms represent single nucleotide polymorphisms (SNPs) encoding amino acid substitutions in either the surface-exposed regions of cell-surface proteins or in the transferase enzymes responsible for oligonucleotide sequences on cell-surface glycoproteins or glycolipids. There are, however, a variety of other genetic mechanisms that are responsible for blood group polymorphisms. The RhD-negative phenotype usually results from homozygosity for a deletion of RHD, from the presence of inactivating mutations within RHD, or from a gene that is a hybrid of RHD and RHCE. The most common O alleles arise from a single nucleotide deletion in a gene that is otherwise the same as that encoding the A-transferase. Duffy-null, which is common in Africans, arises from a SNP in the promoter region of the Duffy gene, which prevents binding of the erythroid-specific transcription factor, GATA-1.

Some rare blood group phenotypes result from mutations in genes other than those encoding the blood group protein. Rhnull and Rhmod may arise from mutations in RHAG, the gene producing the Rh-associated glycoprotein,. One of these mutations, responsible for Rhmod, also causes the expression of a rare antigen, Ola, on the Rh-associated glycoprotein. Heterozygosity for mutations in the erythroid transcription factor gene, KLF1 (aka EKLF), causes the In(Lu) phenotype, the characteristics of which are extremely weak expression of Lutheran antigens and AnWj antigen plus reduced expression of P1 and antigens of the Indian (CD44), Knops (CD55), and Raph (CD155) systems.

Somatic mutations in haemopoietic stem cells may cause acquired changes in blood group phenotypes. This is particularly applicable to X-linked genes, which are subject to X-inactivation. Clonal mutations in the X-linked gene PIGA, which is essential for the biosynthesis of glycosylphosphatidylinositol anchors, gives rise to paroxysmal nocturnal haemoglobinuria and loss of antigens of the Yt (acetylcholinesterase), Dombrock (CD297), Cromer (CD55), and JMH (CD108) blood group systems. The acquired blood group phenotype Tn, which is associated with polyagglutination, results from a somatic mutations in the X-linked C1GALT1C1 gene, the product of which is necessary for expression of T-synthase, a glycosyltransferase essential for correct biosynthesis of O-glycans.

3C-S14-02
A SECOND EXAMPLE OF THE RHCE AGA CODON 229 (NT 685-687) TRIPLET DELETION LEADING TO AN ALTERED E ANTIGEN EXPRESSION

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Background: Three examples of triplet nucleotide deletions were described in humans as a rare mechanism that produces RHCE and RHD proteins variants lacking a single amino acid: RHCE allele ceBP homozygous deletion of AGA codon 229 (nt 685–687) and two partial D phenotypes (deletions of codons 229 and 235 in RHD).

Aims: To describe the serological findings and molecular changes in a Brazilian proband presenting with an e antigen variant.

Methods: The blood sample was obtained from a healthy Brazilian woman at post partum. Her sample was referred for molecular studies due to the phenotyping variance with anti-e using conventional hemaglutination. RHCE*E/e genotyping was performed using genomic DNA and RHCE exon 5 PCR-RFLP with Mnl I restriction digestion. Further, RHCE exon 5 was sequenced to identify nucleotide changes.

Results: The proband was identified because she was discordant for the e antigen with her newborn. The infant's RBCs revealed normal c and e antigen expressions and the proband's RBCs revealed normal c antigen but extremely weak e antigen after 15 min RT incubation only with the Gamma monoclonal anti-e (clones MS-16/21/63). RHCE exon 5 PCR-RFLP with Mnl I demonstrated the expected profile for e allele plus an unexpected band of approximately 200bp. The sequencing of RHCE exon 5 demonstrated a heterozygous AGA deletion (nt 685–687) and a homozygous 676C>G polymorphism; the latter consistent with the absence of the RHCE*E allele. Unfortunately, the newborn's DNA was not available for analysis.

Summary/ conclusions We report a second case in Brazil of an *RHCE* AGA triplet deletion of Arg229 (nt 685–687) that affects e antigen expression. The weakened expression of the e antigen was unexpected given that the proband is homozygous for the *RHCE*e* allele. Either the Rhce-Arg229 del variant affects the expression of the wildtype polypeptide, or the proband is a compound heterozygote for another *RHCE* mutation. The Arg229 deletion likely represents a founder allele since it has been reported previously in Brazil. Our work suggests that the population frequency and the clinical significance of the Rhce-Arg229del variant should be examined closely.

3C-S14-03
GENOMIC STRUCTURAL VARIATIONS IN THE RHD GENE REGION AS A CAUSE OF SEROLOGICALLY RH-NEGATIVE PHENOTYPE REVEALED BY FIBER-FISH ANALYSIS

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Background: The human Rhesus (Rh) blood group system is determined by two highly homologous RH genes, RHD and RHCE. These genes are located on chromosome 1p36.1, a genomic region representing copy number variation. In Rh-positive individuals, both RHD and RHCE genes are present, while in most Rh-negative individuals, RHD genes are missing and are homozygous for a single RHCE gene. Some individuals with a serologically Rh-negative phenotype were diagnosed to be RHD-gene positive by a locus-specific DNA assay using polymerase chain reactions with sequence-specific primers (PCR-SSP). Alterations in the genomic structure of RHD that cause negative gene expressions are difficult to detect by the PCR-based assay because of highly homologous sequences of the two RH genes.

Aims: The genomic structure of the RH gene region in Rh-negative Japanese donors whose genotype was diagnosed as RHD-gene positive by the PCR-SSP assay were examined by using extended chromatin fluorescence in situ hybridization (fiber-FISH) technique to demonstrate that varied sizes of deletion and/or insertion in the RH loci cause the Rh-negative phenotype.
Methods: Peripheral blood mononuclear cells (PBMCs) were obtained from six unrelated healthy Japanese donors who were diagnosed serologically to be Rh-negative by standard techniques and reagents, and were found to be RHD-gene positive by PCR-SSP for the intron 4, exon 7 and exon 10 of the RHD gene. One common Rh-positive and one common Rh-negative blood samples were also prepared as controls. Fiber-FISH with two DNA probes for introns 3 and 7 of the RH genes on extended chromatin fiber preparations derived from the PBMCs was performed. Hybridization signals were detected with a fluorescence microscope equipped with a CCD camera. At least twenty images per sample were captured and processed with the IPLab Spectrum v. 3.5.1 software (Scanalytics Inc.) for analysis. The lengths of signal arrays and intervals between the signals were measured on digitized images.
Results: A control sample with the typical Rh-positive phenotype showed two sets of intron 3 and intron 7 signal arrays arranged in the opposite direction, denoting the following gene arrangement: RH (5'–>3') - RH (3'<–5'). A control sample with the typical Rh-negative phenotype showed one set of the signal array. As for the samples tested in the present work, every donor had alleles with different types of genomic alterations, such as deletion (2.5–11 kb, varying in size among different individuals) in intron 7, 30-kb deletion or 5–15-kb insertions within the region between RHCE intron 7 and RHD intron 7.
Conclusions: We demonstrated by fiber-FISH analyses that varied sizes of deletion and/or insertion in the RH loci caused the Rh-negative phenotype. These large-scale genomic alterations in the RHD gene region are difficult to detect by the current sequence-dependent strategies alone. Therefore, direct physical analyses by fiber-FISH could be crucial for detecting genomic alterations to see the correlation between phenotype and genotype, especially at regions containing copy number variations such as Rh and MNS blood group antigen gene loci.

3C-S14-04
SEROLOGICAL CHARACTERIZATION OF RHD DISCREPANT CASES IN INDIAN POPULATION

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Background: Variants of D antigen can be divided into two groups: quantitative variants characterized by lower or higher number of D antigenic sites and qualitative variants characterized by the absence of one or more number of D epitopes (partial D). If partial D variant subjects are exposed to normal RhD antigen they can produce anti-D antibody against missing epitopes. Hence the identification of partial D variants is clinically important. Partial D and weak D phenotypes give discrepant Rh grouping results when different monoclonal anti-D reagents are used in laboratories. A panel of monoclonal anti-D antibodies are being used in order to identify partial and weak D variants when there are discrepant results.
Aims: To identify and classify partial D variants serologically with panel of monoclonal anti-Ds in RhD discrepant cases in Indian population.
Methods: Thirty nine samples referred to the Institute for discrepancies in RhD grouping were tested with the ALBAclone Advanced Partial RhD Typing Kit (Alba Bioscience Limited, UK) by indirect antiglobulin test (a panel of twelve monoclonal anti-D). The kit can identify and classify weak D type 1 & 2 and partial D variants (DII & DNU, DIII, DIV, DVa, DCS, DVI, DVII, DOL, DFR, DMH, DAR, DHK & DAU-4, DBT and RoHar).
Results: Out of the thirty nine D discrepant samples tested with the ALBAclone Advanced Partial RhD Typing Kit, eleven were found to be DOL, thirteen DFR, three DAR, three DVa, one DVI, two DCS and four were of weak D type. Only two cases could not be classified by the kit.
Conclusions: The ALBAclone Advanced Partial RhD Typing Kit is very useful in confirmation of RhD status when different commercial anti-D reagents give discrepant results. We can also conclude from our results that the kit can reliably interpret the correct RhD status even if further molecular facilities are not available for classification.

3C-S14-05
LONG-TERM FOLLOW UP OF ANTI-HOST A/B ANTIBODY AFTER MINOR ABO-INCOMPATIBLE HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: ABO blood antigen plays a key role in transfusion medicine. In minor ABO-incompatible transplants, production of anti-host A/B antibody by transfused donor B cells occasionally cause hemolysis after 2–3 weeks, known as passengerlymphocyte syndrome. However, there have been few reports concerning the kinetics of anti-host antibody production, particularly late after transplantation.
Patients and methods: We retrospectively reviewed the medical records of 603 patients with various malignancies who underwent allogeneic hematopoietic stem cell transplantation at our hospital between 1999 and 2007. There were 159 minor ABO-incompatible pairs. The inclusion criteria for the evaluation of long-term outcome were as follow: (1) erythrocyte engraftment confirmed by the presence of donor ABO antigen with more than 1% of reticulocytes, and (2) follow-up beyond 100 days. Consequently, 94 cases including 29 bi-directional ABO-incompatible transplants were eligible for this study. The median follow-up was 377 days (range: 100–2704). The median age of the patients was 48 years (range: 4–67), and the diagnoses included AML (n = 37), MDS (n = 14), ML (n = 21), CML (n = 8), ALL (n = 6), solid cancer (n = 5), and others (n = 3). The conditioning regimens used were myeloablative in 45 (TBI-containing 20 and non-TBI 25) and busulfan-based reduced-intensity in 49 cases. GVHD prophylaxis consisted of cyclosporine with (n = 47) or without (n = 25) short-term MTX, and tacrolimus with (n = 14) or without (n = 8) short-term MTX.
Results: Anti-host A/B antibody was detected only in five cases (5.3%) after day 100, including three in whom it was detected only once. In two cases, the antibody kinetics fluctuated during immunosuppression therapy with steroids for graft-versus-host disease. Notably, in a patient with AML who received donor lymphocyte infusion for relapse after transplantation, anti-host antibody was for the first time detected one month later.
Discussion: In most recipients, anti-host antibody was not documented after minor ABO-incompatible transplantation. The reason may include (1) absorption of produced anti-host A/B antibody by host tissue antigen and (2) cell immune tolerance due to exposure to host antigen. Concerning the mechanism of repetitive positive anti-host A/B antibody in the two cases, absorption of anti-host antibody might be attenuated or B-cell immune tolerance might be disrupted due to the potent immunosuppressive treatment. Platelet transfusion containing anti-host antibody might be another reason. Donor lymphocyte infusion may also induce production of anti-host antibody by the mechanism similar to ''passengerlymphocyte syndrome''.
Conclusions: Anti-host antibody was rarely detected in the late phase of ABO-incompatible transplantation, which supports the concern that laboratory blood type mismatch tend to prolong.

Tuesday: Parallel Session S15: TRALI

3C-S15-01

FIGHT AGAINST TRALI RECENT ADVANCES

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Transfusion-related acute lung injury (TRALI) is a life-threatening adverse reaction to transfusion. It is characterized by noncardiogenic pulmonary edema that develops during or within 6h after transfusion. Transfusion of blood products or massive transfusion has long been known as a rare cause of acute lung injury (ALI)/acute respiratory distress syndrome (ARDS). Because the main causes of ALI/ARDS are sepsis and/or pneumonia, little attention has been paid to transfusion as a cause of lung injury. Owing to indifference to and underrecognition of this complication, ALI after transfusion may be attributed to other causes. Moreover, physicians tend to attribute the cause of respiratory failure to more common complications of transfusion such as allergic reaction and circulatory overload. Even if an appropriate diagnosis of noncardiogenic pulmonary edema is made, transfusion may not be identified as its cause. Although anti-leukocyte antibodies have been proved to be one of the main causes of TRALI, the precise mechanism of this syndrome is poorly understood. Anti-HLA and -HNA antibodies in labile blood products are reported to cause TRALI, and animal models of TRALI show that these antibodies induce lung injury under certain experimental conditions. We have recently showed that anti-CD36 antibody caused TRALI in two patients. This finding suggests that as-yet-unknown antibodies other than anti-HLA or -HNA antibodies might cause TRALI. According to current haemovigilance reports and previous look-back studies, the incidence of TRALI is not very high. This finding supports the concept of the two-hit theory of ALI, where certain patients' clinical conditions are prerequisite in addition to transfusion for developing TRALI. A recent study in the ICU of a tertiary care medical center has revealed a high incidence of suspected TRALI cases. This study revealed that female plasma, number of pregnancies in donors, number of donor units positive for anti-granulocyte antibodies and anti-HLA class II antibodies and concentration of lysophophatidylcholine in donor products are associated with developing TRALI. Our recent prospective study has revealed the predominance of male FFP in post-transfusion respiratory function when comparing male only FFP and mixed FFP in surgical patients. The incidence of TRALI in the UK was successfully reduced by using preferentially male plasma. Although TRALI has a relatively better prognosis than ALI with other causes, TRALI has still been the leading cause of transfusion-related death in the United States in recent years. The prevention of TRALI caused by plasma products has been successful, but TRALI caused by apheresis platelets is still problematic. A recent study has suggested that the prevalence of anti-HLA antibodies in female donors increases with the number of pregnancies. Thus, screening for anti-HLA antibodies has emerged as an effective measure to prevent TRALI caused by apheresis platelets. At present, no single measure can thoroughly eliminate TRALI; however, early diagnosis and intervention may improve the outcome for patients with TRALI. Further study is required to elucidate the mechanisms of TRALI, which may lead to the development of preventive measures and treatment.

3C-S15-02

INCREASE IN PERMEABILITY OF HUMAN LUNG MICROVASCULAR ENDOTHELIAL CELLS BY COCULTURE WITH PERIPHERAL BLOOD MONONUCLEAR CELLS IN THE PRESENCE OF ANTI-HLA CLASS II ANTIBODY *IN VITRO*

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Background: The activation of monocytes induced by anti-HLA class II antibodies is thought to play important roles in the etiology of transfusion related acute lung injury (TRALI). Increased permeability of lung microvascular endothelial cells contributes to the pathogenesis of pulmonary edema, which is a hallmark of TRALI. We experienced a TRALI in the recipient of anti-HLA class II antibody-containing platelet concentrate.
Aim: To investigate the involvement of anti-HLA class II antibodies in TRALI, we studied whether permeability of human lung microvascular endothelial cells (HMVECs) could be enhanced by the coculture of HMVECs with peripheral blood mononuclear cells (PBMNCs) in the presence of anti-HLA class II antibodies.
Methods: The anti-HLA class II antibody-containing donor plasma (anti-HLA-DR plasma) that was involved in a TRALI case was used. The antibodies had a broad specificity for DR antigens including DR4 but not for DR8 or DR12. Flow cytometry analysis showed that the anti-HLA-DR plasma reacted with PBMNCs from three subjects with HLA-DR [4, 10], [4, 15], and [4, 4], (cross-match-positive PBMNCs), whereas the same plasma did not react with PBMNCs from two subjects with HLA-DR [8, 12] (cross-match-negative PBMNCs). HMVECs seeded on the transwell were incubated with the cross-match-positive or -negative PBMNCs, anti-HLA-DR plasma (0.5%: vol/vol) and 40-kDa FITC-labeled dextran (0.5 mg/ml) for 6h. As a negative control, HMVECs and PBMNCs were incubated with pooled plasma from blood group AB donors, which contains no antibodies to HLA, HPA, or HNA. As a positive control, those cells were incubated with LPS (1 μg/mL). After the incubation, FITC-labeled dextran in the lower chamber of the transwell was measured. The roles of cytokines, platelet activating factor (PAF), leukotrienes in the anti-HLA-DR plasma-induced enhancement of endothelial permeability were studied by testing the blocking effects of anti-TNF-alpha, anti-IL-1bata, anti-IL-6, anti-IL-8 neutralizing antibodies, PAF receptor antagonist (CV-3988) or cysteinyl-leukotriene (CysLT) receptor antagonist (BAY-u9773) to the coculture.
Results: The coculture of HMVECs with cross-match-positive-PBMNCs in the presence of anti-HLA-DR plasma resulted in the increase of endothelial permeability when compared to incubation with AB plasma. In contrast, the combination of cross-match-negative PBMNCs and the anti-HLA-DR plasma had no significant effect on HMVEC permeability. The coculture of HMVEC with PBMNCs in the presence of 1 μg/mL LPS showed similar increases in HMVEC permeability regardless of cross-matched reaction. Anti-TNF-alpha neutralizing antibody partially reduced the increase in endothelial permeability induced by the coculture, whereas neutralizing antibodies against other cytokines did not exhibit any effect. CV-3988 almost completely suppressed the increase in endothelial permeability, but BAY-u9773 did not.
Conclusion: The coculture of HMVECs with PBMNCs in the presence of anti-HLA-DR plasma caused the enhancement of endothelial permeability in a corresponding antigen-antibody dependent manner. TNF-alpha and PAF, which might be generated during the coculture, mainly participated in this response. The activation of monocytes by the anti-HLA-DR antibodies may contribute to the pathogenesis of pulmonary edema, a hallmark of TRALI.

3C-S15-03
AUTOMATED HIGH-THROUGHPUT SCREENING FOR GRANULOCYTE-SPECIFIC ANTIBODIES IN BLOOD DONORS USING FLOW GIFT

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Background: Granulocyte associated antibodies are considered as being responsible for severe pulmonary transfusion reactions (TRALI). However, investigation of a large number of blood donor samples using the standard granulocyte immunofluorescence (GIFT) and granulocyte agglutination test (GAT) proved to be a time consuming process and the large number of test cells required can be problematic. Using the novel Flow-GIFT method, (flow cytometric granulocyte immunofluorescence) allows a rapid detection of granulocyte antibodies marked by an automation in pipetting of samples and flow cytometric analysis. The prevalence of granulocyte antibodies in blood donors with this novel method has been shown.
Materials and methods: Out of 1140 sera collected from female blood donors, 697 (61.1%) had a history of pregnancy. For testing, MNC from two HNA-typed donors were isolated using cell sedimentation in a ficoll density gradient. Subsequent pipetting steps into 96-deep well plates were automated using the machine Biomek NXp Workstation. Antibody binding to test cells was detected using FITC-conjugated antibodies and analysed on the flow cytometer FC 500 MPL. 7-AAD was used to exclude dead cells. Standard GIFT and GAT were also performed as reference methods. For the detection of HLA class I and II IgG antibodies, AB screen ELISA assay was used.
Results: In 169 (24.2%) of 697 females with a history of pregnancy, specific antibodies against granulocyte-antigens (n = 10; 1.43%), HLA class I (n = 105; 15.1%), HLA class II (n = 25; 3.6%) and HLA class I as well as class II (n = 29; 4.2%) could be detected. However, in 8.8% (n = 39) of 443 females without history of pregnancy antibodies against granulocyte-antigens (n = 3; 0.68%) and HLA class I (n = 38; 8.5%) could be found. The granulocyte-antibodies in females with history of pregnancy were determined as anti-HNA-1b (n = 1), anti-HNA-2a (n = 4), anti-HNA-3a (n = 2); anti-CD16 (n = 2), one antibody with unclear specificity. Interestingly, in three females without history of pregnancy, these granulocyte antibodies with the specificities against HNA-1b (n = 1) and CD16 (n = 2) could also be detected.
Conclusion: Flow-GIFT, as an automated method, allows rapid and simple detection of granulocyte antibodies while less donor test cells are needed than for the current methods. This high throughput method can open the way for screening of granulocyte antibodies in large donor populations. TRALI due to transfusion of FFP and platelet apheresis products can be reduced or avoided in a most effective manner.

3C-S15-04
A PILOT STUDY ON THE INCIDENCE OF HLA CLASS I, CLASS II AND HNA ANTIBODIES IN AUSTRALIAN APHERESIS DONORS

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Background: Demand for both fresh components and plasma for fractionation in Australia continues to increase amidst increasing stringency of donor selection guidelines. In 2008/09, platelet supply increased by 1.5% compared with 2007/08 and clinical fresh frozen plasma (cFFP) supply increased by 4.9%. Total intravenous immunoglobulin supply continues to increase at a rate of 10–12 % annually and with it, the demand for plasma for fractionation. Concurrently, the proportion of apheresis-derived platelet and plasma components required to support clinical need in Australia also continues to increase.
Transfusion-Related Acute Lung Injury (TRALI) is a severe complication of transfusion where antibodies to Human Neutrophil Antigen (HNA), Human Leucocyte Antigen (HLA) class I and class II have been implicated. As multiparous female donors are more likely to develop leucocyte antibodies, many blood services, including Australia from mid-2007, have begun supplying predominant male cFFP as a TRALI risk reduction strategy.
However, extension of this policy to apheresis platelet collection is problematic as this panel is HLA- and HPA-typed and requests for HLA- or HPA-compatible platelets could not be supported by using male donors alone. Thus the place of HLA and HNA antibody testing in apheresis donor selection is currently being considered as a complementary TRALI risk reduction strategy.
Aim: This was a pilot study to determine the frequency of HLA and HNA antibodies in an Australian apheresis donor population.
Method: De-identified serum samples from 331 random donors were collected and screened using Luminex LSM kits (One Lambda Inc) for HLA class I and II antibodies, and granulocyte agglutination test and immunofluorescence test for HNA antibodies. Gender was recorded. No information was collected on history of transfusion in either male or female donors, or the number of pregnancies in female donors.
Results: 108 females and 223 male donors were tested. HLA antibodies were detected in 80 donors (24.1%) and HNA antibodies in six donors (1.8%).
53 (48.1%) of female donors tested had leucocyte antibodies. 28 (25.9%) had HLA class I antibodies only, three (2.8%) HLA class II antibodies only and 19 (17.6%) had both HLA class I and II antibodies. Only one had HNA antibodies alone and another had both HNA and HLA class II antibodies.
Unexpectedly, 35 (15.7%) of male donors tested had leucocyte antibodies. HLA Class I antibodies were detected in 27 (12.1%), HLA class II antibodies in three (1.3%) and HNA antibodies in five (2.2%).
Conclusion: These pilot data indicate that the incidence of HLA antibodies in Australian male apheresis donors is higher than expected. A further study is planned to determine the factors associated with the development of leucocyte antibodies by gathering donor demographic data including gender, age, pregnancy history and transfusion history. This will allow further refinement of donor selection policies in relation to antibody-mediated TRALI risk reduction in the Australian context as well as assess the predictive value and applicability of leucocyte antibody testing platforms to blood donor screening.

3C-S15-05
EFFECTS OF ANTIOXIDANTS AND A PPAR GAMMA LIGAND ON HEMIN-INDUCED HUMAN NEUTROPHIL ACTIVATION – STUDIES WITH FLOW CYTOMETRY, CONFOCAL LASER SCANNING MICROSCOPY AND ELECTRON MICROSCOPY

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Background: TRALI (transfusion-related acute lung injury) is induced by injury of vascular endothelial cells due to neutrophils activated by anti-leukocyte antibodies or physiologically active substances. Previous reports demonstrated activation of neutrophils by hemin (a heme-related molecule) and suggested the involvement of erythrocyte-derived substances in the onset of TRALI.
Aims: The present study was aimed at investigating the effects of heme-related molecules including hemin on the formation of ROS (reactive oxygen species) in neutrophils. The study was additionally designed to explore the mechanism for neutrophil activation by heme-related molecules through evaluating morphological changes of neutrophils under stimulation with hemin and the effects of various antioxidants and PPAR gamma ligands in suppressing ROS formation, in comparison to neutrophils under stimulation with PMA (phorbol myristate acetate).

Methods: Polynucleated cell fractions, isolated from peripheral blood of healthy volunteers, were exposed to APF (Aminophenyl Fluorescein), a fluorescent reagent for detection of ROS. Then, using FCM (flow cytometry), CLSM (confocal laser scanning microscopy) and EM (electron microscopy), ROS formation by the cells under stimulation with heme-related molecules (hemin, hemoglobin, mesoporphyrin) was analyzed. During FCM, CD16b-PE staining was carried out to check for neutrophil fraction.

Some polynucleated cell fractions were treated with one of antixodants (apocynin-NADPH oxidase inhibitor, superoxide dismutase (SOD)) or PPAR gamma ligands (pioglitazone, 15d-prostaglandin J2) before exposure to APF in a way similar to above, to evaluate the effects of these factors in suppressing ROS formation.

Results: FCM analysis revealed that ROS formation in neutrophils was stimulated not only by hemin but also by other heme-related molecules (hemoglobin and mesoporphyrin) to a similar degree. In some samples, ROS formation was stimulated even by low-concentration hemin when combined with fMLP. CLSM revealed formation of reactive oxygen species in neutrophil granules.

When neutrophils were stimulated with heme-related molecules, FCM revealed appearance of many particles with low FSC signals in addition to cells. Minute particles were also detected by CLSM. EM revealed that cells stimulated with hemin released structures akin to vesicles or granules, without showing vacuolation of granules markedly seen under stimulation with PMA.

In evaluation of suppression of ROS formation by antioxidants, apocynin suppressed PMA and hemin, while SOD suppressed only PMA. Pioglitazone suppressed both PMA and hemin, and its effect against hemin was particularly marked.

Summary/conclusions: In this study, not only hemin but also other heme-related molecules stimulated ROS formation in neutrophils, endorsing the view that erythrocyte-derived substances are involved in the onset of TRALI. The features of morphological changes of neutrophils and the effects of antixoidants and PPAR gamma ligands in suppressing ROS formation under stimulation with hemin differed strikingly from those under stimulation with PMA. These differences suggest a difference in the mechanism for neutrophil activation between hemin and PMA. This is another open question for the future.

Tuesday: Parallel Session S16: Novel Developments

3C-S16-01

IN VITRO PRODUCTION OF TRANSFUSABLE RED BLOOD CELLS

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Background: The supply of transfusable red blood cells (RBCs) is not sufficient in many countries. If transfusable RBCs could be produced abundantly from certain resources, it would be very useful. We have developed a method to produce enucleated RBCs efficiently from hematopoietic stem cells present in umbilical cord blood (Miharada et al., Nature Biotechnology 24: p. 1255, 2006). More recently, it was reported that enucleated RBCs could be abundantly produced from human embryonic stem (ES) cells (Lu et al., Blood 112: p. 4475, 2008). The common obstacle for application of these methods is that these methods require very high cost to produce sufficient number of RBCs that are applicable in the clinic.
Aims: If erythroid cell lines (immortalized cell lines) able to produce transfusable RBCs *in vitro* were established, they would be valuable resources. However, such cell lines have not been established. To evaluate the feasibility of establishing useful erythroid cell lines, we attempted to establish such cell lines from mouse ES cells.
Methods: We developed a robust method to obtain differentiated cell lines following the induction of hematopoietic differentiation of mouse ES cells. Briefly, we have used feeder cells and several kinds of humoral factor so as to induce hematopoietic differentiation of mouse ES cells efficiently.
Results: We established five independent hematopoietic cell lines using the method. Three of these lines exhibited characteristics of erythroid cells. Although their precise characteristics varied, each of these lines could differentiate *in vitro* into more mature erythroid cells, including enucleated RBCs. Following transplantation of these erythroid cells into mice suffering from acute anemia, the cells proliferated transiently, subsequently differentiated into functional RBCs, and significantly ameliorated the acute anemia. In addition, we did not observe formation of any tumors following transplantation of these cells (Hiroyama et al., PLoS ONE 3: e1544, 2008).
Summary/conclusions: To the best of our knowledge, this is the first report to show the feasibility of establishing erythroid cell lines able to produce mature RBCs. Considering the number of human ES cell lines that have been established so far and the number of induced pluripotent stem (iPS) cell lines that will be established in future, the intensive testing of a number of these lines for erythroid potential may allow the establishment of human erythroid cell lines similar to the mouse erythroid cell lines.

3C-S16-02

NOVEL THERAPEUTIC STRATEGY IN PATIENTS WHO REQUIRE REPEATED TRANSFUSION OF HUMAN LEUKOCYTE ANTIGEN-MATCHED PLATELETS DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Donated blood-derived platelets are utilized for patients receiving chemotherapy or with various bleeding disorders. Risks of infection, donor shortages, and short shelf-life (< 4 days in Japan) have prompted searches for an alternative source of platelets. In this context, human embryonic stem cells (hESCs) have been proposed as a source of blood products. Repetitive transfusion of platelets that are not human leukocyte antigen (HLA) recipient-matched often generates anti-HLA antibodies in patients, resulting in unresponsiveness to platelet transfusion therapy. To avoid immune reactions following multiple allogeneic platelet transfusions, it is desirable to use HLA-matched or autologous platelets. We have recently established an *in vitro* culture system whereby human embryonic stem cells (hESCs) can be differentiated into 'unique sac-like structures' (ES-sacs) containing hematopoietic progenitors. These hematopoietic progenitors express early hemato-endothelial markers (CD34, CD31, CD41a, and CD45), and coexist with lectin-1 binding activity+ endothelial layers. Upon further cultivation under appropriate conditions, these hematopoietic progenitor cells differentiate into various blood cells such as megakaryocytes, generating platelets with morphology and integrin functions indistinguishable from those of human platelets (Takayama et al., Blood, 2008). Utilizing this culture system, we attempted to generate functional platelets from human induced pluripotent stem (hiPS) cells. All of more than 10 different hiPS cell lines that we examined generated Sac-like structures (iPS sacs) that led, although to varying extents, to production of functional platelets *in vitro* and in an *in vivo* mouse model. We propose that use of HLA-matched hiPS cells may be a useful strategy to generate functional platelets for patients requiring repeated transfusion.

3C-S16-03

EFFECT OF VEGF ON THE EXPRESSION OF CXCR4 AT UNRESTRICTED SOMATIC STEM CELLS

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Background: Umbilical cord blood is an ideal source of stem cells to be used in cell therapy and transplantation in many diseases. Stem cell homing is a vital key for successful engraftment after stem cell transplantation in patients with injured tissues. CXCR4/SDF-1 axis plays an important role in proper homing of stem cell circulating in peripheral blood into damaged site in the body. Unrestricted somatic stem cells (USSCs) are recently isolated and introduced as new human somatic stem cells from umbilical cord blood. Because of the high potentiality of USSCs for stem cell therapy and transplantation, its homing ability and regulation is considered important. So in this study, we evaluate the CXCR4 expression at USSCs and studied the effect of VEGF on its expression.
Materials and methods: Cord blood was collected from the umbilical cord vein with informed consent of the mother and USSCs were separated by density centrifugation over a Ficoll-Hypaque gradient and using dexamethasone. To assess the effect of VEGF on CXCR4 expression, passage 5 was selected and the growth factors added with concentration of 50 ng/ml and 10 ng/ml respectively. CXCR4 expression at mRNA level and expression of surface CXCR4 was evaluated using RT-PCR and FITC- conjugated monoclonal anti CXCR4 respectively.
Result: According to the results, before adding the VEGF in USSCs culture, the population of the cells which expressed CXCR4 on their surface was 30% ± 8%. RT-PCR analysis of CXCR4 gene expression also revealed the active mRNA levels in different passages of USSCs. After using VEGF, the CXCR4 expression was increased from 30% ± 8% to 52% ± 13%.
Conclusion: Increased level of CXCR4 induced by VEGF in USSCs makes them more potent and ideal to be used in stem cell therapy with more efficiency. On the other hands, we can control the surface expression of CXCR4 on USSCs to improve the efficacy of stem cell.
Key words: Cord blood, Unrestricted somatic stem cells, Vascular endothelial growth factor, CXCR4.

3C-S16-04

THE EFFECTS OF OSTEOPONTIN EXPRESSED BY DIFFERENTIATED OSTEOBLASTIC CELLS FROM MESENCHYMAL STEM CELLS AS A FEEDER COMPONENT ON CORD BLOOD DERIVED HEMATOPOIETIC STEM CELLS EXPANSION

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Objective: Bone marrow mesenchymal stem cells (MSCs) reside in the non hematopoietic components in the postnatal bone marrow and provide a suitable hematopoietic microenvironment for the hematopoietic cell population's proliferation and differentiation. MSCs have also been reported to differentiate into osteoblastic (OB) cell types after systemic administration *in vitro* and *in vivo*. OBs synthesis and express osteopontin (Opn), the highly acidic glycoprotein, acts as a potent constraining factor on hematopoietic stem cells (HSCs) proliferation. Comparison of MSCs and OB cells as feeders in expansion of cord blood HSCs and down-regulation of Opn and its consequence of the suppression, in this process were aimed at this study.

Methods: MSCs were isolated and characterized. OB differentiation media was added to the MSCs. After 5 days, Opn-small interfering RNA (siRNA). Real-time PCR and Western blotting were used to quantify the mRNA and Opn protein levels. Early HSCs derived from cord blood co-cultured on MSC, osteoblastic cells with and without Opn down regulated as a feeder. HSCs expansions analyzed by colony assay, apoptosis and cell surface markers.

Results: Sequence-specific siRNA targeting Opn suppressed Opn-RNA expression by 75% and also decreased Opn-protein level by 65% in osteoblastic cells. Expansion of HSCs was higher on OB cells as a feeder layer compared with MSCs. on the other hands; this ability was lowered in the presence of Opn-siRNA ($P < 0.05$).

Conclusion: This study suggested crucial role of OBs in HSCs expansion and key effect of Opn in this interaction These results prompted us to designing a specific *in vitro* feeder containing osteoblastic material for further expansion of HSCs would be helpful in future.

Tuesday: Parallel Session S17: Haemovigilance and Transfusion Reactions

3C-S17-02

TRANSFUSION ERRORS AND THEIR PREVENTION

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The risk of non-infectious hazards, including risks related to hospital-based steps in transfusion care, is at least 100 times greater than the risk of viral transmission through blood components (Dzik, Transfusion 2003;43:1190–8). Mistransfusion, in which the wrong blood is administered to the recipient, is the final outcome of one or more procedural errors or technical failures in the transfusion process, starting with the decision to transfuse a patient and ending with the actual administration of blood components. ABO-incompatible blood transfusion attributable to the incorrect identification (ID) of the patient or the blood unit is one of the most serious transfusion hazards. The Serious Hazards of Transfusion (SHOT) scheme in England showed that approximately 70% of incorrect blood component transfused (IBCT) event errors occur in clinical areas, with the most frequent error being failure to perform the final patient ID check at the bedside (Stainsby et al., Transfus Med Rev 2006;20:273–82). Thus, the pretransfusion check at the bedside is the most critical step for the prevention of mistransfusion. However, a large observational audit revealed the failure to perform the final bedside check for patient ID (Novis et al., Arch Pathol Lab Med 2003;127:541–8). The prevention of human errors is of major significance in transfusion medicine, and reducing the human error rate increases transfusion safety. Machine-readable ID technology, especially a bar code-based ID system, is ideally suited to bedside check requirements. A bar code-based patient-blood unit ID system (Nursing Pass, Olympus Systems, Tokyo, Japan) was implemented in the hospital, including all inpatient wards except for the psychiatric and dermatologic wards, operating rooms, and an outpatient hematology unit in July 2002 (Ohsaka et al., Transfusion 2008;48:1730–8). The transfusion policy for blood administration in our hospital includes a standard two-person (doctor/nurse or two nurses) visual and verbal double-check followed by bar code ID using a hand-held device just prior to blood administration. The bar code-based ID system has worked well over a 7-year period, and approximately 90,000 blood components have been transfused without a single mistransfusion. The overall rate of compliance with 'second' electronic bedside checking for blood components was 99%. Human error was the most frequent cause of errors leading to the failure of the bedside bar code ID (Ohsaka et al., Transfus Med 2008;18:216–22). The implementation of the bar code-based ID system compelled nursing staff to adhere to an additional verification procedure. However, most staff members who responded to the questionnaire survey indicated that the bar code-based ID system was acceptable for the pretransfusion check at the bedside. Because of the limited number of patients who received blood components, the effect of the bar code-based ID system on preventing mistransfusion cannot be clarified based solely on our experience. Further multicenter studies are needed to clarify this issue. We showed that the bar code-based ID system that we used was fully applicable to the bedside pretransfusion check for pediatric transfusion, i.e., blood dispensed in syringes, and preoperative autologous blood donation, as well as regular allogeneic blood transfusion.

3C-S17-03

THREE-YEAR EXPERIENCE OF A NATIONWIDE NETWORK OF UNIVERSITY HOSPITAL TRANSFUSION SERVICES TO INVESTIGATE TRANSFUSION REACTIONS IN JAPAN

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Background: In 1998, a nationwide network of university hospital blood transfusion services was started among national university hospitals in order to investigate transfusion reactions, and public university hospitals and private university hospitals joined in 2005.

Aims: This study assessed ways to improve and standardize the method of investigating transfusion reactions at university hospitals, and to determine the exact risk of transfusion reactions.

Methods: In 2007, 44 national university hospitals, eight public university hospitals, and 32 private university hospitals participated in a nationwide network of university hospital blood transfusion services. Data on all transfusion reactions were collected between April 2005 and March 2008, and analyzed by the Working Group for Transfusion Reactions of our network in cooperation with the Japanese Red Cross Blood Center. Standard methods for investigating transfusion reactions, including collecting residual blood components, were promoted during this period. We proposed a temporary classification of investigations of transfusion reactions at participating hospitals. The amount of blood components transfused was also reported.

Results: Reported non-hemolytic transfusion reactions from a nationwide network of university hospitals from fiscal year 2005 to 2007 are shown in Table 1. In fiscal 2007, among all 84 university hospitals, the total amount of red cells transfused comprised 743,234 units, fresh frozen plasma 681,161 units, and platelet concentrates 1,828,429 units, and the actual number of patients transfused was 87,999. Transfusion-related acute lung injury (TRALI) was reported in 16 cases, transfusion-associated circulatory overload (TACO) in five cases, transfusion-associated dyspnea (TAD) in 22 cases, severe allergic reaction in 143 cases, minor allergic reaction in 4,502 cases, hypotensive transfusion reaction in 68 cases, febrile non-hemolytic transfusion reaction (FNHR) in 638 cases, suspected transfusion-transmitted bacterial infections in three cases, and unclassified transfusion reaction (UCT) in 382 cases. However, there was still a marked difference in the number of reported cases of reactions according to the number of transfusions among university hospitals after the 3 years of experience.

Conclusion: These attempts are useful for improving the accuracy of investigation of transfusion reactions at university hospitals. Further effort to standardize such investigations is necessary.

Financial support: Part of this study was supported by the Ministry of Health, Labor and Welfare (Regulatory Science of Pharmaceuticals and Medical Devices)

Table 1 Reported non-hemolytic transfusion reactions from a nationwide network of university hospitals in Japan

	2005[a]	2006[b]	2007[c]
FNHTR	601	764	638
Minor allergic reaction	3,347	5,528	4,502
Severe allergic reaction	146	278	143
TRALI	15	26	16
TACO	0	5	5
Transfusion-associated dyspnea (TAD)		60	22
Hypotensive transfusion reaction		147	68
Unclassifiable complication of transfusion (UCT)	97	290	382
Suspected transfusion-transmitted bacterial infection	5	11	3

[a]44 national, 8 public, and 21 private university hospitals
[b]44 national, 8 public, and 28 private university hospitals
[c]44 national, 8 public, and 32 private university hospitals

3C-S17-04

ANALYSIS OF TRANSFUSION REACTIONS

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Background: Adverse effects of blood transfusion are serious causes of mortality and morbidity. An active hemovigilance programme is not yet established in many countries particularly, the developing countries and it is important to learn from the experience of the SHOT (Serious Hazards of Transfusion) programme in the UK. Reporting adverse reactions would help in the root cause analysis and prevention of some of the reactions

Aim: To analyze immediate blood transfusion reactions

Materials and methods: A total of 10,281 patients with 51,632 transfusion episodes were analysed during a period of 3 years and 5 months from January 2006 to May 2009. As a routine, all blood transfusions are monitored and documented in the blood transfusion form. The basal temperature, pulse and blood pressure were documented just before starting the transfusion and once in 15 min during the process of transfusion. Patients are not given any prophylactic medication. Red cells and random platelets were prepared by the removing the buffy coat. Blood units causing any adverse transfusion reactions were returned to the blood bank along with two post transfusion samples for work up of the reaction which included grouping & cross matching of the patient with the donor, with both the pre and the post transfusion samples and a DAT of the patient's post - transfusion sample, to rule out hemolytic reactions. The units which caused fever as the major symptom of the reaction were sent for microbiological evaluation, to rule out bacterial contamination. The details collected from the transfusion forms were: patient details, type of blood component transfused, volume transfused before symptoms occurred, symptoms caused because of the transfusion. The reactions were classified according to the symptoms as:

Febrile Non hemolytic transfusion reactions (FNHTR): an increase of one degree from the basal temperature

Allergic: itching, hives or chills without rise in temperature

Others: breathlessness, hypotention

Results: Are indicated in the tables

Table I : Indicates the number of reactions Table II: Represents the different type of reactions caused due to different type of components. Results of pre and post transfusion samples for grouping and crossmatching showed correlation and the patient's post other transfusion DAT was negative in all cases. The 32 blood units causing FNHTR were found to be sterile using the BacT Alert system. Out of the 32 FNHT reactions, 25 occurred in patients undergoing chemotherapy. Overload causing respiratory distress were found in two patients due to errors in bed side blood administration. The reason for hypotension in one case could not be determined.

Table 1: The number of reactions

Components	No. of Transfusions	No. of Reactions	Percentage
Red cells in SAGM	25,083	57	0·23
Random Donor platelets	12,019	06	0·05
Fresh Frozen plasma	13,799	05	0·04
Single donor platelets	724	01	0·14
Total	51,632	69	0·45

Table 2: Type of reactions

Component Type	Allergic	FNHTR	Others		Total
			Overload	Hypotension	
Red cells	22	32	02	01	57
Random donor platelets	05	01	-		06
Single donor platelets	01	—	-		01
Fresh Frozen Plasma	05	-	-		05

Conclusion: Febrile and allergic reactions are the two most common immediate reactions. The use of leucoreduction filters could have prevented febrile reactions in multiply transfused patients on chemotherapy. An active hemovigilance can reduce the number of preventable reactions.

Type of Reactions Tuesday: Parallel Session S18: History

3C-S18-01

THE INTRODUCTION OF WESTERN MEDICINE AND BLOODLETTING TO JAPAN

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The worldwide evolution of science and medicine can be illustrated by the way it developed in two distant parts of the world, Europe and Japan. Differences and similarities show in the Western as well as the Eastern records.

Europe had adopted principles expounded by Galen, a Greek practicing in Rome. He taught that man's life and health depended on four ''humours''or bodily substances: Blood, Yellow Bile, Black Bile and Phlegm. They corresponded to the four elements of Air, Fire, Earth and Water, the four Seasons and the four Ages of Man. Blood was the only one of the humours that man could regulate and that led to the overwhelming importance of bloodletting and the bloodletter in the Western world.

Eastern medicine had taken a different route in the much older Chinese natural philosophy that had five elements of Wood, Earth, Water, Fire and Metal that did not correspond to the humours and were not integral to the principal energy of the natural world called qi. Whereas the West modified and corrected balance among the humours by a cutting into veins and removing blood, qi was generated to nurture regulate body function by acupuncture and moxibustion in the East.

Chinese medicine had spread through Korea and into Japan long before the Portuguese trading ships arrived in 1543. After one hundred years of what the West calls the 'Christian Century', the Portuguese, who wanted to both trade and convert the Japanese to Christianity, were expelled and displaced by the Dutch, whose interest was in trade only and not in religion.

Nevertheless, all barbarian Westerners remained suspect and the Dutch were isolated to an island off Nagasaki called Dejima where they had a trading post for the next two hundred years. Physicians and ''barber surgeons''posted there to shave, pull teeth and bleed were the most educated of the foreigners and had the additional task of teaching language exchange. They used the writings of their trade as texts and in that way Western medical theory, called kampo, became known to the interpreters and through them to the courts of the Shogun and the Emperor. The Japanese then added a modified form of bloodletting to acupuncture to create a hybrid therapy known as shiraku.

Even the most famous of the European physicians working for the Dutch on Dejima such as von Siebold and Thunberg were practicing and teaching the medicine of Galen over the next two hundred of years during which Japan isolated itself from the West. Bloodletting was discarded in Europe and America in the 19th Century and replaced by its opposite, blood transfusion, in the so-called Age of Enlightenment. That change did not penetrate Japan's wall of seclusion. It was not until the reopening of Japan at the time of the Meiji there was a great flood of Western thinking in many fields. Among other things that brought the blood transfusion that had replaced bloodletting as the established therapy.

3C-S18-02

TRANSFUSION MEDICINE AND BLOOD PROGRAM IN JAPAN SINCE WORLD WAR II

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In 1948, two women developed syphilis due to transfusion of fresh blood immediately after collection from paid donors. Due to these incidents, the Government established a supply system for stored blood. In 1951, the first commercial blood bank was opened to collect blood from paid donors and to provide stored blood. The next year, the Japanese Red Cross (JRC) started to collect blood from voluntary non-remunerated donors, but commercial blood banks continued to thrive even more. However, around the 1960s, this prosperity had not only caused many paid donors to develop severe anemia due to frequent blood donation, but also caused post-transfusion hepatitis (PTH) in more than half of the patients receiving the blood. In 1964, the Government was forced to change the policy concerning blood collection from paid to voluntary non-remunerated donors, and allowed JRC to take almost monopolistic control of the management of the blood program. Although the number of blood units collected had remarkably decreased by about 30% in the following year, the donation rates increased by about 10% each year thereafter, reaching more than 8% in 1985. This reform also resulted in a dramatic decrease in the incidence of PTH to 16%. HBV screening was introduced in 1972, but PTH did not decrease as much as expected. Blood component therapy was promoted in the latter half of 1970s, which resulted in a large volume of FFP consumption without an increase in the source plasma and the discarding of many surplus units of RCC. Since around1980, blood products and source plasma were increasingly imported, and many hemophiliacs developed AIDS (about 40%) following treatment with imported unheated concentrated anti-hemophilic factors (AHF). In order to resolve these problems, the Government introduced new blood policies in 1985. These policies included 400 ml whole blood collection and apheresis (plasma and/or platelet), as well as guidelines for appropriate use of red cell concentrates (RCC), human serum albumin (HSA) and fresh frozen plasma (FFP). The following year, HIV and HTLV-1 screening were introduced. In 1989, a guideline for appropriate transfusion practices was issued, and HCV screening was introduced, which resulted in a remarkable decrease in PTH.

In 1996, several civil suits filed by hemophiliacs who had developed AIDS following treatment with plasma-derived AHF were settled, which led to the enactment of the ''Blood Law''in 2002. In 1999, NAT testing for HBV, HCV and HIV were introduced, and while residual risks were recently estimated to be 10?15 cases for HBV and one case for HCV per year, that for HIV was estimated to be one case per 2?3 years and there have not been any cases of HTLV-1 reported since 1986. Self-sufficiencies for HSA and intravenous immunoglobulin (IVIG) have gradually been increasing each year since 1986, and reached 64% and 95%, respectively, in 2008.

A high level of safety for blood and blood products provided in Japan has been secured.

3C-S18-03

INFLUENCE OF WESTERN MEDICINE IN JAPANESE BLOODLETTING AND BLOOD TRANSFUSION THERAPY

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The ancient therapy of blood letting that was universal in the West traveled to Japan five hundred years ago on the trading vessels that carried physicians and barber-surgeons to care for the body and Christian missionaries to care for the soul. At that time, the West used the bloodletting of Galenic medicine to 'restore balance to the humours'. As early as 1670 one Japanese doctor noted that the Dutch were ''hot''and needed ''bloodletting from a basilic vein''whereas for Japanese, moxibustion sufficed. During this period, bloodletting was not a therapy in the East. The Japanese were using both moxibustion and the acupuncture of the Chinese to 'restore the balance of flowing energy'.

By 1641 Japan had adopted a rigid policy of national seclusion and suppression of Christianity. The shogunate granted a trading monopoly to the Dutch East India Company that restricted it to the island of Deshima (Dejima), Nagasaki. However, Western medications and especially surgery continued to be used in Japan. The importation of Dutch books was authorized in 1720 excluding those referencing Christianity. One of the Deshima interpreters Kogyu Yoshio (1724–1800) developed a major interest in medical texts as they arrived and became an eager student of

the Company doctors and an advocate and teacher of Western medicine. His name appears frequently in the early Japanese story of Western medicine and he taught its bloodletting to his students. The Japanese blended blood letting into their use of Chinese acupuncture in the unique treatment form called Shiraku. A book 'Shiraku-hen' (1771) by Gengai Ogino illustrates bloodletting tools as well as Japanese needles. Ogino describes European cupping and leeching and the use of a ceramic bowl to collect blood in the manner of the barber-surgeons. Such bowls had been produced in the famous Arita porcelain for export to the West since late in the 17th century. In the late the 18th century, Shiraku became popular among the masses.

Kogyu obtained a book 'Chirurgie' by Lorenz Heister in a Dutch translation. Others translated it partially into Japanese and in 1819 a Japanese book was published with the figures and legends of Heister, showing not only bloodletting but also early concepts of blood transfusion. Thus, Japanese in Edo period already knew of transfusion therapy.

In 1868, Japan opened itself to the West and specialists were invited to assist in the modernization of Japan. An 1876 book on Pathology by Henry Hartshorne, a Professor at the University of Pennsylvania, illustrates transfusion methods. However, the first successful blood transfusion in Japan was only performed in 1919 and from there its history will be described.

An understanding of those transitions in medicine affords an understanding of the exchanges that occur between world cultures. In Japan the transition continues and has led to advanced practice of blood transfusion and apheresis today.

3C-S18-04
HISTORY OF HEMOPHILIA

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Background: If a woman has her first son circumcised and he dies from bleeding and then has her second son circumcised who also dies from bleeding then she need not have her third son circumcised (Rabbi Judah the Patriarch - 2nd century AD).

Aim: To study the history of diagnosis and treatment of hemophilia

Methods: Search through different web sites to compare the different aspect of bleeding disorders.

Results: The first modern description of hemophilia is attributed to Dr. John Conrad Otto, a physician in Philadelphia, who in 1803 published a treatise entitled 'An account of a hemorrhagic disposition existing in certain families.' He clearly appreciated the three cardinal features of hemophilia: an inherited tendency of males to bleed. However, the first use of the word 'hemophilia' appears in an account of the condition written in 1828 by Hopff, a pupil of Schönlein at the University of Zurich.

Early treatments: - The first hint of success came with the report from R.G. Macfarlane in 1934 that snake venoms could accelerate the clotting of haemophilic blood, and he reported success in controlling superficial bleeds in people with hemophilia after topical application

Blood transfusion A report from a surgeon, Samuel Lane, in The Lancet in 1840 described the control of post-operative bleeding with fresh blood in a boy with severe hemophilia. However, a lack of understanding of blood groups and basic transfusion methods hindered further development at that time

Plasma concentrates In the early 1950s, plasma from animals was used for treatment.

The work of Dr. Edwin Cohn in developing fractionation of plasma with variation of temperature and concentrations of saline and alcohol led to the development of fairly crude plasma concentrates of human factor VIII in a number of centers ('antihemophilic globulin').

A truly major advance was the discovery by Dr. Judith Pool in 1965 that slow thawing of plasma to around 4 CO led to the appearance of a brown sediment which was rich in factor VIII, which she called cryoprecipitate. The availability of such products facilitated home treatment. Another landmark was the recognition by Prof. Pier Mannucci in 1977 that desmopressin (DDAVP) could boost levels of both factor VIII and von Willebrand factor, and this remains a useful option in mild patients

Recombinant products and gene therapy The structure of the factor VIII gene was cloned in 1984. This led to the availability of recombinant (genetically engineered) factor VIII a decade later. The availability of safe products has stimulated the growth of prophylactic treatment

Summary The rate of progress continues apace. However, we do not forget that many people with hemophilia around the world still receive absolutely no treatment. Perhaps the current position can best be expressed in words paraphrased from Sir Winston Churchill: this is not the end of our struggle to conquer hemophilia, and not even the beginning of the end. However, we can at least say that this is the end of the beginning of our campaign.

Wednesday: Parallel Session S19: Massive Transfusion and Major Haemorrhage

4A-S19-02

EVALUATION OF TRANSFUSION PRACTICE IN MASSIVE HAEMORRHAGE

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Background: Massive transfusion is a predictable response to uncontrollable haemorrhage in various clinical settings. Transfusion management policy in patients with massive blood loss is often empirical although recent studies indicate that red cells, plasma and platelets should be administered in a 1:1:1 ratio to mimic the composition of fresh whole blood. It is not known whether this approach is better than patient specific transfusion protocols based on clinical parameters guided by laboratory investigations.
Aim: We evaluated, retrospectively, our transfusion policy which was not based on any pre-defined protocol for massive haemorrhage.
Methods: An electronically linked database of blood and blood products usage with clinical outcome of patients who had massive haemorrhage was developed for the period 1998 to 2006. During the study period there was no formal massive transfusion protocol. Patients were managed following individual clinical and laboratory assessment. Three time periods, years 1998–2000, 2001–2003 and 2004–2006 were compared to evaluate the transfusion practice and outcome. The prevalence of microvascular bleeding (MVB), a clinical indicator of severe coagulopathy and its correlation with coagulation parameters was also determined.
Results: Three hundred and seven patients who had received more than 10 units of red cells in the first 24h were identified. The median number of units of red cells transfused during the first 24h was 15 (interquartile range 12–21), units of fresh frozen plasma 6 (interquartile range 4–11), and platelet units was 2 (interquartile range 1–3). Thirty patients received cryoprecipitate and five patients received recombinant factor VIIa. The overall prevalence of MVB was 31% and was statistically associated with abnormal activated partial thromboplastin time and thrombocytopenia. The prevalence of MVB was 36%, 22% and 35% and mortality was 37%, 31% and 19% respectively during the three time periods. The overall fresh frozen plasma : red cells (FFP: RC) ratio at 6h was 1:2.8 and at 24h was 1:2.3. The pre-intensive care FFP: RC ratio was 1:3 as compared to the intensive care FFP: RC ratio of 1:1.
Conclusions: With a transfusion policy based on clinical parameters and guided by serial monitoring of laboratory tests, there has been improved survival over the nine year review period. Introduction of a specific massive transfusion protocol to enhance easy access to blood products in conjunction with the use of rapid point-of-care coagulopathy screening tests and judicious use of recombinant factor VIIa may further improve the outcome.

4A-S19-03

RECOMBINANT FACTOR VIII CONCENTRATES: A COMPARATIVE PROTEOMIC ANALYSIS

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Background: Haemophilia A (HA) is an inherited blood coagulation disorder whose replacement therapy is based on plasma-derived (pd) or recombinant factor VIII (rFVIII) concentrates (CS).
Aims: The objectives of this study were to compare the heterogeneity of high-purity rFVIII preparations of commercially available products used in the treatment of HA.
Methods: Helixate NexGen (HN) [octocog alfa, CSL-Behring], ReFacto (RF) [moroctocog alfa, Wyeth] and Advate (AD) [octocog alfa, Baxter] were submitted to comparative proteomic analysis. 4–12% mono-dimensional gel electrophoresis (1D SDS-PAGE) was performed on rFVIII CS and rFVIII fragments generated by thrombin (Th) digestion. rFVIII CS were analyzed also by two dimensional gel electrophoresis using for the first dimension 17 cm pH 4–7 IPG strips with the BioRad' Protean IEF Cell isoelectric focusing system. For the second dimension, 11% polyacrylamide large gels were used.
Differentially expressed spots were excised and digested with trypsin, peptide mixtures were separated using a nanoflow-HPLC system. Peptides were eluted directly into a High Capacity ion Trap (model HCTplus, Bruker-Daltonik, Germany). The scan range used was from 300 to 1800 m/z. Proteins were identified through the National Centre for Biotechnology Information non-redundant database using the Mascot program.
Results: Initial 1D SDS-PAGE comparison of rFVIII samples revealed similar banding patterns between recombinant factors, showing three major species at approximately 90 kDa, 80 kDa and above 170 kDa. The fragments generated by Th digestion from all the rFVIII CS, showed a different SDS-PAGE profile in comparison to those deriving from pdFVIII (data not shown). In particular rFVIII samples showed two different bands at 40 and 43 kDa, identified as the A2 domain. Interestingly, the 40 kDa band (absent in the pdFVIII - data not shown) seems to be peculiar to rFVIII. In addition 2D IEF-SDS-PAGE followed by electrospray ionization tandem mass spectrometry was performed to look for impurities deriving from the manufacturing process of rFVIII CS. Two proteins were identified from 2DE maps of HN: Human Haptoglobin (HH) and Hsp70. HH is a cell culture medium component present in the fermenter [Jiang R et al, Haemophilia 2002], whereas the Hsp70 is a molecular chaperone overexpressed to increase the rFVIII secretions from the baby hamster kidney cells [Ishaque A et al, Biotechnol Bioeng 2007]. On the contrary RF and AD 2D maps did not show the presence of these contaminants.
Conclusion: Our preliminary data demonstrate that proteomic approach may allow the quality control and the molecular characterisation of different rFVIII CS, thus providing a tool for valuable insights into the mechanisms underlying their immunogenic potential. Specifically, our preliminary results show that the purification steps of biotech manufacturing processes of HN seem to be less effective in comparison to those of RF and AD.

4A-S19-04

ANALYSING PATTERNS OF BLOOD UTILISATION FOR EARLY IDENTIFICATION OF A MAJOR HAEMORRHAGE IN A TERTIARY TEACHING HOSPITAL

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Timely management of a major haemorrhage is vital to improve probability of survival. Early recognition is key to subsequent intervention, where the sooner the event is managed then the total sum of blood components required is generally less. The term 'Massive Transfusion' is commonly accepted as meaning a transfusion of 10 units of packed red cells (pRBC) within 24h. Identifying a major bleed with the potential to develop into massive haemorrhage would lead to early blood component dispatch times and thus blood component support. As a large tertiary referral centre the Royal Free hospital has approximately three acute major blood loss events per month. We decided to determine whether a request for 6 units of red cells could be used as a trigger or adjunct to initiate a massive transfusion protocol (MTP).
We looked at retrospective data showing blood component usage per patient for the year 2008. We collected all cases where a request for six, or a total of six, pRBC units had been made within 6h, and the units had been transfused within 24h.

Only 1/34 (3%) cases had begun with an initial request for 10 pRBC units; in contrast most cases 20/34 (59%) began with a request for 6 units.
In 62% (21/34) of the cases there were massive transfusions i.e. patient transfused 10 or more pRBC units. The largest proportion of these cases 62% (13/21) occurred when the initial request was for 6 units.
To see if the amount requested reflected the actual amount transfused the data was divided into two categories of less than 10 units pRBC used versus 10 or more pRBC units used. The amount used relative to the total requested was slightly greater in the higher user group when 10 units or more had been requested (83%) compared to when less than 10 units had been requested (74%).
The data clearly shows initial requests for 10 units of pRBC for bleeding patients are not as common as calls for less than 10. Triggering a MTP based upon an initial request for 10 units of pRBC would have missed the opportunity for early intervention of blood component support in 97% of cases.
Regarding amount of units 'requested' compared to 'used', this was only slightly different between the high and low user groups. Measures to reduce potential wastage can be used such as the use of validated cool boxes.
To conclude, the request for 6 units of pRBC for a bleeding patient is a useful alert for identifying a potential major haemorrhage within the hospital, and the local massive transfusion policy should be adapted to incorporate this trigger.

4A-S19-05

CLINICAL USE OF CRYOPRECIPITATE OR FIBRINOGEN CONCENTRATE TO MASSIVE HEMORRHAGE DURING SURGERY FOR THORACIC AORTIC ANEURYSM, LIVER TRANSPLANTATION, AND HEPATOMA/PERIHILAR CHOLANGIOCARCINOMA

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Background and aims: Massive hemorrhage during surgery may often come from diluted coagulopathy due to loss of coagulation factors (e.g., fibrinogen), especially in cases of thoracic aortic aneurysm, liver transplantation, and hepatoma/perihilar cholangiocarcinoma. The most important issue to prevent massive hemorrhage during surgery would be a transfusion therapy for hemostasis. This study analyzed the hemostatic efficacy of cryoprecipitate or fibrinogen concentrate during surgery when massive hemorrhage occurred.
Patients and methods: When massive hemorrhage occurred in cases of thoracic aortic aneurysm, liver transplantation, and hepatoma/perihilar cholangiocarcinoma, we measured the fibrinogen level in plasma, and then, administered cryoprecipitate (i.e., corresponding to 15 units of fresh frozen plasma) or 3g of fibrinogen concentrate to the patient when the fibrinogen level was below 150 mg/dl. The hemostatic efficacy of this treatment was evaluated by counting volume of blood loss and transfusion units in comparison with cases of treatment with fresh frozen plasma.
Results: We observed rapid increases in plasma fibrinogen level and the following improvement of hemostasis after cryoprecipitate or fibrinogen concentrate was administered. The average of blood loss decreased by 30% and the average of transfusion units was reduced about 30 to 60% when those agents were given to the patients with severe hypofibrinogenemia during surgery. The number of cases of early death after surgery due to massive hemorrhage decreased by 75% since fibrinogen concentrate was used.
Conclusions: When the patient shows severe hypofibrinogenemia (i.e. <150 mg/dl) during surgery, the administration with fibrinogen concentrate should be effective for good hemostatsis, and therefore, that results in decreases in blood loss and transfusion units. This treatment should contribute to better prognosis of patients of surgery for thoracic aortic aneurysm, liver transplantation, and hepatoma/perihilar cholangiocarcinoma and to reduction of transfusion volume as well.

Wednesday: Parallel Session S20: Novel Developments

4A-S20-02

ARTIFICIAL PLATELETS AND PLATELET SUBSTITUTES

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Transfusion of platelet concentrates from blood donation is currently the only means of efficiently treating or preventing bleeding caused by quantitative platelet disorders. Particularly for platelets among blood components, the product supply is always in a critical situation because of the short shelf life (4 days in Japan, 5-7 days in the United States and Europe) under stringent storage conditions. Moreover, the risk of blood-borne infections (particularly bacterial sepsis) and acute immune reactions remains a major concern for allogeneic platelet transfusion.

To overcome the limitations and drawbacks inherent to standard platelet products, a number of rigorous attempts have been made to develop alternative substitutes to fresh platelets, including the use of materials derived from outdated platelets such as fixed or lyophilized whole cells or cell membranes, and platelet function-supporting synthetic products (artificial platelets) such as ligand-coated albumin particles or liposomes.

In 1992, Coller et al conducted an extensive *in vitro* study of fibrinogen-derived synthetic peptide, arginine-glycine-asparatic acid (RGD)-coated erythrocytes (thromboerythrocytes) as a possible platelet substitute. platelet aggregation is mediated by fibrinogen by bridging adjacent platelets through integrin $\alpha IIb\beta 3$ (GPIIb/IIIa) in an activation-dependent manner. Several sequences in fibrinogen have been designated as GPIIb/IIIa recognition sites: the RGD-based sequences 95RGDF98 and 572RGDS575 in the Aα chains; and 400HHLGGAKQAGDV411 (H12, the fibrinogen γ-chain dodecapeptide) in the carboxyl-terminal of the γ-chain. In fact, thromboerythrocytes have been shown to form mixed aggregates *in vitro* with platelets via preferential interaction with GPIIb/IIIa on activated platelets suitable for a prototype platelet substitute. The next generation of artificial platelets was then developed using human albumin particles as carriers of fibrinogen or fibrinogen peptides. In 1995, Yen et al produced fibrinogen-coated albumin microspheres (Thrombospheres; mean diameter, 1.2 μm), which were shown to be hemostatically active in thrombocytopenic rabbits. In 1999, Levi et al extensively tested their fibrinogen-coated albumin microcapsules (Synthocytes; mean diameter, 3.5–4.5 mm) as a platelet substitute, showing hemostatic efficacy *in vivo* in severely thrombocytopenic rabbit models.

In 2003, we started to develop synthetic platelet substitutes based on a strategy of using liposomes (mean diameter, 0.22–0.26 mm for each) as a carrier vehicle and synthetic H12 peptides as a surface-coating ligand to target activated platelets. To strengthen the hemostatic ability of H12-coated vesicles as a platelet substitute, we exploited installation of a drug delivery function by encapsulating a potent platelet agonist, adenosine diphosphate (ADP) into liposomes.

In this talk, with an overview on the current status of platelet substitutes including artificial platelets, the successful development of a novel synthetic platelet substitute, H12-coated, ADP-encapsulated liposome will be reported, which behaves like fibrinogen and platelets to exert hemostatic functions at sites of vascular injury, but not in circulation.

4A-S20-03

RANDOMIZED, DOUBLE BLIND, PLACEBO-CONTROLLED PHASE III STUDY OF WEEKLY ADMINISTRATION OF DARBEPOETIN ALFA IN ANEMIC PATIENTS WITH LUNG OR GYNECOLOGIC CANCER RECEIVING PLATINUM-CONTAINING CHEMOTHERAPY

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Background: Anemia is a frequent complication in cancer patients receiving chemotherapy. Darbepoetin alfa (DA) has hematological efficacy with less frequent treatment for patients with chemotherapy induced anemia.

Aims: The purpose of this randomized, double-blind, placebo controlled study was to evaluate the efficacy and safety of weekly administration of DA in anemic patients with lung or gynecologic cancer receiving platinum-containing chemotherapy.

Methods: Anemic patients (hemoglobin [Hb] $\leq$ 11.0 g/dL) with lung or gynecologic cancer receiving platinum-containing chemotherapy were randomized in a 1:1 ratio to receive DA 2.25 μg/kg or placebo, administered subcutaneously once a week for up to 12 weeks. Study drug was withheld if a patient's Hb concentration increased to greater than 13.0 g/dL (amended to 12.0 g/dL during the study). We evaluated the proportion of patients who reached the transfusion trigger (Hb $\leq$ 8.0 g/dL or received a red blood cell [RBC] transfusion) from week 5 to the end of the treatment phase as a primary endpoint. The hematological endpoints, adverse events, and survival were also assessed for all patients who received at least one dose of the study drug. Proportion of endpoints was calculated using Kaplan-Meier method.

Results: 207 patients (103 DA vs. 104 placebo) received at least one dose of the study drug. Baseline patient characteristics were similar in both treatment groups. Fewer patients receiving DA reached the transfusion trigger (28.5% vs. 60.1%, difference = 31.6%; 95%CI = 18.2% to 45.0%; P < 001) than patient receiving the placebo. The subpopulation analysis suggested that DA showed more efficient in the population who had a baseline Hb range of 8–10 g/dL compared with the whole population. The proportion of the patients who received RBC transfusions were lower in DA group compared with placebo group (7.2% vs. 19.2%, difference = 12.0%; 95%CI = 2.5% to 21.5%; P = .015). The proportion of patients with a hematopoietic response (ΔHb $\geq$ 2.0 g/dL or Hb $\geq$ 12.0 g/dL) was higher in the DA group (55.0% vs. 20.5%, difference = 34.5%; 95%CI = 22.2% to 46.9%; P<0.001) than placebo group. The safety profiles were similar between the groups. There was no difference on survival between the groups.

Conclusions: DA decreased the incidence of anemia with Hb concentration less than or equal to 8.0 g/dL or requiring transfusion. DA can support anemic patients with cancer receiving platinum-containing chemotherapy without adverse clinical effects and influence on survival.

4A-S20-04

EXOSOMAL-LIKE VESICLES WITH IMMUNE MODULATORY FESTURES ARE PRESENT IN HUMAN PLASMA

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Background: Exosomes are small membrane vesicles that are secreted from a variety of cell types into various body fluids including the blood,

urine, breast milk. These vesicles are thought to play a role in cell-cell interactions.

Aims: In the present study we investigated whether exosomes from human plasma might contribute to immune regulatory function.

Methods: We isolated vesicles from human plasma by differential ultracentrifugation and ultrafilter. The vesicles were identified both by electron microscopy (EM) and phenotypically by western blot. Then we examined the immune modulatory ability of exosomes incubated with CD4+T cells.

Results: We found that these vesicles display similar shape, size as those of previously described exosomes and packed CD63, CD81 molecules. They also expressed molecules such as MHC-?, co-stimulate molecules CD80 and CD86, Intracellular Signal Molecules Wnt3a, Wnt5a and FasL. We performed the biochemical characterization by flow cytometry after immunoisolation with CD63-coated latex beads. Furthermore, function analysis show that exosomes from plasma suppress proliferation of resting CD4+T cells or activated CD4+T cells and induce them apoptosis. Moreover, CD4+T cells with low concentration were inhabited by plasma exosomes with or without IL-2.

Conclusion: human plasma contains exosomes with the capacity to influence immune responses.

Wednesday: Parallel Session S21: Blood Donation

4A-S21-01

DONOR COMPLICATION AND DONOR CARE -VASOVAGAL REACTION AND PREVENTIVE MEASURES-

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Vasovagal reaction (VVR) is the most common adverse reaction associated with blood donation, and we have analyzed risk factors and suggest preventive measures for VVR.

Donor complications In Japan, there were 5,137,612 donations in 2008, and the incidence of all donor complications was 1.16% (male 0.87%, female 1.76%). The distribution of complications was; VVR 76.8%, hematoma 16.5%, local pain 2.3%, citrate reaction 0.9%, nerve injury 0.8% and others 2.8%.

Risk factors of VVR The incidence of VVR with respect to donation types, sex and ages was evaluated. The incidence was significantly higher for donors age 16 to 29 than for older donors for both sexes and all donation types, except for the female apheresis group in which the incidence was higher also for donors age 45 and older. The multivariate statistical analysis was performed to assess risk factors for donors at the JRC Tokyo Metropolitan Blood Center. The VVR risk was significantly higher for younger donors, female donors, those with a low BMI, predonation tachycardia, and having had less sleep. The risk was lower with 200 mL donations than with other donations.

Late phase VVR We also studied the incidence of VVR after leaving donation sites for 400 mL whole blood donors using a questionnaire. We found that 5.22% of donors (3.6% for males, 9.6% for females) felt symptoms such as discomfort, dullness or dizziness. Syncope occurred in 0.13%. At higher risk for late phase VVR were younger, female, and first time donors, similar to immediate type VVR. Donors with late phase VVR were significantly more likely to have had a previous incidence of VVR than donors without late phase VVR.

VVR and autonomic nerve function We studied changes in autonomic nerve function by inducing VVR in volunteers using the Schellong test, and analyzed heart rate variability with electrocardiographs. The Schellong test after blood donation induced VVR in 45% (9/20) of the volunteers. There was a tendency for the vagal nerve function to be more dominant in the VVR group than in the non-VVR group, but not significantly. It is difficult to recognize donors with a higher risk of VVR before blood donation.

Preventative measures for VVR A decrease in circulating blood volume by phlebotomy and an imbalance in autonomic nerve function inducing hypotension and peripheral ischemia are considered to be causes of VVR. Thus, drinking water before and after donation is a universal practice. As stress and tension make the autonomic nerve function unstable, then the vagal nerve function may become dominant and induce VVR.

Consideration shown to the donor with explanations in a relaxed atmosphere before the donation, and enough rest afterwards are necessary, particularly for first time and younger donors. It is also necessary to inform donors of the possibility of fainting, the causes of it and measures which should be taken to avoid it, including what to do should symptoms occur, to avoid serious incidents. Donors who have experienced VVR repeatedly should refrain from making blood donations.

4A-S21-02

DONOR CARE, THE CHANGE WE NEED!

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Background: Safe and adequate blood supply is the ultimate goal of blood transfusion services. Apparently, donor retention is more cost-effective than donor recruitment. Furthermore, repeated blood donors showed lower prevalence of transfusion transmitted infection than first time donors. At present, approximately 70% percent of blood donors, at National Blood Centre, the Thai Red Cross Society were repeated blood donors. Therefore, donor retention is considered the key success of blood safety and adequacy.

Aims: To focus on ''donor care, the change we need''by decreasing the number of two main adverse effects of blood donation which were fainting and anemia in blood donors.

Materials and methods: 1.) To prevent blood donors from fainting, donors were asked to drink approximately 400 mL (four glasses of 100 mL) of water within 20 min. prior to blood donation. The donors were closely observed during and after blood donation for any adverse effects. Information and education regarding the useful effect of water on blood donation were provided to the donors by poster, brief presentation and VDO presentation. The findings were recorded and analyzed.

2.) To prevent blood donors from anemia, the study group of 435 subjects were deferred from blood donation due to anemia (Hemoglobin < 12 g/dL, for females and < 13 g/dL for males).

Among these, there were 370 females and 65 males and 80% were repeated donors. 100 tablets of ferrous fumarate (66mg elemental iron) 3 tab/day were prescribed for each subject. Hemoglobin was determined using Automatic hematology analyzer (Cell Dyn 1700, Abbott Diagnostic) and serum ferritin was determined using Immunoassay (AxSYM Ferritin Reagent Pack, Abbott Diagnostic). Counseling on nutrition and iron supplementation was provided to each individual.

3.) Both were in the period of months.

Results: 1.) It was observed that the percentage of fainting after donation was decreased from 11.01% to 0.16% within 8 months in the study group.

2.) Baseline, 80 (18.39%) of 370 of females and 7(1.61%) of 65 males were first time donors while the rest were repeated donors. One hundred and seventy-one cases (40.52%) were iron deficiency anemia (female, Hb<12 g/dL, male, Hb<13 g/dL, serum ferritin< 12 ug/L), while 29 cases (6.87%) were non-anemic iron deficiency (normal Hb, serum ferritin< 12ug/L).

After iron supplementation: Hb and serum ferritin in females and males were significantly increased. Among these, 41.15% were able to return for blood donation after one month onward.

Conclusion and recommendation: 1. Sufficient water intake before blood donation will significantly decrease fainting due to vasovagal adverse reaction in blood donation.

2. There is an increase risk of iron deficiency anemia due to blood donation which is more evident in females. Iron supplement of 100 tablets (66mg elemental iron) is usually sufficient per one donation. However, Serum ferritin should be monitored once a year in order to maintain serum ferritin level up to 60 ug/L.

4A-S21-03

IMPACT OF CHIKUNGUNYA INFECTION ON BLOOD SUPPLY IN SINGAPORE

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Background: The Chikungunya virus (CHIKV) is transmitted by Aedes mosquito. The name ''chikungunya'', comes from the Swahili for stooped walk, reflecting the physique of a person suffering from the disease. The disease has been described in Africa, Asia, Italy and occurs mainly during the rainy season.

Aim: To study the impact of CHIKV fever on the collection of blood in Singapore from Sep to Dec 08.

Materials and methods: There was a rise in cases of CHIKV fever in Singapore and steps were taken to identify the source, mode of transmission and the dynamics of the disease. Blood donation criteria were amended and precautionary blood safety and self-sufficiency measures were adopted by the ministry of health, National Blood Centre, to minimize its spread and transmission.

A supplementary questionnaire was introduced where donors had to tell their travel history to the endemic areas in the past 2 weeks and whether

they had any fever or flu or whether they came in contact with any person suffering from it

Donors who had a travel history to the affected areas, and staying there overnight were deferred for a period of 2 weeks, whereas, those coming back within the same day were allowed to donate but the units were quarantined. Donors who had flu or fever were deferred for 2 weeks and 3 weeks respectively.

Later, donation criteria were modified and donors who have visited overseas hotspots and were well were stopped being deferred. However, those unwell, were deferred for 2 weeks from the date of recovery (3 weeks if fever). Donors who had a history of close contact with a person with CHIKV were deferred for 2 weeks from the last date of contact and finally, donors who had a history of CHIKV fever were deferred for 6 months after complete symptom resolution and cessation of treatment.

Results: The precautionary measures adopted in the blood system affected the collection of blood components. Total collection of blood over the period from Sep 09 to Dec 09 was 30159 units, and the total deferrals made were 7409. Out of these, 810 units were deferred for CHIKV due to travelling history to affected areas and approximately 1589 units were quarantined, 400 cases were deferred with symptoms of flu, 400 cases with fever, and two cases with diagnosed CHIKV fever earlier. Out of the units quarantined, 112 units were discarded because either the donors called back for development of fever or they were diagnosed with CHIKV. % deferral for CHIKV was 10.93% and % lost due to same was 2.68%.

Conclusion: CHIKV outbreak poses considerable problems for public health authorities, who need to take measures for vector control and record epidemiological surveillance to deal promptly with the potentially severe effects of an outbreak and for national emergency plans to sustain the blood supply, so as to target for adequate blood stocks and, when affected areas require a significant blood inventory to meet with the demands.

4A-S21-04
ALTERATION OF HBA1C LEVEL WITH AUTOLOGOUS BLOOD COLLECTION

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Background: Hemoglobin A1c (HbA1c) is the marker of diabetes mellitus (DM), and it is usually used as the following index. HbA1c is well known to decrease in the situation of large bleeding or the shortage of red blood cell life span. Autologous blood (auto-blood) collection may affect the HbA1c level; and give rise to the bias in the management of DM patients. However, the precise alteration level of HbA1c by collecting auto-blood is not well understood.

Aims: We investigate the alteration of HbA1c level with auto-blood collection to find out the bias level.

Methods: We carried out the prospective analysis at the Blood Transfusion Division, Kobe University Hospital from the January to June of 2009. Among all patients to collect the auto-blood during the above period, we select the patients fulfill the following criteria: 1) planning to collect auto-blood in more than two times, 2)blood sugar > 125 mg/dl, or HbA1c > 5.8 % at the first auto-blood collection time. Hemoglobin (Hb), HbA1c, glycoalbumin (GA) was measured at the point of each auto-blood collection. The alteration of Hb, HbA1c, GA was expressed as ?Hb, ?HbA1c, ?GA. The correlation with?Hb, and ?HbA1c was expressed as ?(GA/HbA1c). The influence of the intake of ferrous (Fe) medicine (six patients) on ?(GA/HbA1c) was analyzed.

Results: Nine patients (male; six, female; three) were selected. The mean age was 64.9 (52–73), the mean volume of collection blood was 506 (200–800) mL, and the mean collection interval was 14.7 (7-25) days. The mean value of ?Hb, ?HbA1c, ?GA were -0.87(decrease), -0.3(decrease), and 0.26 (increase), respectively. The mean ?(GA/HbA1c) in all nine patients or Fe-intake six patients were 0.17(increase), and 0.24(increase), respectively. Although the student's t-test analysis between Fe-intake and non Fe-intake groups was not significant (=0.079).

Summary/conclusions: Auto-blood collection affects the decrease of HbA1c. We find out the trend to decrease ?(GA/HbA1c) extensively in Fe-intake patients. When standing operation with collecting auto-blood, GA seems to be better for the management of DM.

Wednesday: Plenary Session Clinical Transfusion

4B-PL5
PATIENT BLOOD MANAGEMENT – A NEW PARADIGM FOR TRANSFUSION?

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The development of many modern therapies such as high dose chemotherapy, transplantation and complex surgery has been facilitated by the availability of blood product support. For many decades the focus has been on ensuring the safety and quality of the product. Much of the use of blood has not been based upon science, but on tradition and anecdotal experience. A variety of factors are, however, combining to challenge current practice.

Blood is a precious resource and pressure on supplies will only heighten in future due to the ageing population. Costs have continually increased due to advances in collection, testing and processing. The costs of the administration of transfusion to patients are often overlooked. It is estimated that the overall costs of transfusion make up 5% of the total health service budget. Risk of infection following transfusion has been dramatically reduced; however there has been little change in the recognised non infectious adverse consequences such as transfusion associated acute lung injury, haemolytic and allergic reactions. It is sobering to see that the largest category of significant hazards of transfusion is still due to human error. Most worrying though, is the increasing literature demonstrating a strong association between transfusion and adverse outcomes. These include increased length of stay, post operative infection, morbidity and mortality. A dose response relationship also appears to be present. A recent international consensus conference on transfusion outcomes (ICCTO) concluded that there was little evidence of efficacy of transfusion: alternatives should be adopted and transfusion avoided wherever possible. Ethically patients must be made aware of the potential hazards of transfusion and its risks and benefits for them so that they can make informed choice about their management.

Consensus guidelines for blood component therapy have been developed to assist clinicians and these have long advocated more conservative 'triggers' for transfusion. However significant variation in practice has still been demonstrated, with likely inappropriate transfusion. The 'blood must always be good philosophy' continues to permeate clinical practice.

An alternative approach, however, is being adopted in an increasing number of centres. Experience in managing Jehovah's Witness patients showed that complex care without transfusion was possible and results were equal, if not better, than those of transfused patients. The approach of 'blood management' grew from here. Principles include optimizing erythropoeisis, reducing surgical blood loss and harnessing the patient's physiological tolerance of anaemia. Treatment is tailored to the patient, requires a whole team approach and employs a combination of modalities. Results have demonstrated reduction of transfusion, improved patient outcomes and patient satisfaction. Significant healthcare cost savings have also followed.

Despite the success of patient blood management programmes and calls for practice change, the potential and actual harm to patients caused through inappropriate transfusion is still not sufficiently tangible for the public and many clinicians. This has to change. The medical, ethical, legal and economic evidence cannot be ignored. Patient blood management is good medical practice and more importantly, good medical common sense.

4B-PL6
TRANSLATIONAL RESEARCH: AN IMPORTANT INTEGRATED PARADIGM FOR TRANSFUSION MEDICINE

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Translational research (TR) has many different potential definitions. There are two broad elements: one is research that serves as a bridge between basic science and the development/use of a therapeutic product/intervention; the second (also known as knowledge translation) explores why a clinically established product is not used more widely and/or further characterizes its optimal use. The first TR element has always been an integral part of transfusion medicine and has previously been referred to as ''applied research''or ''development''. TR includes animal model research, and Phase I, II, and I/II clinical trials using human volunteers to determine safety and start evaluating efficacy of a novel therapy; some TR definitions also include definitive Phase III randomized controlled trials. TR usually needs a different infrastructure than basic research; funding may require public (government or university) and private (commercial companies) partnerships. Conducting TR requires a strong understanding of regulatory requirements and often necessitates the establishment of national or international multi-center research programs supported by sophisticated information technology and a clinical coordinating center well versed in these disciplines. To this end, the National Heart, Lung, and Blood Institute in the U.S. not only offers its usual funding mechanisms [e.g., R01s, R21s, P01s)] to investigators interested in conducting TR but has also developed several other funding mechanisms [e.g., the Specialized Centers of Clinically Oriented Research (SCCOR) and Clinical Trials Networks (such as the Transfusion Medicine/Hemostasis Clinical Trials Network) to better support and coordinate such activities. There are multiple examples of successful TR in transfusion medicine. An historical example is the licensure and clinical use of RBCs in different preservative solutions. More contemporary examples of successful TR efforts in the area of technological advances are nucleic acid testing (NAT) for infectious disease agents and the use of monoclonal antibodies in immunohematology. Scientific questions addressed both historically and currently by animal model research include red cell alloimmunization, graft versus host disease (GVHD), transfusion related immunomodulation (TRIM), and transfusion related acute lung injury (TRALI). In the area of product development, pathogen inactivation (PI) of blood components has been the subject of recent TR efforts leading to the use of PI platelet and plasma products in many countries. Conversely, TR into blood substitutes has spanned more than two decades with disappointing early phase clinical trial results and the need to return to early phase TR (animal model research). A new area of TR in transfusion medicine is the production of functional mature RBCs from cultures of embryonic stem cells. The issue of how to best conduct TR still remains; challenges include finding mechanisms to adequately fund technological and product development, overcoming regulatory obstacles to the licensing of new technologies, and designing and funding large clinical trials to answer many of the remaining questions about transfusion triggers and best transfusion practices. The knowledge translation component of TR offers additional challenges; these require a different set of investigator skills and infrastructure in order to evaluate the effectiveness of interventions that can change clinical practice.

POSTERS
1.1. Management and Organisation
Blood Transfusion as an integral part of national health systems

P-001

PROFILE OF BLOOD TRANSFUSION SERVICE BY INDONESIAN RED CROSS

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Background: Indonesia has a big five island (Sumatra, Java & Bali-NTT-NTB, Kalimantan, Sulawesi & Maluku, Papua) which amount of width 5.500 km and height 1.880 km, for blood service its established and controlled by Indonesian Red Cross (IRC) then supported by government.
Aim: Blood service is one of health service in Indonesia, the activity containing of recruitment donor, collection, blood processing, blood storage and distribution. All Blood Transfusion Service (BTS) gives the activity report every three month to Central Blood Transfusion Service (CBTS), then CBTS to do the audit and evaluation for the activity.
Method: Indonesia has 212 BTS and one CBTS, a number percentage of BTS is 20% in Sumatra, 57% in Java & Bali-NTT-NTB, 10% in Kalimantan, 11% in Sulawesi & Maluku, and 2% in Papua. From all report will be appear the profile of blood service.
Result: Place of BTS 76% outside of hospital and 24% inside of hospital, for the personnel is, 3% recruitment donor, 12% medical doctor, 45% blood transfusion technician, and 40% administration. The location for collection blood is 45% on inside BTS, 52% by mobile unit on outside BTS, and 3% on inside government hospital. Totally donation by voluntary donor and replacement donor on 2006 was 1.560.919, increased the number every year around 15%. Blood donation based on blood group on 2006 was 25% A, 28% B, 39% O, 8% AB then based on the Rhesus system was 99.95% positive and 0.045% negative. In 212 BTS 24.5% has already to produce blood component ± 2.4 million blood bag per year. For used of blood on 2006 was 17% surgery, 34% antenatal case, 41% internist, 8% others case. For the Transfusion Transmitted Infection (TTI's) BTS do the test about Syphilis, HBsAg, anti-HCV and anti-HIV by elisa method & rapid test and also Malaria for some area endemic, BTS using the WHO strategy, its screening only with reagent has 99% sensitivity and 98% specificity, then for reactive result must be confirm to national reference laboratory. Transmitted Transfusion Infections (reactive result) percentage on 2003-2006 was ±0,67% syphilis, ±1,8% HBsAg, ±0,48% anti-HCV, ±0,08% anti-HIV. For blood cost its responsibility the patient, its around USD12 up to USD25 and the government help for some TTI's reagent. The main problem of blood service in Indonesia is less of support by government about financing and management.
Conclusion: "Safety blood will be created only with good quality and good management". Blood service is one of health service of government responsibility, to establish a good blood service is not easy because its must be accountable and professional then following the new technology. Supported by government is important, and also all stake holders who using the blood. Good relationship makes a good quality of blood service, therefore will be decrease of number mortality that caused of blood service.

P-002

STUDY OF PRESENT INFRASTRUCTURE SYSTEMS IN IRANIAN BLOOD TRANSFUSION ORGANIZATION (IBTO) CENTERS

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Background: Human societies have always been exposed to natural disasters and unnatural events that have caused irreparable damages to these societies. The role of infrastructure systems like electrical energy, emergency electrical energy supply, water supply, cooling, heating, lighting and telecommunication systems in human life and specially those centers and organizations established to serve people are not ignorable. Therefore, as the health centers and hospitals are responsible for protection and promotion of physical and psychological health of human people after each disaster especially in Iran as it is placed under such area, the importance of infrastructure systems get prominence.
Aim: The aim was to collect all major information about infrastructure systems like electrical energy, emergency electrical energy supply, water supply, cooling, heating, lighting and telecommunication in 30 regional blood bank centers.
Methods: Iranian Blood Transfusion Organization (IBTO) was established in May 1974 and presently is functioning with 30 regional blood centers throughout the country to ensure a safe and sufficient blood supply. IBTO within a period of 12 months (from 21 Mar 2008 to 20 Mar 2009) had a total collection rate of 1,731,238 blood units. In order to achieve the major information about the present condition of the mentioned systems to prevent any malfunction in activities in blood centers during each event, the data of 38 important factors (16 factors pertaining emergency electrical energy supply, 10 to water, nine to cooling, heating and lighting and three to telecommunication systems) that could be essential at each event; thereafter the data are collected for at least 22 centers analyzed by SPSS.
Results: The results obtained for 28 blood centers showed that only 15.3% (six centers) contained the secondary supply stoker and 96.6% of centers have emergency electrical energy generator. There were no emergency electrical energy generators equipped with secondary supply stoker. 73.2% of blood centers were equipped with portable emergency electrical energy generator. 17.2% of blood centers were equipped with cool chain blood using services other than electrical energy, 37.4% with emergency water tanks, only 14.2% with more emergency water tanks, 11.6% with transportation equipment for emergency water tanks, 6.9% with access to underground waters. 10.3% of blood centers' heating and cooling systems were equipped with secondary supply stoker. 79.7% of cool chain blood centers with warning devices were equipped with emergency electrical energy and 52.4% of telecommunication and telephony networks with emergency electrical energy.
Conclusion: Investigation on the status of blood centers' infrastructure covering 28 regional blood centers are under process and the findings will be presented with poster in Nagoya, Japan. The preliminary studies about these systems in 28 centers provided us a good knowledge on how to apply them in natural and unnatural disasters.

P-003

JICA-KENYA NBTS BLOOD SAFETY PROJECT; OVERVIEW

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Background: To address the issue of blood safety, coordination and blood utilization, the Ministry of Medical Services through the NBTS in collaboration with Japan International Cooperation Agency (JICA) started implementing the project known as "Blood Safety Project"in October 2006.
Project purpose: Approaches for safe,, appropriate and efficient use of blood products are developed, demonstrated and applied as national standard.

Methods: The Project started in October 2006 and will end in October 2009. The Project expects four main outputs during the three-year implementation.
(1) The linkage, communication and information sharing between BTS institution and hospitals are strengthened. (2) Small volume packed red cell for children are safely prepared at RBTC (Regional Blood Transfusion Center) Nakuru (3) Improve logistics and inventory management of blood products in RBTC Nakuru, model hospitals and non-model hospitals in Nakuru Region and the system is introduced to other regions in Kenya. (4) Blood products are safely and appropriately used in model hospitals.
Results: (1) Project Implementation Meetings and RBTC supervisory visit to hospitals for logistic management have strengthened the linkage, communication and information sharing among NBTS (National Blood Transfusion Services), RBTS Nakuru and three model hospitals. HTC (Hospital Transfusion Committee) has promoted them among a hospital lab and wards in each hospital. (2)A decision was taken to prepare one single small volume (125 mL) of packed red cells (PRC) for use in children. The pilot for the preparation and use of small volume packed red cells was started in 2007 at Nakuru PGH and now utilized in three model hospitals in Nakuru. Several SOPs and charts have been developed to assist the clinicians better prepare the PRC. (3)The concept and theory of logistics and inventory management, as maximum/minimum stock levels by blood group, and many kinds of manuals and checklists, have been introduced for the first time in Kenya and validated through supervisory visits by RBTC Nakuru staff. (4) The project has established HTCs in all model hospitals. Some of the achievements of the HTC are establishing and meeting regularly, having action plans, developing tools for monitoring blood use and discussing adverse reaction of blood transfusion. The HTCs have managed to develop the following tools to facilitate proper management of transfusions at the hospital; Cross-match register, Blood requisition form, Blood transfusion register and Blood transfusion observation chart. A hemovigilance manual was drafted in April, 2009 and is still in process of authorization. Supply of PRC was started from RBTC Nakuru to Provincial General Hospital in February, 2009 and whole blood used previously has been almost replaced by PRC.
Summary and conclusions: JICA/Kenya NBTS Blood Safety Project was successful in achievement of each output in the model site. In near future, it is expected to sustain these achievements to expand in nation-wide scale of Kenya.

P-004
INFORMATION TECHNOLOGY FRAMEWORK FOR TERRITORY WIDE BLOOD UTILIZATION – A CASE STORY IN HEALTH CARE PLANNING

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Ageing population in developed countries together with low birth rate emerges as an important determining factor in health service planning. It is well observed that demand for geriatric health service has been growing rapidly with longer life span. Simultaneously, blood demand is also noted to be increasing despite attempts in blood conservation and technological improvements. Unfortunately, expansion in blood collection is not an easy task as blood donor pool is in general shrinking with increasing difficulties in blood collection under today blood safety standards. Therefore, it is pertinent to develop strategies to ensure adequate and safe blood supply and these in turn should rely on timely and accurate information obtained from the existing health care system.
In Hong Kong, Hospital authority provides > 90% public health service. Over the past 3 years from 2006 to 2008, the blood demand has been significantly increased by 8.6%. With this, we present here our initiatives in the development of an information technology framework for analysis of the territory-wide blood utilization and provide a modeling platform in determining future blood demand.
In short, the information technology framework pools data from different information system (patients' demographic, disease categories, relevant haematological data, surgical operation related data and transfusion records) into a consolidated database for analysis. With this, we are able to identify a significant increase in utilization trends among elderly medical patients. However, even in a small city like Hong Kong, it is noted that elderly surgical and orthopaedic patients also accounts for a significant rise in certain regional hospitals.
In summary, the information technology framework enables health care provider in timely analyzing blood utilization trends and provides important information for demand modeling. Further explorative analysis is being undertaken to identify variation of clinical practice and its role in future auditing.

P-005
EXPERIENCE OF THE NATIONAL MICROBIOLOGY REFERENCE LABORATORY IN PREPARING QUALITY CONTROL SAMPLES

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Background: It is mandatory for any Blood Transfusion Service (BTS) to develop a quality control (QC) system that is required to ensure the overall effectiveness of the blood screening program. Quality Control is considered a corner stone in implementing an effective quality system; it refers to the measures that must be included during each test run to verify that the test is working properly. Commercial QC samples are available, but poor delivery logistics and limited financial resources might present an obstacle for continuous supply especially in developing countries; thus, the alternative solution is to prepare homemade samples.
Aim: Producing sufficient QC samples to be continuously available for evaluation of different screening assays that are in use within the NBTS.
Methods: For each screening assay that is in use, a specific QC sample should be prepared as follows:
1. Selection of positive blood units:
   Confirmed positive (4–5) blood units in the specific required marker, which are either high positive for its plasma to be used by serial dilutions or low positive to be used without dilution.
2. Preparation of diluents:
   Confirmed seronegative blood units, its plasma units are selected to be used as diluents by pooling, noting that plasma units used in preparation of HBsAg QC diluents have to be screened for Anti HBs.
3. Preparation of the QC samples:
   Serial dilutions are prepared from selected high positive units. The prepared dilutions are then processed using specific screening assay in order to select the matched 2–3 positive blood units. Samples from the matched units are pooled together, processed without dilution and with serial dilutions using the same assay. The dilution at which the Signal/Cutoff (S/CO) ratio is around 3 is selected to prepare the QC samples specific for this screening assay.
4. Calculation of the results:
   Prepared QC sample has to be tested using the specific screening assay for at least 40 runs. The ratio is calculated in each run, then the mean and +/− 3SD are calculated and plotted on westgard chart.
5. Stability testing:
   Stability testing of the prepared QC samples is done by keeping it in different storage conditions, the samples are processed in at least two runs per week for one month, if any sample turned to be negative, then stop testing and stability should be considered at the last reasonable S/CO result.

Results: In the period from June 2006 till May 2009, the prepared QC samples were as follows:
- For HBsAg screening assays, seven lots were prepared.
- For HCV screening assays, six lots were prepared.
- For HIV screening assays, six lots were prepared.
- For syphilis screening assays, three lots were prepared for particle agglutination assays and another three lots were prepared for EIAs and

Trponema Pallidum Hemoagglutination assays. Each lot of prepared QC samples consists of 500 aliquots.

Conclusion: Our attempt for producing the homemade QC samples helped us to bypass the obstacles of purchasing commercial ones and the consistency in the production helped competent implementation of a good QC system.

P-006

THE IMPACT OF GOOD LABORATORY PRACTICE IN TRANSFUSION MEDICINE

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Background: Blood transfusion service in our country was mainly hospital based pattern, due to the fact that some blood donations in remote parts of the country were tested using poor quality kits, blood testing centralization was declared by the ministry of health on March 2007,Thus there were 27 testing centers all over the country instead of 199. Consequently the blood testing practice will follow the same standards, strategy & protocol.

The serology department (SD) & the quality assurance unit of the National Blood Transfusion Center (NBTC) in Giza as the headquarters of the Egyptian National Blood Transfusion Services (NBTS) played a key roll in full & consistent implementation of such a plan.

Aim: To monitor overall laboratory performance and to assure that the test results generated by the laboratory are accurate and precise.

Methods: Establishing a laboratory quality system, extending the quality system to all tires (central, regional and district blood banks of the service network).

- Laboratory technical manual & manual for maintenance and calibration of equipments is to be followed.
- Extensive training program for all staff categories, The training program was planned at a basic level on weekly basis (5 days/week, 15 participants/course). The programs are conducted in theoretical, practical & on job training.
- Implementing a scientific based evaluation strategy for TTIs screening kits by the Microbiology reference laboratory (MRL) and ABO/D typing and antibody screening reagents by the Red cell reference laboratory (RCRL) at NBTC to be effective in proper assay selection determining the relative sensitivity and specificity.
- Also the MRL & RCRL provide a local quality control (QC) program to be used as go -no go controls in every assay, results were plotted on charts and were sent back to the reference laboratories to be analyzed.
- Regular internal audits (twice / year) a report with any non-conformance will be forwarded to the laboratory for action plan correction & scheduling a date for re-inspection.

Results: - The NBTC has gained credibility by World Health Organization & became center of excellence for education & training for the Easter Mediterranean Region.

- Audit reports of the SD revealed that there were 35 non-conformances in December 2006, 18 in May 2007, 8 in December 2007& 6 in April 2009 which indicates that the general quality status of the SD has been improved.
- 40 lots of different TTIs kits have been validated since 2006 till current.
- 22 lots of TTIs QC & 88 lots of ABO/D typing & antibody screening QC were made & distributed within the service network from year 2006 till current.

Conclusion: Implementation of a testing laboratory quality management improves not only quality of blood, blood products and services, but also helps to set free novel motivation in our employees, also it raises community confidence in the services provided by the blood transfusion services. Finally it offers the services a cost-effective way of saving money for research and development in future fields of transfusion.

P-007

ORGANIZATIONAL ISSUES AND OUTCOMES IN INDIAN BLOOD SERVICES: A SYSTEMS APPROACH

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Aims: (1)To describe the present blood banking system in India and understand its working using a 'systems' approach.

(2)To suggest an alternative model which alone will deliver the desired outcomes

(3) To suggest a framework action plan to initiate the alternative model.

Methods: Data about blood collection and issue is taken from published sources. A systems approach [Study of the nature of complex systems in nature, society, and science, based upon the idea that the whole is different from sum of the individual parts] is employed to understand the working of the present system, and to predict its behaviour.

The alternate model is proposed and its working explained using the systems approach, to bring out the way it will respond to the needs of the healthcare system.

Aim(2): For framework action plan [Aim (iii)], Government officials in two South Indian States have been initiated into a dialog to establish State Blood Services. Advocacy and concepts of change leadership were used to motivate Government officials into action.

Results: Aim (1) We found that the present system will behave in such a manner that will prevent the objectives of a good blood service from being achieved. That is due to the principle ''structure determines behaviour''.

Aim (2) We tested the proposed system using the same approach, and found that it will deliver the desired outcomes in an efficient and cost-effective manner. This is not surprising since similar systems are working well in many countries.

Aim (3) Since retro-fitting the new system into the existing system will be problematic, we conducted exercises with two State Government Health Services ie Kerala and Andhra Pradesh, to understand the issues involved. A feasible approach has emerged which can be employed in the country. The Armed Forces, hematologisits, hospital administrators, donor groups, representatives from the Disaster Management Agency and policy makers were invited to a workshop to discuss why blood services are a national priority. The outcome of the workshop was a collective decision that a Community Blood Center [CBC] catering to a certain geographical area be set up, one in each district of the State and a Blood Processing Center (BPC) for every three districts. An extensive distribution and logistic management system would link the CBC, BPC and nearby Hospitals. The patient would get the blood directly from the hospital and be saved the trouble of replacing every unit of blood used. A self sustaining system like this would ensure a steady stream of voluntary blood donors, who are deemed the most reliable source of safe blood the world over.

Conclusions: A National Blood Service ensuring blood security for every citizen is a basic right that should be ensured by the State. In India, the present structure is such that this is not possible. Citizens have to find the donors to get the blood transfusion they need! Unless the reasons for this are understood, spending more money will not solve the problem, as has been proved by the experience of the past couple of decades.

1.2. Management and Organisation Information technology

P-008

EFFECTIVENESS OF INTEGRATED BLOOD DONOR COMPUTER MANAGEMENT SYSTEM ON PROMOTION OF BLOOD SAFETY IN FIVE BLOOD TRANSFUSION CENTERS IN IRAN

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Background: The main duty of all blood services around the world is to ensure safe and adequate blood supply. To fulfill this goal, it is necessary to implement an Integrated Blood Donor Computer Management System in order to assimilate activities of all central databases of Iranian Blood Transfusion Organization (IBTO) across the country. Therefore, the use of an integrated database is necessary to make sure all procedures of blood collection and preparation are appropriate. It also enables us to trace all blood units through the collection, preparation, and distribution processes.
Aim: The purpose of this research was to identify the role and effectiveness of Integrated Blood Donor Computer Management System in five blood centers prior and after its establishment. In this research, we mainly focused on positive effects of registration of records of blood donors in databases.
Methods: In this research, 538669 blood donors before and 516914 after the implementation of the management system were tested by Elisa and confirmatory methods in five blood centers and their affiliated collection centers within the same city where the blood centers are located. The findings obtained were analyzed using Chi-square test ($P<0.05$).
Results: The percentage rates of HCV and HBS positive blood donors with Elisa significantly decreased from 0.48 and 0.55 before the system implementation to 0.31 and 0.45 afterwards, respectively; with confirmatory test these rates again showed a decrease from 0.12 and 0.47 to 0.10 and 0.34, respectively ($P < 0.05$). The percentage rate of HIV positive blood donors also decreased, but this decline was not significant ($P < 0.05$).
Conclusion: The above statistics indicate that Integrated Blood Donor Computer Management System work well in detection of permanent or temporary deferred donors who attempt to return for blood donation during their deferral period. Thus, this management system can act as one of the factors effective in ensuring blood safety. The use of this system in all blood centers across the country would certainly raise the blood safety index.

P-009

THE GATHERING OF THE ADVERSE EFFECTS INFORMATION USING THE ELECTRONIC MEDICAL RECORD

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From June 2002 we started to use the transfusion verification system by PDA. In this system we got the information of the adverse effects by handwriting report. After the first checklist report on the compatibility conformation sheets which should be return to the division of Blood transfusion medicine. In case of the occurrence of the adverse effects the detailed reports were made by the physician in charge and should be send to the division of Blood transfusion medicine. This report was occasionally rate and incomplete.
We have been using the Electronic Medical Record (EMR) in our hospital from JAN 2007.
In this EMR system we have gathered the information of the adverse effects by the electronic input system using the template forms. After adoption of this new system we acquainted the 100% of detailed information of the adverse effects instantly. Additionally the information was easy to read and be understood than before. [Conclusion] EMR system is useful to gather and analyze the adverse effects information. Because the template form can be made or revised by the EMR office in our institution, we do not need extra fee of EMR programming by the software house. In future we could construct the good Haemovigilance using EMR.

1.3. Management and Organisation Cost/effectiveness in BTS

P-010

COST OF UMBILICAL CORD BLOOD UNITS FOR MEDICINE AT THE OCCASION OF APPLYING GMP

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Background: Medical efficacy of transplantation of umbilical cord blood is acknowledged. However, it has not received the treatment as medicine. For medicinization, a level fixed about quality is maintained and security of safety is also required for it. Moreover, it is necessary to evaluate of the validity as the medical treatment method or economical efficiency. The occasion when umbilical cord blood units were medicinized and GMP (Good Manufacturing Practice) was applied, we calculated about the cost and considered economically for construction of the stable supply organization.
Methods: Based on data of 'Analysis investigation about the enterprise and management evaluation of the cord blood bank and the Japanese cord blood bank network' (2002 to 2003: K.Kawahara), and 'Surveillance study report about medicinization of umbilical cord blood (Inc. Nomura Research Institute 2006), we re-calculated the cost about exhibition of umbilical cord blood units and examined the cost of which was added to the present cost.
Results: To the cost of per umbilical cord blood unit in the present cord blood bank was 851,540 yen, at the occasion of applying GMP, the cost of per umbilical cord blood unit changed to 4981,061 yen. The Cost leaped up about 5.8 times.
Conclusion: The cost of per umbilical cord blood unit leaped up about 5.8 times by applying GMP. Strengthening of staff assignment and increase of the amount of office work, etc. were considered as a reason for a jump of the cost. In considering medicinization of umbilical cord blood, an economic factor is important and it is necessary to make a system efficient with streamlining of the cord blood banks

P-011

CURRENT BLOOD ORDERING PRACTICE AT THE NATIONAL HOSPITAL OF SRI LANKA: STEP TOWARDS A MAXIMUM SURGICAL BLOOD ORDER SCHEDULE (MSBOS)

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Background: At present a MSBOS is not implemented in Sri Lanka. Ordering for blood is frequently based on subjective anticipation of blood loss instead of evidence based estimates of average requirement. The excess ordering of blood affects the blood stock management, cost and the efficiency of the blood bank staff.
Aims: To assess the current practice of blood ordering for elective surgeries, at the National Hospital of Sri Lanka, and to justify formulating and implementing a MSBOS.
Method: We analyzed blood ordering and transfusion practice in 1185 elective surgeries done sequentially at the National Hospital of Sri Lanka. Crossmatch to Transfusion ratio (C:T) and Transfusion Index (TI) were used to analyze the data. C:T ratio of 3 and TI of 0.5 were considered to be significant.
Results: Out of 1825 crossmatches for 1185 surgeries (59 procedures), 363 (20%) were transfused. Laparoscopic cholecystectomy and total abdominal hysterectomy had a TI of zero. Amongst the other lowest blood utilizing procedures were thyroidectomy (C:T=42:1, TI=0.02), uretero/pyelolithotomy (C:T=23:1, TI=0.04), transurethral resection of the prostate/ bladder (C:T=16:1, TI=0.06), aspiration of chronic subdural haematoma (C:T=15.5:1, TI=0.06), vaginal hysterectomy & repair (C:T=15:1, TI=0.06) and laminectomy (C:T=13.3:1, TI=0.08). Also nephrectomy, mastectomy, ventricular peritoneal shunt, laparotomy and dynamic hip screw had a TI below 0.25. Altogether 18 common procedures with a TI < 0.5 were responsible for 51% (n = 605) of the total surgeries.
Summary and conclusions: Without a MSBOS, 80% of crossmatched blood is nonutilized. Common procedures has a low TI (TI<0.5). By implementing a type and screen (T&S) policy for procedures with a low TI more than 50% of the blood orders for elective surgeries could be managed without crossmatching. Formulating and implementing a MSBOS could avoid the over ordering and maximize the utilization of blood.

P-012

PREPARATION OF HOMEMADE EVALUATION AND VALIDATION PANELS OF TTIS TEST KITS

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Background: Accurate assessment during evaluation of the analytical sensitivity of TTIs test kits in the process of evaluation and validation requires specific commercial panels, which are expensive and of little volume aliquots. It is essential to any resource limited country to find an alternative to these commercial panels that provide adequate supply of evaluation and validation panels, and to be accessible to staff, to insure a quality focused approach to the provision of TTIs screening assays systems and reagents in the Egyptian national blood transfusion service. TTIs screening assays are systematically evaluated and selected; validation is done to every lot used from the selected assays.
Aim:
- Preparation of homemade panels which contain negative, positive, serial dilutions and Quality control (QC) specimens for use in the evaluation and validation of screening assays.
- Decrease the expenses needed in the evaluation and validation.

Methods: Panels are prepared to all markers (HCVAb, HBsAg, HIVAgAb and SyphilisAb); each panel is specified to one marker, composed of 120 samples and is divided into evaluation panel and stability panel.
Steps of preparation:
Step 1: plasma units are chosen after screening by ELISA and\or NAT tested according to the following criteria:
- Non reactive units for (HCVAb, HBsAg, HIVAgAb and SyphilisAb); they are used as negative panels and diluents for serial dilutions of positive units.
- Reactive units for (HCVAb, HBsAg, HIVAgAb and SyphilisAb) including strong, weak reactive.

Step 2: All samples are repeatedly tested by at least five different EIA test kits, chemiluminesence for (HCV Ab, HBsAg, HIVAb and/or HIV Ag Ab, and syphilisAb)
Step 3: Confirmation by line and blot assays is done for HIV and HCV reactive units and neutralization for HBsAg reactive units
Step 4: Serial dilutions of strong positive units were done.
Step 5: The evaluation panel contains 80 samples (24 negative samples, 16 dilutions, 2 QC samples, 18 weak positive and 20 strong positives)
Step 6: The stability panel contains 40 samples (12 negative samples, 8 dilutions, 2 QC samples, 8 weak positive and 10 strong positives)
Step 7: Coding is done according to year, marker, type of panel, and lab. Number of panel.
Results: Accurate assessment and evaluation of different screening assays took place in the Microbiology Reference Laboratory (MRL) of the NBTC since Jan2007 till June2009 using well characteristic homemade panels. Seven evaluation reports were developed, three of which were evaluation reports for screening kits that will be used in the NBTS, two evaluation reports were done for kits used in the primary and secondary confirmation done by the MRL of the NBTC. Two reports requested by the WHO for screening kits used by the Yemen Republic. Evaluation of new systems

introduced by the NBTS in screening or confirmation took place during the past two years.
Conclusion: Home made evaluation and validation panels prepared by well trained authorized staff of MRL are suitable for evaluating different test kits. The use of the Reference Panels provides a convenient resource to assess analytical sensitivity of different assays and different lots of the same assay.

P-013

CENTRALIZED NAT TESTING AT APOLLO HOSPITALS, CHENNAI, AND CONSOLIDATION OF NAT TESTING FOR OTHER APOLLO GROUP HOSPITALS IN SOUTH INDIA

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Background: Apollo Hospitals located in Chennai has adopted routine NAT testing for HIV-1, HCV, and HBV on all blood donations since January 2008. The Procleix Ultrio assay based on TMA technology and the semi-automated Procleix platform (Chiron-Novartis, Emeryville, CA) is used in individual donor testing (ID-NAT) format.
Aim: To extend NAT testing facility to other blood banks of Apollo group for maximum utilization of NAT laboratory set up at our center and to provide the additional layer of blood safety in the Apollo hospitals of the region
Method: After successful implementation of Ultrio assay in ID-NAT format, we proposed to extend the NAT facility to the transfusion services of Apollo Hospital, Bangalore, and Apollo Specialty Hospital, Teynampet, Chennai. Samples are collected and labeled in EDTA vacutainer tubes in duplicate at the blood donor collection site, adequately packed with coolants and sent by approved couriers to the Apollo hospitals, Chennai. The samples are transported at 2–10ordm; C. Samples received in the evening are processed the next day and those received in the morning are tested the same day. NAT reactive results are informed immediately by text message to the respective blood banks for immediate product quarantine. Detailed monthly reports are submitted electronically. Recently, Apollo Hospital, Madurai, has also decided to send their samples for NAT testing.
Results: From Jan 2008 to April 2009, we have tested a total of 24,530 samples, including 18,144 from Apollo Hospitals, Chennai, 4,697 from Apollo Specialty Hospitals, and 1,689 from Apollo Hospitals, Bangalore. In general, we found samples reached us in proper condition and were processed according to schedule. Overall, nine HBV NAT yield samples were detected so far, for a yield rate of 1 in 2,726. No HIV-1 or HCV yield case had been identified yet.
Conclusion: In our experience, NAT was effective in detecting window period and occult HBV infections, and the Procleix system had adequate throughput to manage 200 samples per day. We could consolidate NAT testing for Apollo Group hospitals in the southern region and maintain high level of standards. After 16 months of routine NAT testing, we can conclude that ID-NAT platform using the Procleix system is more than adequate for centralized NAT screening. It makes NAT screening cost effective, provides greater reach of the facility to small and medium sized transfusion services institutions in the region and ensures optimal utilization of the investment and resources. The easy and early availability of results after blood collection is critical for product inventory management. The centralized blood screening facility also contributes to better infrastructure, and more efficient operational and workforce management.

P-014

A DESCRIPTIVE STUDY TO ASSESS THE COST-EFFECTIVENESS OF ROUTINE BLOOD GROUPING IN ALL MOTHERS ATTENDING ANTENATAL CLINICS IN A TERTIARY CARE MATERNITY HOSPITAL IN SRI LANKA

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Background: Blood grouping is an essential antenatal investigation and routing blood grouping is done in all mothers attending De Soysa Maternity Hospital (DMH), Colombo which has an average of 1100 booking visits per month. Availability of documented blood group is not sought before carrying out the investigation. Average time spent by one doctor for grouping of all clinic patients is 4h per day and the average monthly expenses amount to Rs 60,000/= excluding the pay for the Staff.
Most of these mothers have a previous record of their blood group and it was felt that the assessment of blood group in all mothers attending the antenatal clinic was an unnecessary repetition.
Aims: Objective of the study was to evaluate the cost-effectiveness of limiting the routine blood grouping only to mothers without proper documentation of blood group and to evaluate the reliability of such records.
Method: A descriptive cross sectional study was conducted over a period of two weeks in De Soyza Maternity Hospital and data was collected from 580 consecutive mothers attending DMH antenatal clinics for the booking visit using an interviewer administered questionnaire. Patient's ability to recall the blood group and the details of previous records were documented. This was compared against the blood grouping done at DMH blood bank during this visit.
Results: 394 (67.9%) patients had a previous record of the blood group. Among the documents carrying patient's blood group 33.5% were original reports issued by the blood bank, 25.4% were original reports from private laboratories and 41.1% were diagnosis cards or local antenatal clinic cards with the blood group documented. In two patients this record was different from the grouping at DMH in this clinic visit ($P > 0.05$). 386 (66.2%) patients could recall blood group and only one patient recalled a wrong blood group ($P > 0.05$). 320 (55.2%) patients could recall the Rhesus factor and 3 patients were wrong in their answer ($P > 0.05$).
Conclusion: Blood grouping at the booking visit at DMH is required only in 32.1% of patients and most of the other patients are unnecessarily undergoing this investigation. An average monthly sum of Rs. 40,000.00 could be saved from changing over to the policy of performing blood grouping only on mothers without a reliable record of the blood group.
Therefore it is recommended that previous records of the blood group need to be reviewed at the booking visit and blood grouping should be done only in the mothers who do not have a reliable previous record of the blood group which will save a significant expenditure on unnecessary regrouping.

P-015

DEVELOPING EGYPTIAN NATIONL BLOOD TRANSFUSION STANDARDS

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Background:
- Previously the Egyptian Blood Transfusion Services (BTS) had not had specific standards to regulate the activities inside the blood banks.
- BTS had only one presidential law published since 1960 & not updated also they had some of ministerial clauses
- At the beginning of 2nd phase of the Egyptian Swiss project for restricting the blood transfusion services in Egypt, an audit was

conducted by Swiss Red Cross's (SRC) External Expert on the blood transfusion services (2005)
- At this time the need for developing Egyptian National standard became apparent & we started to develop our own standard

Aim: To assist blood transfusion services in their implementation of GMP requirements. The provision of the standard is applicable to all procedures in the blood transfusion centers that may affect product quality.

Methods:
- Work shop was conducted by SRC's External Expert with Key persons in National Blood Transfusion Center (NBTC) on how to develop standards.
- Quality staff started internet search to get copy of the international standards (AABB,GMP& South Africa Standards)
- Quality staff selected the main elements to be included in the standard and distributed these elements to Key persons.
- The Key persons put the details of these elements to be included as the minimum required for the different work area.
- Meeting of the Key persons was conducted for discussing the 1st draft of the standards.
- Adjusting the 1st draft by including all the comments of the Key persons & distribution of the 2nd drafts to all Regional Blood Transfusion centers(RBTCs) Directors
- Adjusting the 2nd draft by including the comments of Regional Blood Transfusion center's (RBTCs) Director
- The same sequence occurs several times & distribution of the drafts to:

All Stake holders for their comments
External Expert:
linguistic expert for English language
- Adjusting the 5th draft by including all the comments
- Printing the needed number of copies to be distributed
- Conducting one day workshop for promoting the Egyptian National Standards for blood transfusion practices to all stakeholders involved in blood transfusion practice, RBTCs Directors and NBTS Core Management Team.
- Publishing the Egyptian National Blood Transfusions Standards to all workers in blood transfusion
- Ministerial decree to reinforce the Standards
- Continuous program to advocate

Results:
- The document consists of 10 sections, 5 Annex and Glossary of QMT in 72 pages (A4 size)
- After 2 years of continuous work and discussions, The national standards for blood transfusion services were created and reviewed by many international and national experts, which is subjected to be reviewed every 3 years to be updated & works well
- All the staff in Blood Transfusions Services use the National Standards as a reference to them in their activities

Conclusions:
- The Egyptian National Blood Transfusion Standards were created to standardize performance in blood banks, which is the basis for the provision of safe blood
- National Standards were designed to form a basis for assessment of the Blood Transfusion Services in Egypt

P-016
COST ANALYSIS IN BLOOD TRANSFUSION. IS IT ESSENTIAL!

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National Blood Transfusion Service (NBTS) of Sri Lanka (SL) is a centrally coordinated transfusion service composed of the National Blood Centre and 75 Hospital Blood Bank. It is the provider of transfusion support to the both private and state sector. The cost per unit of blood and other blood products contribute immensely to the direct healthcare budget of Sri Lanka. This highlights the importance of conducting the operation of NBTS with a view towards cost effectiveness. Cost recovery analysis play a major role in the sustainability of the organization, as there is significant cost associated with various procedures in Transfusion Service from Donor recruitment up to Transfusion.

The study is being conducted with the aim of reviewing current average cost for a unit of blood and blood products transfused to a patient. (RCC 4.60 US $, Platele tApheresis Unit 180 US $, FFP US $ 4.60, RDP US $ 4.60, Buffy US $ 4.6, Cryo US $23, CM US $ 1.15), using available accounts or other financial records such as profit and lost statements, budgets, planning documents, audit reports and expenditure reports Classification of all activities is a critical step in creating a costing method in order to estimate cost of specific activities in blood transfusion service and hospital based blood banks.

Two main steps in designing a costing study are (1) Budget Planning and Financial Accountability (2) Evaluation and Cost effectiveness analysis. Following cost determinant factors to be considered.

(1) Cost of the blood bag
(2) Cost of the Reagents
(3) Cost for conduction a mobile drive
(4) Cost of the Critical consumables
(5) Cost for staff & staff training
(6) Cost of the TTI Screening assay
(7) Cost for infrastructure facilities
(8) Cost for vehicle & transportation
(9) Management cost

In conclusion we are aiming at ''non profitable''Blood Transfusion Service which is sustainable through achieving partial cost recovery via effective cost analysis and 100% voluntary non remunerated donor base.

P-017
LABORATORY COSTS OF PROVIDING BLOOD AND COMPONENTS IN A PUBLIC HOSPITAL BASED BLOOD BANK IN DELHI

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Background: The blood safety programme in India funded largely by donor agencies runs as vertically organized programme. The public and private hospital-based replacement donor system is widespread, supplemented by few NGO run blood banks. Different types of blood banks coexist, belonging to different organizations, with different sources of funding leading to wide disparity in cost. The private blood banks charge for cost recovery, the NGO blood banks take processing charges and the public hospital based blood banks provide blood and components free of cost. Evidence suggests that integration of the programme into the existing public health institutions would lower running costs. For an economically sustainable programme especially in the context of low-income countries, both options, i.e. of centralized services, or integration into the existing public health care system, need careful consideration. Therefore it is essential to estimate the cost incurred by these public institutions in providing safe blood and components.

Aims: This study was undertaken to evaluate the cost of laboratory resources needed to provide a unit of whole blood and component in a public hospital based blood bank in Delhi.

Methods: Cost of providing a unit of whole blood and component was evaluated in a retrospective observational study for the year 2008, in a 1000 bedded public hospital based blood bank. The total collection was 9.586 units, with 49.5% of the collection being separated into components. As the supply depended on replacement donors, and blood was utilized within the hospital, costs incurred by the activities of recruitment and distribution was not included. The methodology used had some similarities to the costing guidelines provided by WHO. The unit of output was the cost of a single unit of blood or component and it was assumed that all components had equivalent costs. The capital and the recurring costs were enumerated separately. The cost of building and maintenance were not included in the capital cost as the blood bank was part of a tertiary care public hospital. The recurring expenditure included staff salaries, consumables and miscellaneous items.

Results: Out of 11,894 donors screened, 9,586 were found to be fit for donation. A total of 16,790 units were issued which consisted of whole blood, packed cells, fresh frozen plasma and platelets. The cost of each unit of component was INR 935, equivalent to USD 19.5. Out of the total recurring expenditure, 60% was spent on staff salary, and 30% was spent on consumables.

Conclusions: The hospital-based system has an advantage over the centralized system as only the costs incurred by the activities of collection and processing are only involved. Cost related to donor recruitment and distribution is not included. In the absence of data on activity wise costs of blood transfusion services from India, it is difficult to ascertain the economic advantage between a centralized system and the hospital-based system. But it appears that despite policies advocating centralized transfusion services, the hospital-based replacement donor system is a cost efficient option in the context of low-income countries like India.

1.4. Management and Organisation Training and education

P-018

THE ROLE OF EDUCATION AND RESEARCH IN TRANSFUSION MEDICINE IMPROVEMENT

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Ongoing training and continuing education of the staff are crucial elements of an efficient blood transfusion service As there have been significant advances in the field of transfusion medicine i.e. blood components, advances in immunology, blood group serology, coagulation, microbiology and clinical application of blood transfusion, there is a strong need or regular educational programs for different categories of staff working in the blood transfusion centre (Boyce N, et al 2005). The most remarkable root causes of transfusion events during 1995–2004 were the lack of training and orientation (Elaine Osier Hathaway 2005). The image today of transfusion medicine personnel suffers for several reasons, with the lack of education and training of medical personnel being a major contributor. Thus, the most important goals IBTO pursues through its research and education activities are to make all the staff involved in technical affairs in all blood centers across the country informed on the most recent specialized scientific developments in the fields of transfusion medicine and transfusion sciences as well as to raise the knowledge of specialists, physicians, and paramedics about these sciences, pave the necessary ground for fundamental and applied research relevant to the causes of IBTO to be conducted, and make the suitable atmosphere for participation of IBTO staff in outstanding international and national scientific congresses. The most important research- and education-oriented endeavors in IBTO are put forward below.

Education Department

- establishment of training courses including educational target-oriented programs, workshops, and scientific-specialized seminars,
- preparation of scientific-specialized handouts, brochures and pamphlets aiming to upgrade the scientific and practical capabilities of IBTO staff at different levels,
- involvement in the educational programs of medical and paramedical students including residents and fellows of universities of medical sciences in the fields of transfusion medicine and transfusion sciences,
- initiative to hold MPH course within the domain of transfusion medicine.

Research Department

- Orientation of research projects,
- organization of regular research sessions,
- assessment and follow up of research projects,
- plans to make regular visits to blood centers across the country to exert control and surveillance,
- arrangement of research workshops,
- preparation of the necessary research grounds,
- research prioritization,
- plans to make research findings practical for the purpose of innovation and accomplishment in the domain of transfusion medicine and transfusion sciences,
- authorship, compilation, and translation of specialty books with the contribution of IBTO faculty members, specialists, physicians, and experts,
- preparation and incorporation of the most up-to-date scientific books and resources in IBTO central library with access to electronic books and journals.

P-019

ADHERENCE TO TRANSFUSION PROTOCOLS AND THE USE OF RECOMBINANT ACTIVATED FACTOR VII

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Background: Most hospitals have clinical guidelines for the off-label use of recombinant activated Factor VII (rFVIIa, Novoseven), primarily as part of a massive transfusion protocol. Over the past years rFVIIa has increasingly been used outside the approved indications in haemophilia with inhibitors and Glanzmann's Thrombasthenia, particularly in trauma, cardiac surgery and other critical bleeding episodes. Use in these areas remains controversial.

Methods: Monash University established the Haemostasis Registry in 2005 (with an educational grant from NovoNordisk Pharmaceuticals) to monitor the use of rFVIIa throughout Australia and New Zealand. More than 95 hospitals are contributing data to the Registry including all major users of rFVIIa in Australia and New Zealand. As part of the process of joining the Registry project, participating hospitals are asked to supply copies of their protocols for use of rFVIIa.

Results: Approximately 3000 cases of rFVIIa use have been reported to the Register. Major areas of use are cardiac surgery (~41%), other surgery (~17%) and trauma (~15%). The majority (77.3%) of hospitals have documented protocols for rFVIIa use. Many of these are similar and are centred around situations of massive transfusion. However, 64.5% of cases of rFVIIa use submitted to the Haemostasis Registry, from hospitals where protocols exist, do not conform with the numerical components of these protocols.

Conclusions: This is the largest dataset of rFVIIa cases published to date and can now provide greater insight into the actual rather than theoretical use of rFVIIa in Australia and New Zealand. Lack of compliance with hospital protocols for rFVIIa use indicates either that the protocols do not reflect actual and appropriate use or that clinicians need to be further educated regarding what is currently considered appropriate use. In the absence of sound clinical trial evidence, consensus regarding appropriate use has not been achieved. In these circumstances, data from the Haemostasis Registry continues to be important in elucidating the safety and efficacy of rFVIIa and providing important feedback to doctors and hospitals.

The Haemostasis Registry is funded through an unrestricted Educational Grant from NovoNordisk Pharmaceuticals Pty Ltd. None of the authors have a financial interest in the outcomes of the study.

P-020

THE ROLE OF MEDICAL TECHNOLOGISTS ON THE EDUCATION OF TRANSFUSION MEDICINE: RESULTS OF MULTI-INSTITUTIONAL SURVEYS FROM THE 21ST TRANSFUSION CONFERENCE OF UNIVERSITY HOSPITALS IN JAPAN

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Background: The education of medical students and residents is important to carry out blood transfusion safely and appropriately. However, the number of educational instructors is limited in medical departments in Japan. The current roles of medical technologists in the education of blood transfusion have not been well elucidated, therefore, it is relevant to examine the current situation in transfusion medicine in university hospitals.

Method: Inquiry surveys were designed for staff in transfusion units of university hospitals. The surveys were done as a part of the collaborative studies for the 21st Transfusion Conference of University Hospitals in Japan. They included the number of instructors and the roles of medical technologists as well as content and time period of education. A self assessment of the educational outcomes was also reported. Additionally, problems in the education of transfusion medicine were filled out in free style.
Results: We got returns from 58 institutions out of 89 institutions as of July 1, 2009. In addition to lecture style education, small group training of medical students and residents was adopted in 51 (88%) and 31 (61%) institutions, respectively. Medical technologists play varying roles in the small group training of students and residents in 31 (53%) and 25 (43%) institutions, respectively. The training time period of students and residents were 4.3 +/− 3.7 hours (mean +/− SD) (range, 1–22h) and 1.8 +/− 0.8 hours (0.5–4 hours), respectively. The small group training of students and residents includes blood typing (96% and 97%, respectively), cross-matching (78% and 67%, respectively) and analyses of erythrocyte irregular antibodies (22% and 13%, respectively).
Conclusions: This study revealed that medical technologists in transfusion units have taken a considerable role in the education of transfusion medicine in Japan. There are several problems including training time and number of staff. Due to limited human resources and time, we believe that a greater collaboration between educational instructors and members of other medical centers is needed to improve the standard of transfusion medicine in Japan.

P-021

UPDATE ON TRAINING IN CLINICAL RESEARCH RELEVANT TO INTERNATIONAL TRANSFUSION SAFETY

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Background: In lower income countries, research in epidemiology, virology, and immunology relevant to blood safety has been limited by a lack of trained clinical research personnel. While this may be addressed by longer-term training in higher income countries, the number of such trainees is limited by cost and long absence from primary job responsibilities. We reasoned that an in-country short course could help to develop methodological skills on study design and data analysis relevant to blood safety.
Aims: Develop and implement a 2-week research training course with the following objectives: a) give an overview of research in blood donor selection and laboratory testing methodologies currently utilized to minimize the risk of TTI's; and b) provide practical training in epidemiology and clinical research methods which will allow the trainees to design and conduct studies to improve transfusion safety at their own blood centers.
Methods: The 2-week course is intended for professionals already working in blood transfusion who wish to pursue clinical research in their own blood center. Experts in transfusion medicine and epidemiology teach the epidemiology of TTI's, as well as the principles of clinical research. Lessons are linked with readings from a textbook and illustrated with examples from the experts own research or relevant literature. Each trainee must bring to the course a research question relevant to his/her local blood bank. During the afternoon workshops, the trainees develop this research question into a 5- or 6-page research protocol. After the course, trainees are encouraged to submit applications for funding and/or accomplish their projects with the support of their local blood bank.
Results: The table summarizes locations and number of trainees for research training courses conducted from 2004–2009. A total of 95 trainees have participated in the courses, with each producing a finished research protocol by the end of the course. Nineteen trainees have obtained funding for their project, either through their local blood center, North-South collaborations or a small grant program. Several projects have been completed, with at least 10 abstracts presented at international meetings. Five trainees have participated in a subsequent manuscript writing course at UCSF and at least six papers have already been published or in press at peer-reviewed journals.

Course History and Number of Trainees

Date	Place	Trainees
May 2004	Sao Paulo, Brazil	9
Sep 2005	Buenos Aires, Argentina	13
Oct 2006	Tegucigalpa, Honduras	7
May 2007	Paris, France*	7
Oct 2007	Johannesburg, South Africa	12
May 2008	Paris, France*	10
Sept 2008	Belo Horizonte, Brazil	16
Oct 2008	Durban, South Africa	10
May 2009	Paris, France*	11
	TOTAL	95

* Institut Pasteur; trainees from Francophone Africa

Conclusions: After only 5 years, a short course in clinical and epidemiological research has already been instrumental in stimulating blood safety research in developing countries, and in promoting North-South collaborations. Future plans include obtaining funding for masters and doctoral level training of promising candidates, and starting a web-based system for networking and communication.
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P-022

EVALUATION OF RESULTS OF NATIONAL EXTERNAL QUALITY ASSESSMENT SCHEME FOR BLOOD GROUP SEROLOGY IN SRI LANKA

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Background: National Blood Transfusion Services (NBTS) of Sri Lanka (SL) provide an essential and life saving support within the Government and private health care system in SL. Effective Quality Management System is a must to ensure sustainability and provide safe, appropriate and compatible transfusions. Quality Management Section (QMS) of NBTS of Sri Lanka has taken initial steps to establish the National External Quality Assessment Scheme for Blood Group Serology (NEQAS-BGS) by carrying out the pilot study in 2004. Thereafter the panels prepared by QMS are distributed annually to laboratories in Hospital Based Blood Banks (HBB'S). NEQAS-BGS is an effective way to maintain the quality in Blood Transfusion serology. The scheme aim to improve the standards of participating HBB laboratories by providing individual performance details, overall analysis of the results and education through reports and workshops.
Aim: Analysis of compliance and error rates of the NEQAS-BGS results obtained by participants in year 2005, 2007 and 2008.
Methods: Retrospective study was conducted using result sheets and performance evaluation reports of NEQAS-BGS 2005, 2007 and 2008. Following seven parameters were taken to compare the compliance and error rates: (1) Testing Gap. (2) ABO typing. (3) D typing. (4) Direct Antiglobulin Test(DAT). (5) Antibody Screening(AS). (6) Cross matching(XM). (7) Percentage(%) participants.
Results: Testing Gap is 4.36 days, 4.43 days and 3.57 days respectively in year 2005, 2007 and 2008 respectively. Results are encouraging and

demonstrating the gradual decrease in testing time. The highest participation rate is in 2005(100%). Overall error rates for ABO typing is 13 03%, 26.99%and 37.55% respectively. Results for D typing are gradually improved giving the best result in 2008(error rate 0%). Error rates for DAT is 10.78 %(2005), 3.44(2007) and 6.6 %(2008). Highest error rate for AS is in 2005(26.5%) and the lowest is in 2008(16.37%). Error rates in XM is 22.84%, 15.32% and 21.64% respectively.

Conclusion: Although the compliance to the NEQAS-BGS program has increased with respect to the testing gap, percentage participants has been reduced over time. Educational message through reports and workshops appeared to have an effect on the certain tests that are carried out, such as D typing, DAT and Antibody screening.

This analysis highlights: (1) Importance of motivating, the participating laboratories.

(2) Importance of conducting more workshops and discussions for HBB's to improve their techniques, procedures and understanding about subgroups and ABO discrepancy resolution.

(3) Quality assurance and Quality monitoring of locally prepared blood group reagents and NEQAS samples.

Year	Testing Gap(Days)	% Participants	Overall Error rate				
			ABO	D	DAT	AS	XM
2005	4·36	100	13·03%	0·06%	10·78%	26·50%	22·84%
2007	4·43	93·33	26·99%	2·26%	3·44%	17·34%	15·32%
2008	3·57	75	37·55%	0%	6·60%	16·37%	21·64%

NEQAS (BGS) PERFORMANCE ANALYSIS

P-023

BLOOD SAMPLING PRACTICES FOR TRANSFUSION STUDIES – A CROSS SECTIONAL STUDY AMONG THE NURSING OFFICERS OF THE NATIONAL HOSPITAL OF SRI LANKA (NHSL)

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Introduction: Collection of blood for transfusion studies is an important initial step in the process of blood transfusion as misidentification of patients and inappropriate sampling can lead to fatal immunohaemolytic reactions and waste of resources. Taking this in to consideration a comprehensive set of guidelines on blood transfusion practices including specimen collection was developed by National Blood transfusion Service (NBTS) of Sri Lanka in 2006.

Aims: To asses the knowledge and practice of specimen collection for transfusion studies among nursing officers (NOs) of NHSL.

Method: A cross sectional descriptive study was among 200 randomly selected nursing officers from all medical and surgical wards of NHSL was done using a pre-tested self administered questionnaire on specimen collection practices based on the clinical guidelines by the NBTS.

Results: Prior to collection of samples positive patient identification by name was done by 72% while age and address were checked only by 58% and 50%. Cross checking and confirmation of the above details with the bed head ticket was done by 94%. All three criteria for positive patient identification were fulfilled only by 25%. Nurses with a formal training in transfusion practices fulfilled the criteria more than those who were not trained, but this difference was significant only when checking the address and the age ($P < 0.05$)

Although 94% did the phlebotomy at the bedside a previously labeled bottle was used in all occasions. 20% of the NOs admitted that sometimes the labeling was done by a person other than the phlebotomist. 94% of the NOs knew that blood for cross matching was collected to plain bottles, but 28% and 14% were not aware of the type of bottle used for neonatal grouping and investigation of transfusion reactions. Only 50% of the NOs correctly knew the minimum amount of blood needed for the routine cross matching and only less than 48% correctly knew the amount of blood to be collected for neonatal grouping and investigation of transfusion reactions. Only 36% of the NOs have received a formal training in collection of blood for transfusion investigations and out of them 72% have received it in the form of lectures while only 28% had ward teaching or workshop training. 36% of the NOs have gained knowledge from co-workers while only 10% were trained by blood bank medical officers.

Majority (52%) agreed that formal training in blood collection and transfusion practices is needed.

Conclusion: Positive patient identification before phlebotomy and knowledge in specimen collection was poor and adherence to the guidelines by NBTS is minimal. Formal training programmes by the blood banks will be useful for improvements.

P-024

TRANSFUSING MEDICAL SCIENCE INTO MEDICAL PRACTICE: THE ESTM EXPERIENCE

Rossi U

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Scientific progresses in Transfusion Medicine (TM) are often reported in many international and national congresses and journals.

Professional 'transfer' of the inherent scientific knowledge, however, hasn't reached all concerned medical specialists yet. Provision of basic information on TM at medical undergraduate level is urgent in most European countries, being often still not considered in the medical curriculum. Recognition of TM as an own postgraduate speciality teaching, and inclusion of adequate knowledge of clinical transfusion practice in all curricula of other postgraduate specialisations, are absolutely necessary measures to ensure a satisfactory professional level. TM training of nurses is often neglected.

However, the initiatives taken in the last two decades in the whole of Europe, by the ISBT, by some National Scientific Societies and by the ESTM have considerably helped establishing some network of TM professional communication in Europe: acting also as a stimulus for countries of other continents.

Out of 79 ESTM courses performed in 28 countries in the last seventeen years, on several topics of TM, 49 were dedicated to clinical transfusion practice.

Agreement was reached on basic requirements, essential to build an acceptable safety of blood donation and transfusion: clear definition of TM; sufficient number of dedicated TM specialists; presence of TM competence in other specialists, nurses and technicians; well functioning organisation of voluntary donation; feeling of belonging to a national and European medical community; proper cultural approach to blood safety and risk management.

It became clear that all medical and transfusion problems of Europe need to be addressed not only by national regulations, but also by a common 'transversal', regional approach. Measures are needed to ensure that countries with limited resources could develop faster, to reduce the 'quality gap' as soon as possible.

Migrations, and business and tourist travelling from high-income to low-income countries are increasingly frequent in Europe, and the occurrence of travellers receiving unsafe emergency transfusion has become correspondingly higher: potentially disrupting, once back, the expensive epidemiological barrier erected in their high-income home-country. No decision on blood safety in any European country should then anymore be taken without considering its reflexes on the whole of Europe: a generalised minimal blood safety is far more important than an isolated maximal but vulnerable one.

One cannot see any moral, medical or political meaning in the fact that sophisticated technological investments could absorb most of the available financial resources, at the same time leaving no funding for essential investments in far more important basic requirements for constructing a

safe transfusion system: such as promoting voluntary blood donation, educating donors, informing the public, training TM specialists, rationalising a cost-effective organisation of Blood Transfusion Services.
Appreciation has been reached of the critical relevance of the contribution of clinical medicine to transfusion safety: phasing out replacement donations, publicly promoting voluntary donation, contributing to scientifically clarify medical misconceptions hindering blood donation.
It is being felt all over Europe that 'Transfusing medical science into medical practice''is the real present challenge and common responsibility in TM, requiring a long-lasting human commitment by anyone involved.

1.6. Management and Organisation New techniques

P-025

IDENTIFYING AREAS FOR INCREASED SAFETY AND OPERATIONAL EFFECTIVENESS WITH LEAN SIX SIGMA TOOLS AND AUTOVUE? INNOVA INSTRUMENTATION IN AN ACUTE GENERAL HOSPITAL TRANSFUSION SERVICE IN HONG KONG

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Background: Key concerns for transfusion services are similar, no matter in which country those transfusion services are located. Customer focus, service levels, quality, errors, and costs are all shared concerns, and most are looking to automation to help consolidate testing platforms.
Aims: The study was aimed at using tools from Lean Six Sigma to uncover process improvement opportunities with the current work processes as well as with the application of the ORTHO AutoVue® Innova instrumentation for pretransfusion testing.
Method: Lean Six Sigma tools were used to assess work practices and identify improvement opportunities with and without ORTHO AutoVue® Innova automation (AVI). The main tools used were process mapping, product and operator value flow analysis, defect and error potential analysis and FMEA construction. Work practice assessment included the people, processes, equipment, physical space, and reagents and disposables required. The operations associated with tile, test tube and reagent preparation for ABO/D, antibody screen and identification by ORTHO BioVue®. Also captured and analyzed were specimen receipt, electronic compatibility testing, and blood product issue.
Results: Value analysis and error potential analysis revealed multiple areas for improvement that could be achieved by a simple layout redesign and consolidation of testing platforms with and without automation. Projected improvements with testing consolidation included 57% reduction in test result cycle times, 95% reduction in operator time, 84% reduction in process steps, and 98% reduction in error potential and total FMEA RPN (Risk Priority Number).
Summary: Lean Six Sigma tools offer an effective way to uncover significant opportunities for process improvement. When coupled with the application of the ORTHO AutoVue® Innova instrumentation, exponential improvements can be identified.

Summary of Projected Improvement Opportunities

Category	Test Cycle Time	Operator Time	Process Steps	Defect Opportunities	Total RPN
Current Process	70 min	752 sec	37	507	7,633
ORTHO AutoVue Innova	30 min	40 sec	6	10	129
Percentage Reduction	57%	95%	84%	98%	98%

P-026

EXPONENTIAL IMPROVEMENTS IDENTIFIED WITH LEAN SIX SIGMA TOOLS AND ORTHO AUTOVUE? INNOVA INSTRUMENTATION IN LARGE HONG KONG HOSPITAL TRANSFUSION SERVICE

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Background: Automation alone is never enough for highest quality pretransfusion testing without exploring process context and identifying ways to deliver value to customers faster and with less error potential. Lean Six Sigma tools offer ways to explore context and identify process improvements for increased efficiencies and process effectiveness.
Aims: The aim of this study was to evaluate the effectiveness of Lean Six Sigma tools to identify improvement opportunities in current processes as well as processes that incorporate ORTHO AutoVue® Innova instrumentation for pretransfusion testing.
Method: Lean Six Sigma tools were used to evaluate work practices and identify improvement opportunities with and without ORTHO AutoVue® Innova automation (AVI). The main tools used were process mapping, product and operator value flow analysis, defect and error potential analysis and FMEA construction. Work practice assessment included the people, processes, equipment, physical space, reagent preparation and the disposables required. Processes associated with tile preparation (ABO/D) and test tube reagent and tube preparation (antibody screening and identification) testing were captured and analyzed as well as order entry and specimen receipt, column agglutination technology testing, and compatibility testing, and blood product issue.
Results: Multiple areas for process improvement were uncovered with value analysis and error potential evaluation. Most of the improvements were achievable with layout redesign and consolidation of testing platforms with and without ORTHO AutoVue® Innova automation. Projected improvements with testing consolidation included 75% reduction in test result cycle times, 97% reduction in operator time, 88% reduction in process steps, and 99% reduction in error potential and total FMEA RPN (Risk Priority Number).
Conclusion: Lean Six Sigma tools were shown to be effective in identifying important process improvement opportunities. Exponential process improvements were identified with the application of Lean Six Sigma tools and the AutoVue® Innova instrumentation.

Category	Test Cycle Time	Operator Time	Process Steps	Defect Opportunities	Total RPN
Current Process	120 min	24 min	32	119	8,073
AutoVue Innova	30 min	40 sec	4	4	94
Percentage Reduction	75%	97%	88%	97%	99%

Summary of Projected Improvement Opportunities

P-027

INVESTIGATION OF THE QUICK AND QUALITATIVE STERILITY TEST FOR BLOOD COMPONENTS

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Introduction: In Japanese blood program, the sterility test for blood is proceeding with the random sampling by the culture method. But this method has a demerit that is time consuming as 2 weeks testing period. For example, the period of validity for PC is 4 days, consequently, the test result is able to obtain after its transfusion. Therefore, the development of

quick sterility method is expected. The other hands, the DNA analysis is not time consuming, but it has a demerit to detect dead bacteria. Then, we investigated the combined method with the culture method and the DNA analysis, tried at solving these problems, and obtained following results.
Methods: Suspend the several concentrations (about 1, 10, 100 CFU/mL) of bacteria (S. aureus, E. coli, B. cereus, S. marcescens, P. acnes) in blood sample for the test sample. Mix 5 mL of blood sample or the test sample and 50 mL of culture medium well. Transfer 0.75 mL of this blood sample mixture into a tube as control. Culture the test sample for 0, 6, 13, and 24h. Use 0.75 mL of cultured mixture, lysis and crush with beads for bacteria. Use 0.2 mL of the supernatant and extract nucleic acid by the automatic machine, and amplify the nucleic acid by RT-PCR method, check the Ct value, and judge a positive or a negative for the blood sample.
Results: The test sample with 10 CFU/mL of E. coli or B. cereus, became positive after 6 hour culture. However, P. acnes, was not positive with same conditions. The test sample with 100 CFU/mL of P. acnes finally became positive after 24h culture. Discussion:
In this method, obtaining most sensitive results, the culture condition must be changed for each species. But samples with 100 CFU/mL or less bacteria became positive within 24h or less. Then, the testing time of this method is shorter than the culture method. Furthermore, the sensitivity of this method is almost same as the culture method.

P-030

CLONING AND EXPRESSION OF HUMAN TISSUE FACTOR IN CHINESE HAMSTER OVARY (CHO) CELLS

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Introduction: Tissue factor (TF), a 47-kDa transmembrane glycoprotein, is the major cellular initiator of the coagulation protease cascade. TF activates the extrinsic pathway of blood coagulation in the presence of factor VII and calcium. Prothrombin Time (PT) is the test that evaluates extrinsic pathway of coagulation. Thromboplastin that used in this test is mostly prepared of rabbit brain. Thus it causes variation in the PT results. There is an important advantage of using recombinant TF in PT test to get more reproducible results.
Material and methods: TF mRNA was isolated from human lung fibroblast cells. After preparation of cDNA it has cloned in pcDNA3 plasmid. CHO cells were transfected with recombinant plasmid. Transfected cells were grown in presence of Geneticin. Total proteins were extracted from CHO cells and separated with SDS/PAGE electrophoresis and analyzed by western blotting technique. CHO cells expressing TF were added to citrated plasma in presence of CaCl2 and clotting time was measured. In addition, factor VII activation by recombinant TF in the plasma was assessed by ELISA method.
Results: Results of the restriction enzyme map, PCR and sequencing were shown TF cDNA has correctly cloned. Sufficiently grown transfected cells in presence of Geneticin were selected. Extracted proteins separated on SDS/PAGE showed an approximately 40-kDa band that verified with a monoclonal antibody against TF on western blot analysis. Adding transfected cells (106cell/ml) to the citrated plasma in presence of CaCl2 could decrease clotting time from 3 min to 20 s in compare with untransfected CHO cells. Also the addition of these cells to the plasma in presence of CaCl2 could active factor VII. Factor VIIa was measured by ELISA.
Conclusion: Recombinant TF expressed in CHO cells was able to clot citrated plasma and to active factor VII.

P-031

USING DRIED BLOOD SPOT SAMPLES FOR EPIDEMIOLOGICAL SURVEY AND SENTINEL SURVEILLANCE OF TRANSFUSION TRANSMITTED DISEASES IN CHINA SOME REMOTE AREAS

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Background: Dried blood spot (DBS) samples have been used for epidemiological survey and sentinel surveillance of infections in many parts of the world since 1980's. The investigation of whether DBS sample is a substitute of plasma sample when used in HIV antibody screening was begun in China since 2004.
Objective: To investigate the possibility of the use of DBS samples in epidemiological survey and sentinel surveillance of transfusion transmitted diseases.
Methods: Peripheral blood is collected by piercing the skin of a finger of commercial sex service male customer (CSMC), and blotted onto a filter paper (Whatman Blood Stain Card) where 1 inch circles that when filled with blood will hold about 300 μl (2~4 drops) each. DBS sample was then prepared, and should be allowed to natural drying at ambient temperature, stored in low gas permeable bags that contain desiccant to reduce humidity, shipped to the laboratory at ambient temperature. Be care of avoiding moisture and insolation. DBS sample in the circle was cut into patches, and put into a EP pipe of 1.5 mL. The blood was eluted out in 0.01 mol/L phosphate buffered saline containing 0.05% Tween 80 and 0.005% sodium azide. The resultant EP pipe was put on a free oscillation surface, overnight at ambient temperature. The eluate in the EP pipe was screened for HIV, HCV and TP by Enzyme Immunoassay, and confirmed for HIV by Western Blot.
Results: By using DBS samples, HIV screening positive for CSMCs in three areas (Zigong, Leshan, Xichang, Sichuan province, China) was 5.56%, HIV confirmative testing positive was 1.63%, HCV screening positive was 8.665 and TP screening positive was 5.23%. (Table 1)

Table 1 Results of 612 DBS samples testing for HIV, HCV and TP

		Zigong	Leshan	Xichang	Sum
Collected Samples		204	202	206	612
HIV	Screening Positive	16 (7·84%)	9 (4·46%)	9 (4·37%)	34 (5·56%)
	Confirmative Testing Positive	1 (0·49%)	4* (1·98%)	5 (2·43%)	10 (1·63%)
HCV	Screening Positive	2 (0·98%)	28 (13·86%)	23 (11·17%)	53 (8·66%)
TP	Screening Positive	7 (3·43%)	16 (7·92%)	9 (4·37%)	32 (5·23%)

*One sample was inconclusive.

Conclusions: In comparison with traditional fluid samples, DBS samples are collected easily, prepared simply, shipped conveniently with higher stability and lower biohazard, and can be used in epidemiological survey and sentinel surveillance of transfusion transmitted diseases, especially in remote area or difficulty in blood collection of persons.

P-032

ASSESSMENT OF COBAS S201 SYSTEM AND INCIDENCE OF VIRAL INFECTIONS IN BLOOD SUPPLY IN GREECE

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Background and aims: Blood screening of infectious agents including human immunodeficiency virus (HIV), hepatitis virus C (HCV) and

hepatitis virus B (HBV) is of vital importance in the process of blood transfusion. To reinforce safety, nucleic acid testing (NAT) is strictly implemented. Here, we emphasize in presenting data collected from George Gennimatas, one of the biggest blood transfusion centres in Greece, following the installation of the cobas s201 molecular system, for the period between the 1st of January 2009 and the 31st of May 2009. By assessing cobas s201 in relation to automation, safety and efficiency, the aim of this study was to investigate the incidence of viral infections transmitted through transfusion and to demonstrate the necessity for accurate and precise monitoring.

Methods: The centre receives blood for screening from 12 different hospitals from several compartments of Greece. The cobas s201 is a modular and fully automated system, with a configuration for screening pools of one. The procedure is performed in three basic steps: sample pipetting and archiving, extraction and purification of nucleic acids and finally amplification and detection by means of real-time polymerase chain reaction (RT-PCR). Initial screening performed on cobas s201 system utilizes a multiplex test allowing for simultaneous detection against five viruses: two HIV-1 viruses (M and O group), HIV-2, HCV and HBV. The sample is then tagged as reactive or non-reactive. Following a positive result, the sample is manually processed on a second system (cobas Amplicor) for qualitative discrimination against the three viruses (HIV-1, HBV, HCV) using end-point PCR with the aid of target specific probes.

Results: In total, 41,386 blood donations were processed and investigated for the presence of HIV, HCV and HBV for a time period of five months. Of these, 207 were found reactive in the cobas s201 system. Following discrimination, 132 blood donations were confirmed as reactive. There were 123 cases tested positive for HBV, 17 for HCV and 2 for HIV-1. Data from serological testing reveals that of the 113 cases of HBV infected donators, 14 were found negative to the presence of the surface HBV antigen. Similar results were obtained for two HCV cases where HCV markers could not be detected. Out of the total number of donations, 0.181% of cases appear to be false-positive. No false-negative cases were documented. Given the input and the time needed for processing, overall, the system's efficiency reached 0.965.

Conclusions: NAT has played a fundamental role in reducing the risk of viral infection during blood transfusions. Its use allows for early detection, minimizing the window period of seroconversion, as also evidenced by the results. A high efficiency along with an increased analytical sensitivity and a minimum of hands on time, make cobas s201 a reliable tool for blood screening. To our knowledge, this is the biggest study conducted for testing blood donations in Greece so far. Due to its large size it also offers insight to the incidence of viral infections not only in transfusion medicine but also in the general naïve population.

P-033

IMPROVED DETECTION AND IDENTIFICATION OF RED CELL ANTIBODIES COMMONLY FOUND IN ASIAN POPULATIONS INCLUDING VMNS (FORMERLY MILTENBERGER) USING NOVEL KODETM TECHNOLOGY

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Background: Many laboratories testing samples from Asian populations routinely utilise antibody screening panels made for Caucasian populations. These panels are designed to detect antibodies commonly found in Caucasian populations like anti-D and anti-K but are generally incapable of detecting important, clinically relevant antibodies common in Asian populations such as vMNS antibodies and Dia. While careful Indirect Antiglobulin based crossmatching can prevent or reduce the incidence of transfusion reactions, Caucasian designed screening cells will fail when used for antenatal screening to detect antibodies likely to cause haemolytic Disease of the fetus and Newborn (HDFN)

Methods: Antibody screening cells and a complimentary identification panel were created using careful donor selection and the addition of the vMNS antigens MUT and Mur utilising the novel KODETM technology. KODETM technology is used to add peptide based specific epitopes to screening cells that allow serological detection of IgG vMNS alloantibodies.

Results: Testing with a range of monoclonal antibodies and polyclonal sera demonstrates comparable sensitivity and specificity for red cell alloantibodies of Rh, Kell, Fy, Jk, MNS, Le, Lu and Dia specificities. Testing with four anti-vMNS monoclonal antibodies and demonstrated strong and expected epitope specific serological reactions. Testing with characterised human polyclonal vMNS antisera from a number of different Asian ethnic sources also demonstrated strong and expected serological reactions with IgG class vMNS antibodies. KODETM vMNS cells show no reactivity with IgM class antibodies therefore avoiding the detection of clinically irrelevant IgM class non-red cell immune antibodies. The screening cells therefore demonstrate improved performance over screening and identification systems designed for Caucasian populations. The antibody screening and identification cells were tested and are useable on current testing platforms such as tube and column based methods.

Summary: The use of screening cells containing KODETM technology can improve the detection of clinically relevant red cell antibodies prevalent in Asian populations. This can lead to safety improvements in pretransfusion testing and significantly improve the detection of antibodies likely to cause HDFN.

2.1. Blood Donation Blood donor and donation

P-034

WHY PEOPLE DON'T GIVE BLOOD? A TYPOLOGY OF OBSTACLES TO BLOOD DONATION

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Background: Although Italy is a self-sufficient country in terms of its blood supply, the problem of recruiting new voluntary and non-remunerated donors occurs periodically and is relevant also in light of recent outbreaks of emerging viruses, which can have a negative impact on the blood inventory. In order to develop new strategies for recruitment of new donors, the National Blood Center, in collaboration with a non-profit cultural association (Observa, Vicenza, Italy) has conducted research to investigate non-donors' perception of blood donation, with the aim of developing a map of potential obstacles to donation.
Methods: The research was conducted through five focus groups in different Italian cities, four of which were conducted with non-donors, while one final control focus group was conducted with blood donors. During the focus groups with non-donors, initial brainstorming sessions were conducted, asking participants to indicate the key words that in their opinion better described the practice of blood donation.
Results: The quantitative analysis of the results (by the count of keywords), showed that the concept of solidarity and gift is most immediately associated with the practice of blood donation, with a total of 45 keywords related to this concept reported in the four focus groups, for a total of 41 participants. After this initial brainstorming, focus groups were dedicated to the discussion and exploration of the dimensions along which obstacles to donate blood are articulated, in order to develop a typology of possible obstacles to donation. Data collected made it possible to divide the obstacles into three types. The first is constituted by the fears and emotional obstacles to donating blood. Emotional non-donors are frightened of needles and the sight of blood and feel they are being 'violated' or that donating blood may have a negative effect on their health. A second type refers to the motivation obstacles to blood donation, especially to laziness and to difficulties in finding easy and convenient opportunities to donate blood. For these ''lazy''non-donors, it is crucial to provide the right incentives and the appropriate opportunities for donation. The third type of obstacle to donation refers to manifest skepticism by some non-donors towards the practice of donation, including mistrust against medical facilities and health personnel.
Conclusion: As can be seen from the results of the study, non-donors' perception of blood donation differs greatly, consequently any campaign to promote voluntary and non-remunerated blood donation should also be diversified

P-035

COMMUNICATION OF BLOOD DONATION AND TRANSFUSION IN ITALY

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Background: The Italian National Blood Center, which represents the actual hub of the national blood network and is responsible for technical and scientific control on all transfusion medicine issues ruled by national laws and European directives, in collaboration with a non-profit cultural association (Observa, Vicenza, Italy) which aims at promoting the study and the discussion of the interaction between science and society has conducted a study in order to investigate the features of the blood donation and transfusion therapy communication in Italy.
Methods: The research was conducted through five focus groups and through quantitative content analysis of media coverage of the blood donation and transfusion issues in the two major Italian newspapers ('La Repubblica''and ''Il Corriere della Sera'') from 1998 to 2008.
Results: Focus-group analysis highlights how the issue of blood donation is perceived as being communicated through the use of two main frames. In particular, it is possible to identify a first 'positive' and 'proactive' frame for the practice of blood donation, emphasizing solidarity and gratuity and preventing the blaming of non-donors, and a second frame dealing with emergency communication and describing donation as a practice related to situations of crisis and lack of blood. With regard to blood transfusion, data emerging from focus groups show how this topic is represented through the use of an emergency frame, particularly with reference to the possibility of accidents, use and transmission of infected blood. In addition, analysis of the media - based on 158 articles collected from the online database of the two newspapers for the period considered - has revealed how 41% of news reported accidents in transfusion medicine, 13% reported blood shortage crises, 13% referred to discrimination and group identity issues related to blood donation, while 16% of the news articles reported events and initiatives promoting blood donation and 12% scientific discoveries or advances in transfusion medicine.
Conclusion: Our study shows how communication and perception of donation and transfusion in Italy is characterized by a complex interrelation between communication frames and perceptions. In this context, transfusion is most frequently associated in the media with possible accidents, and in particular with the possibility of infected blood transmission, even if the main real risks are now related to human errors.

P-036

TO ESTIMATE PREVALENCE OF DROP OUT AMONG VOLUNTARY BLOOD DONORS AND FACTORS ASSOCIATED WITH THE DROP OUT AMONG THEM IN SHIMLA BLOOD BANK, INDIA

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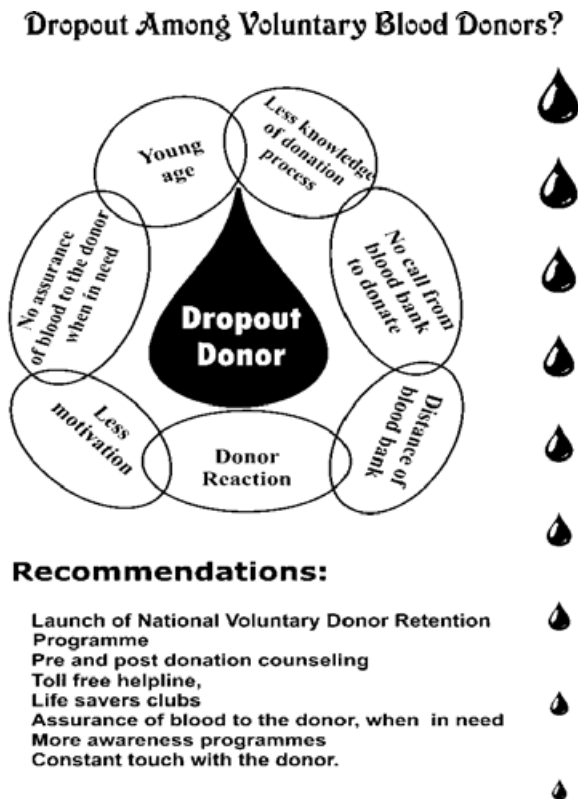
Background: In South East Asia, most blood donors donate blood once in lifetime and only 5–10 % are repeat donors as there is no emphasis on donor retention. In India, against the blood requirement of 8.5 million units annually, the availability of blood is only 4.4 million units.
Objectives: There is no study to know about the prevalence of drop out of voluntary blood donors and factors associated with their drop out in the country. This study would help know the reasons for dropout and take corrective measures to retain donors and increase voluntary donation and help ensure blood safety.
Methods: A line list of voluntary blood donors was prepared for the 2006 cohort of 763 voluntary blood donors from blood bank records. We could recruit 80 dropouts and equal number of regular donors. To know the factors associated with high dropout, we interviewed the donors using a pre-tested semi structured questionnaire prepared based on factors identified on literature review. A case control analysis was conducted, OR and 95% CI were computed.
Results: The prevalence of dropout among voluntary blood donors was 73% (CI 70–76%). The factors significantly associated with dropout included ; No opportunity to know in detail about blood donation, (OR= 5.3, CI=1.9–15.5); donors did not know the right age for donation, (OR= 4.4, CI=1.8–10.7); Not called to donate Blood by the blood bank, (OR= 3.5, CI=1.7–7.1); distance of blood bank >10 km, (OR= 3.1, CI=1.3–7.1), Age less than 25 years, (OR= 3, CI=1.4–6.4); donor not given blood preferentially when in need, (OR= 5.8, CI=1.6–22.9). With increasing distance the proportion of drop out increased significantly (Chi Square for trend is

8.604, P = 0.0034). Other factors like the knowledge of blood group, behaviour of blood bank staff and preference for donation in camp or to individual patient were not significantly associated with dropout of voluntary donor. 49% of dropout and 41% of regular donors have some fears of blood donation while 68% of dropout and 75% of regular donors had some expectation or the other from the blood bank staff.
Conclusion: Better and knowledge based IEC material that addresses the issue of age of donation and the process of donation, need to be developed. We need to address the fears of donors and their expectations for better compliance. For the pre donation and post donation counseling to the donors at the very first contact with the blood bank, we need counsellors. We need to create donor-clubs to remain in touch for calling them to donate, on the lines of successful model of pledge 25 clubs (Zimbabwe) through ''red ribbon clubs''& ''Life Savers Clubs''in all colleges. A toll free number for information of the donors can be of help. On the basis of this study we also recommend to initiate a ''National Donor Retention Programme''by the national and state blood transfusion councils. A poster was designed to educate blood bank officers of the factors identified here.



Poster to educate doctors based on this study

P-037

EVALUATION OF INTEGRATED NETWORK COMPUTERIZED DONOR PROCESSING IN REJECTION, ERROR RATE AND SATISFACTION OF BLOOD DONORS, ISFAHAN, IRAN 2005

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Background: The use of electronic registers generates new perspectives for the studies of transfusion practices.
Aims: We traced blood components via the network system. This study took place with the computerized donor processing system running in parallel the manual operations with loss of inventory in Isfahan, IRAN during 2005.
Methods: This Analytic Descriptive study carried out in Isfahan Blood Transfusion Organization with census method in 2005. Error incidence due to loss of inventory and Behavioral deferral rate in all blood donors at Khajoo (station 1) that uses computerized network donor processing compared with Shariati (station 2) with data entering after blood donation. Donor satisfaction in 100 donors in center 1 compared with the same as at center 2 using the Questionnaire.
Results: Deferral rate (0.2% vs. 1%) and error incidence (0.00 vs. 0.62 percent) in center 1 are lower (CI: 95%, P < 0.05). Donor satisfaction scores due to blood donation procedures showed it was lower than Shariati center significantly (t = 2.5).
Conclusions: Computerized network donor processing system is effective for minimizing error and increasing deferral rate. It contributes to safe and efficient blood but several computerized donor session procedures may cause dissatisfaction and disturbance to private donor counseling.

P-038

STUDY ON THE MOTIVATIONS OF THE BLOOD DONORS

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Background: The study that we have led has the objective to understand the motivations that induce a subject in good health to the donation of blood without remuneration and to identify that is the emotional push that does repeat in the time this attitude and the causes that prevent it.
Methods: The questionnaire is composed of two pages and it contain seven questions including the socio-demographic information (sex, age, profession, title of study) and has been supplied to a champion of 198 donors is usual (77,27 % of which 7,84% gives from months and 92,16% from years) that occasional (22,73%) of inclusive age among the 18 and the 60 years.
Results: The first question is turned to know the motivations that have induced the people to donate for the first time.The motivational factors that have included in our questionnaire are 5:

1 The value is the motive that refers on the empathy and to the altruism in the voluntary service.
2 Social are the motive that reflects the influence of the friends, of the family or of a social group that motivates the people to offer voluntarily.
3 The respect for anyone does voluntary service represent feel well with if same helping the other.
4 The moral appointment and its maintenance.
5 The personal utility.

From the answers to the first question is emerged that they give great importance to altruistic and empathetic motivations in fact, 68,18% of the subjects have answered of have donated for the first turned to help the other, 12.12% to strengthen their self-esteem and for family tradition, 3,03% has donated it because influenced by friends or relatives, finally, 4.55% of the subjects have answered 'other'.
Has been interesting verify that the greatest part of usual donors (45.10 %) continues to donate for altruism, while 29.41% does it to moral commitment, 19.61% to strengthen their self-esteem and 5.88% for personal utility, while any donor has answered of be influenced by friends or relatives.
Across a turned question exclusively to the occasional donors deduces that the greatest part of they don't donate with frequency on account of prejudices (53.33 %) concern above all the really style of life that thinks a few regulate for the donation.
Besides is emerged that 26.67% don't donate through lack of time, 13,33% through lack of information and 6,67% for difficulty to reach the center.
Is important to underline that while the influence of friends or relatives is not considered fundamental by the donors, is usual that occasional, as motivation to continue to donate, it is the principal source that them has induced to do it (48.48 %).

The second channel of recruitment, in orders of importance, are the associations (16.67 %) following by the suggestion of the sanitary personnel (15.15 %) and finally the information of the media (9.09 %), the brochures (6.06 %) and the manifests (4.55 %).
Conclusions: To guess think that this study would be able be extremely useful to identifying the motivations that have to constitute the target of promotional countries.

P-039

EVALUATION OF CORRELATION IN BLOOD DONORS' REJECTION AND TREND OF VIRAL MARKERS FROM 2004 TO 2007, ISFAHAN, IRAN

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Background: Recently, Blood Transfusion Services select and screen blood donors stringently, multi layer and highly controlled therefore, it is necessary to evaluate prevalence and correlation with viral markers trends. This study presents, Do recent donor selection procedures lead to decreasing of viral markers?
Aims: This study presents, Do recent donor selection procedures lead to decreasing of viral markers?
Methods: All of eligible blood donors from 2004 to 2007 referred to Isfahan Blood Transfusion Organization included to the study. Data related to rejection prevalence due to potential TTDs and viral markers prevalence and correlation were accounted using IBTO software.
Results: Blood donors populations from 2004 to 2007 were 84275, 95879,100290 individuals and overall rejection frequencies were 23%, 32%, 29% respectively. Rejection frequencies due to potential TTDs were 4.1%, 5.4%, 5.2% and viral markers prevalence was 0.16%, 0.13%, 0.12% respectively.
Summary and conclusions: Recently, overall rejection prevalence increased and rejection prevalence in blood donors due to potential TTDs correlated reversely to decreasing of viral markers prevalence. It may be due to stringent procedures of IBTO.

P-040

A STUDY OF SERUM PROTEIN AND GLOBULIN LEVELS OF APHERESIS DONORS WHO HAVE DONATED 12 TIMES PER YEAR

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Background: Serum protein and immunoglobulin in apheresis platelet or plasma donors can be depleted during the process of apheresis. Healthy individual may have transient reduction in serum protein and immunoglobulin levels after a session of apheresis.
Aims: The purpose of this study was to determine the effect of regular apheresis (defined in the study as 12 times per year) on donors' serum protein and globulin levels.
Serum protein and globulin levels of regular apheresis donors (who have donated 12 times per year) were determined whether such regular apheresis donations would affect the donors' protein and immunoglobulin levels. This may potentially influence our guidelines for apheresis donation in our future practice.
Methods: Regular apheresis donors who have donated 12 times in the year 2007 were identified and the study population consisted of 152 male donors. Mean time interval between donations was not more than 30 days. Serum levels of total protein, albumin and immunoglobulins were measured after their twelfth donation. The apheresis collection was performed according to automatic standard procedure along with 300 mL of concurrent plasma.
Results: Of the 152 donors, 71% (108) of them had normal serum levels of either protein, albumin and immunoglobulins, while (29%) 44 donors had high or low values. Of the 44 donors, 34.1% (15) had elevations in Alpha 2 globulins levels and another 31.8% (14) had high Beta globulin levels. However 4.5% (2) donors had decreased Beta globulin levels. A reduction in Gamma globulin levels was observed in (11.4%) five donors. Serum albumin levels were also reduced in another (9.1%) four donors. Elevation in both Alpha 2 and Beta globulins levels were observed in (9.1%) four donors.
Conclusion: Results did not show significant decrease in protein and immunoglobulin levels to warrant any deferment of donors from the programme. This study concludes that removal of 500–600 mLs of plasma at 4-weekly intervals involves little, if any risk of total protein or immunoglobulin depletion in donors who satisfy current criteria.

P-041

USING AUTOMATION AND SIX-SIGMA PRINCIPLES TO IMPROVE DONOR RECRUITMENT AND RETENTION

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Background: This large center (172,000 whole blood collections per year) seeks collection self-sufficiency. We use both automated platelet and red cell apheresis (RCA) to achieve this goal.
Aims: Since most blood collections are on mobile blood drives, we must motivate blood donors to donate on apheresis equipment and remain as apheresis donors, even though apheresis is a longer procedure. Previously we found that 32% of mobile RCA donors returned to donate apheresis on subsequent visits. We found this to be unacceptable.
Methods: Working with our apheresis provider, we used 6-Sigma principles to resolve this problem. The 6-Sigma method involves 1) problem definition; 2) measurement; 3) analysis; 4) improvement and 5) controlling the process. Using these steps, several changes were made in the recruitment process: a) the marketing message to donors was changed; b) donor collection staff were trained to use this message with whole blood donors; c) donors were also contacted using specific email messages; d) once an automated donor donated, there was improved targeting of these donors for future automated donor blood drives; e) automated donor goals were established. Donor recruitment and collections costs were tracked. Statistical differences were measured using a paired T-test.
Results: From January to May 2009, the repeat automated donor rate improved by 10.38% (P = 0.008). A total of 339 additional donors were retained in the automated donor pool. Over this 5 month period, $26,000 USD was saved as a result.
Conclusion: Using apheresis collection and a data-driven, focused approach to donor recruitment, additional units of blood were collected, financial savings achieved and donor loyalty increased.

P-042

PSYCHOLOGICAL EFFECTS OF BLOOD DONATION ON REPEAT AND FIRST TIME DONORS IN TEHRAN BLOOD TRANSFUSION CENTER

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Background and objectives: Provision of adequate and safe blood is the main purpare of Iranian Blood Transfusion Organization. One of the most important and vital strategies to deal with the issue is to replace once donors by repeat donors. Psychological effects of blood donation on frequent and infrequent clients having referred to different blood collection centers in Tehran were considered to be the aim of this study.
Materials and methods: In the course of this analytic cross-sectional study the standard questionnaire of GHQ was utilized to measure mental health of the participants. All of the donors who had referred to blood collection centers in Tehran during 2004–2005 formed the participants of this research. Data were analyzed with Chi-square, t-test and Man Withney test.

Results: Nine hundred thirty five frequent and 749 infrequent donors filled the questionnaire. The level of anxiety and depression in repeated donors was also lower than the other group.
Conclusions: The individuals who attempt on blood donation for the first time report their health and physical conditions at a completely acceptable level in order to be accepted as an eligible candidate; however, it is not the same for constant donors, as they do not benefit from this. The return rate of blood donors is very low in case of individuals suffering from high stress. Religious beliefs and the feeling of being beneficial to the society are considered to be important factors both in motivating people to repeat blood donation and protecting against depression. The results of the present study upon publication will also encourage donors to repeat their behavior.

P-043

DONOR RECRUITMENT FOR PLATELETAPHERESIS IN YOGYAKARTA BLOOD TRANSFUSION SERVICE, INDONESIA

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In Indonesia, plateletapheresis is a new technology in the blood transfusion service. Until November 2006, all blood components were achieved by whole blood donations from voluntary, family or replacement donors. In November 2006, the first procedures to collect single donor platelets by apheresis techniques applying MCS3p (Haemonetics®) were started. In our experience, donor recruitment for plateletapheresis remains a problem.
To start our plateletapheresis program, we recruited volunteer regular whole blood donors from the Indonesian Red Cross of Yogyakarta as apheresis donor. Donors were given information regarding the risks of plateletapheresis, the beneficial aspects, and the important role of apheresis platelets. Volunteer donors who gave informed consent were marked in our documentation system and placed in groups based on their AB0 blood group.
Plateletapheresis products are expensive. Since health insurance is not covering the costs for apheresis platelets, not all of the patients can afford this product. Therefore, plateletapheresis procedures are only performed if there is a request from clinicians. Because of this, apheresis platelets can not be held in stock, e.g. for acute needs. As a consequence, patient AB0 compatible volunteer donors will be selected and promptly invited to donate a platelet product by apheresis. This works rather successful. However, in some circumstances, e.g. at night, finding volunteer donors willing to travel to the donor centre for a 2h plateletapheresis procedure is very difficult. In these situations, patient's family members are asked to donate by plateletapheresis.
In periods of relative shortage of volunteer donors, e.g. Dengue season and Ramadan (fasting month), plateletapheresis is very helpful to cover the shortage of whole blood derived platelets. Because of Dengue, there is an increased request for blood components. Meanwhile, during Ramadan and Dengue season the number of available volunteer donors decreases with more than 50% compared to that that in the other months.
Also, in Yogyakarta, it's still common that patients only want to receive blood components donated by family or relatives (replacement donor). However, because of higher risks (blood borne diseases, transfusion associated GvHD) we try to discourage this. An effort to cover apheresis products by health insurance and increase the number of volunteer donors is needed.
In 29 months, 126 plateletapheresis procedures were performed on 126 donors. Of them, 60 (48%) were volunteer and 66 (52%) replacement donors. Informed consent was achieved from every donor prior to the apheresis procedure. After the plateletapheresis procedure, every replacement donor was requested to become a regular volunteer donor. As result, 7/66 (11%) replacement donors became volunteer donor and can be called for unrelated patients.

P-044

MOTIVATION - TERM FOR CHANEGE OF STRUCTURE OF BLOOD DONORS IN THE TRANSFUSION SERVICE – TETOVO

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Introduction: Supplying enough blood quantities for the patients and the injured is a basic condition of every transfusion service. In the period when big state firms existed and there was awareness about blood donating this was easier to accomplish. After the conflict in 2001, these kinds of firms in our municipality extinguished and transferred into private. Entering in them is getting more and more difficult. A solution out of this situation we sought in fieldwork and motivation in schools and universities. This succeeded and brought a significant change in the structure of donors - family donating (donating blood for family members) and voluntary donating (for an unknown user).
Purpose: A purpose for this effort is to show the relationship of the family and voluntary blood donating in the period from 2002 to 2008 in the transfusion service - Clinical hospital, Tetovo.
We have presented voluntary field donating, family donating and voluntary donating in the service.
Material and methods: Diaries for blood donating were used on the field and in the service as well as questionnaires which were filled in by the blood donors.
Results: In the period from January to December 2002, there were 136 units (11.35%) of blood collected on the field. 995 units (83.05%) were family donations and 67 (5.59%) were voluntary donations on the service. There were 1198 donations total. In 2003, 1527 units of blood were collected, 537 (35.14%) were collected from fieldwork action, 123 (8.05%) were voluntary donations in the service 867 (56.77%) were family donations.
In 2004 out of total 1445 units of blood, 401 (27.75%) were fieldwork, 152 (10.05%) were voluntary donations in the service and 892 (61.73%) were family donations.
In 2005, 1750 units were collected. From them 549 (31.37%) were fieldwork actions, 198 (11,31%) voluntary donations in the service and 1003 (57,31%) were family donations.
In 2006 out of 1724 units, 494 (28.65%) were fieldwork actions, 197 (11.42%) were voluntary donations in the service and 1033 (59.9%) were family donations.
In the last 2008, 473 (23.8%) were fieldwork actions, 301 (16.42%) were voluntary donations in the service, 1095 (59.73%) family donations or in total 1833 units of blood.
Conclusion: In our region the percentage of family donations is still high throughout which we easily get the necessary quantities of blood. The reducing of this number is evident (2002–83.5%); (2008–59.73%). There is a noticeable increasing of the fieldwork donors (2002–11.35%); (2008–23.8%) and a permanent increasing of the voluntary donors in this service (2002–5.59%); (2008–16.42%).
We accomplished this by motivational lectures before every donation, neat evidence of the donors and struggle for proper evidencing of voluntary donors in the service (not rarely written down as family).

P-045

RESULTS OF DONOR ACQUISITION PROGRAMME CONDUCTED BY ERCIYES UNVERSITY MEDICAL FACULTY BLOOD BANKING

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It is an important issue to maintain recruitment and retain of sufficient numbers of regular, volunteer blood donors for adequate and safe blood supply. Good donor care, personal satisfaction experienced during the first donation and ending the procedure without any complication affects the retention of safe donors. Adverse reactions and complications negatively affect the likelihood of volunteer donor recruitment and retain. World Health Organization and the Council of Europe recommend collecting

blood, plasma and other blood components only from voluntary and non-remunerated donors in order to maintain safety blood products. It is important to prevent the transmission of diseases through blood. Volunteer blood donation recruitment and retain strategies include all the activities that increase the number of volunteer donors. Poster, bulletin, media, internet are the most used methods which promote poeple for blood donation. It seems better to inform the candidate of blood donor directly by face to face. Educational and social status and the prior false believes of the person is important in blood donation.

Aim: In this study we aimed to report the results of donor acquisition programme conducted by Erciyes University Medical Faculty Blood Banking between November 2006-August 2008.

Patients and methods: Annualy 25 thousands of total blood units are being used by separating them into its components in our blood bank center. Donor acquisition program was started firstly in November 2006. One of our blood bank staff was employed as a donor motivation and acquisition member to reach the volunteers effectively and to rise the awareness about blood donation. Thus, training programs about blood donation was started in the university; various media including electronic and print versions such as; email, brochures and posters were utilized.

Results: Mobile blood banking teams organized blood donation campaings by making educational programmes 2 or 3 times a week. 29614 poeple were informed about blood donation. Blood banking teams reached to 8730 persons from university and 6036 (69%) of these people applied for blood donation. 6398 of the 20884 the people (31%) from outside the university applied as volunteer donors. Volunteer donor candidates were deferred for several reasons; 22% of them were from outside university and 12% of them were from university. Adverse reactions during the donation were seen in 1% of the donors from outside university and in 0.4% of the donors from university. HBsAg was detected in 1.7% of the donors from outside university and 1.03% of the donors from university. Anti-HCV antibody was detected in approximately 0.02% of the donors both from university and outside from university.

In this study it is showed that volunteer donor populations from university are at low risk for transfusion-transmissible infections than the donors outside from university. Deferral reasons and adverse reactions were low in the donors from university. After donor acquisition program it was seen that the number of application for blood donation was higher in university population.

As a conclusion; efforts to increase the number of volunteer donors for adequate and safety blood supply is of greate importance.

P-046

DEMOGRAPHIC STUDY OF POPULATION REFFERED TO BEHBAHAN BLOOD CENTERS FOR BLOOD DONATION DURING MAY-SEP2008

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With the developing of medical science and increase life potency and blood transfusion demand, the blood transfusion finds his great role in public health. The blood donations are based on the health of blood donors and blood recipients. Recognize of population and their demographic status helps us to planning for blood donor's recruitment and providing safe blood supply.

This descriptive study included all people referred to Behbahan blood transfusion centers during May to Sep 2008. All data analyzed with SPSS14.

In this period 4888 people referred to Behbahan centers (61.37 % under 36 years old; 91.8 % male, 8.2 % female; 69.9 % married, 30.1 % single; 27.2 % were university graduated, 37.2 % diploma; 46.7 % referred to head office, 21.8 % and 21.1 % admitted to Dhdasht and Gach saran respectively). 87.5 % of referral population accepted as blood donors and 13.5 % rejected permanent or temporary. The main reject causes were medication, hypo or hypertension and anemia.

Our finding showed that the main of peoples that admitted to our centers was young and married people, because the low rate of high risk behavior in married population, their recruitment as repeated donors more safe and it's so cost benefit to educate them for provide blood supply. The rate of rejection was high, but because the main causes avoidable we can to re entry them as blood donors by using educational program.

P-047

EFFECT OF SPECIFIC DONOR VARIABLES ON YIELD IN SINGLE DONOR PLATELETPHERESIS

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Background: Apheresis technique for harvesting single donor platelets (SDP) was introduced to National Blood Transfusion Service (NBTS) Sri Lanka in 2002. The rapid developments of NBTS, academic improvement of the knowledge of transfusion medicine and the increased medical and surgical demand for platelets have initiated the use of SDP. Single donor plateletpheresis is done in NBTS specially to collect platelets from rare blood group donors and when there is a shortage of random donor platelets. The transfused dose of platelets influences the platelet recovery of the recipient which in turn is dependent on the yield of SDP. Therefore it is very important to have a good yield from the donor.

Aim: To evaluate the effects of specific donor variables such as age, weight, pre donation platelet counts and pre donation haemoglobin levels on platelet yield in single donor plateletpheresis

Methods: A retrospective study was done on apharesis donors attended to the National Blood Center and National Hospital Blood Bank, Sri Lanka during the period from March 2006 to May 2007. In this present study, both Amicus and MCS cell separators have been used for plateletpheresis. But it has not been separately documented the pack numbers to identify the machine used to harvest it. A convenient sample of 137donors was taken. Demographic data such as age and weight were collected from donor declaration forms filled by the aphaeresis donors. Predonation haemoglobin values and platelet counts were collected from full blood count reports attached to the declaration forms, Volume of the unit and the platelet yield were obtained from aphaeresis donor registry, and platelet quality control reports collected from quality control department. Haemoglobin and platelet counts were measured on automated analyzers. The collected data and test results were analyzed.

Results: A total of 137 male donors with a mean age of 31.6 y ±7.2 y and with a mean weight of 67.9kg were analyzed. There mean pre donation Hb was 14.2 ± 1.2 g/dl and their mean pre donation platelet count was 251.3 ± 58.4 x 109/l. The mean value of platelet yield of this donor population was 179,028 ± 68,675/pack. In the study sample there was a negative correlation ($r = -0.122$) between age and platelet yield but it was not a statistically significant correlation ($P = 0.155$) Between weights of the donors and platelet yield there was a positive correlation. ($r = -0.014$) but it was also not statistically significant. There was a positive correlation ($r = 0.038$) between Hb and platelet yield but it was not statistically significant ($P = 0.394$). In this study there was a positive correlation ($r = 0.390$) between pre platelet count and platelet yield and it was a statistically significant correlation ($P < 0.001$)

Conclusion: A positive correlation was observed between pre donation platelet count and the platelet yield in this study population. There was no any correlation found between donors' weight, age and pre donation haemoglobin and platelet yield.

P-048

PRE DONATION COUNSELING TRIAL AT JAKARTA BLOOD TRANSFUSION SERVICE

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A Blood Transfusion Service (BTS) must provide a safe blood for transfusion. To get a safe blood, it is started from recruiting a healthy

and low-risk donor. Then, all blood must be screened for transmitted transfusion infection. However, there is a tendency that reactive result is increasing every year. To overwhelm this situation, we tried to arrange a pre donation counseling which is done by trained counselor to help high-risk donor make a decision not to be a donor (self deferral).
The pre donation counseling was held on December 2004 at Jakarta BTS using a questionnaire. Among 600 participants; there were 8.3% new donor, 91.7% routine donor. Their reasons being a donor are for helping others (76.6%), for their own-health (10.8%), to check health-status(1.5%), et cetera (11.6%). Seventy point eight percent donors have understood about Transfusion Transmitted Infection (TTI) before counseling. There were 12.2% high-risk donors. Ten from 600 donors decided not to donate their blood (1.7%). After screening test for TTI, 10 donors which have reactive result (1.7%), five are new donors and the rest are routine donors. Pre donation counseling needs well-trained counselor with good interpersonal and public relations skills because they must covey information and eliminate high-risk donors.

P-049

CAN DISPLAYING BLOOD TRANSFUSION INFORMATION HELP INCREASE BLOOD DONOR PARTICIPATION?

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Background: In order to meet the demand of blood and blood products in Japan, a sustainable number of blood donors are necessary. It is important to analyze the factors of why donors donate their blood for transfusions repeatedly.
Aim: It is essential to concentrate on the recruitment of new donors while also focusing on how to keep the donors who frequently donate their blood to sustain blood donations. This study evaluated how many people returned to donate their blood when they read stories of the patients who had received transfusions in their serious conditions.
Methods: A self-administered questionnaire that was accessible through a secured web site was given to 600 blood donors (between the ages of 19 to 26) at 2008. Participants were asked their age, sex, occupations as well as their motivations to donate blood. The stories of blood transfusions such as traumatic accidents and neonatal jaundice were presented to the participants at random on web site. Half of the participants read the stories and the other half did not. On the web page, all of the participants read the terms and conditions of this survey stating that participation was voluntary and all of them were able to withdraw from the survey freely at anytime. After six months from the first questionnaire period, the participants were asked whether they return to be a donor or not.
Results: We have received 400 answered questionnaires. One hundred and sixty nine answers are male and 225 people have returned to be donor (Table 1). We defined two types of donation experiences. The occasional donor was defined as a donor who had already given blood less than five times before this survey and the repeat donor was defined as someone who had donated more equal five times. The repeat donor became return donor significantly than occasional donor ($P < 0.0001$, Chi-square test). There was no significant difference between return donor and non return donor for scene condition. Multivariate logistic regression was used to calculate the odds ratio (OR) and 95 percent confidence intervals (CI) to assess potential relationships between donor demographics, donation experience, reasons for donation and who became a return donor or non-return donor. The person who donated their blood at the donation center or mobile bus during their commute or holiday time were more likely to return to be donors (OR, 1.85: 95% CI. 1.23–2.82).
The calculated OR, adjusted for sex and age show that individuals who already had motivated by free health check (OR, 2.85: 95% CI. 1.75–4.73), effective use of free time (OR, 1.93: 95% CI. 1.28–2.92), and repeat donor (OR, 3.52: 95% CI. 2.30–5.45) seems significantly to be return donor than non–return donor. However, the occasional donors were more likely go to blood donations ($P = 0.0645$, t-test) when they read the transfusion stories.
Conclusion: Collection agencies should consider appealing to occasional donors by displaying the adequate blood transfusion or blood products information. These strategies may encourage more return donors.

Table 1: Characteristics of 400 donors

Characteristics		Return Donor (N= 225)	Non-Return Donor (N= 175)	p
Mean(±SE) age - yr		23·4±0·15	23·3±0·17	0·713
Male sex - no.(%)		98 (43·6)	71 (40·6)	0·549
Meet the donation center or mobile bus during commuting or holiday - no.(%)		124(50·6)	101(65·2)	0·0043
Donation experience - no(%)	Occasional Donor[a]	68 (30·2)	103 (58·9)	<0·0001
	Repeat Donor[b]	157 (69·8)	72 (41·1)	
Scene condition - no(%)	Transfusion Scene	111 (49·3)	92 (52·6)	0·521
	None	114 (50·7)	83 (47·4)	
No. of donations (Mean±SE) in occasional donors	Transfusion Scene	40 (1·65±0·20)	-	0·0645
	None	28 (1·07±0·23)	-	
No. of donations (Mean±SE) in repeat donors	Transfusion Scene	71 (3·4±0·36)	-	0·412
	None	86 (3·01±0·32)	-	

a) Ocasional donors already had experienced the blood donation less than 5 times.
b) Repeat donors already had experienced the blood donation more equal 5 times.

P-050

PREVALENCE AND TRENDS OF HUMAN IMMUNODEFICIENCY VIRUS, HEPATITIS B AND C VIRUS AMONG BLOOD DONORS IN IRAN, 2004–2007

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Background: Evaluation and monitoring the prevalence of transfusion transmissible viral infections in blood donors is a valuable index of donor selection and blood safety.
Aim: This study analyzed the trends of blood-borne infections among Iranian blood donations during 4 years.
Methods: Viral screening results of 6,499,851 allogenic donations from 2004 to 2007 were analyzed. All of donations were screened for hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV) and syphilis. Prevalence of HBV, HCV and HIV infections per 100,000 donations and 95% confidence interval is calculated. P value is estimated by chi-square test.
Results: The prevalence of HBV, HCV and HIV were decreased during 4 years study from 2004 to 2007. The overall prevalence was 0.56% for HBV, 0.004% for HIV and 0.13% for HCV. There was a significant and impressive drop in HBsAg prevalence from 0.73% in 2004 to 0.41% in 2007. The prevalence of HIV was decreased from 0.005% in 2004 to 0.004 in 2007. HCV prevalence shows a slight decline in blood donations from 0.13% in 2004 to 0.12% in 2007. For HBsAg in all of repeat, lapsed and first time donations, prevalence rate was decreased. The HBsAg frequency was slightly higher in male than female. It was increased by age in male and female. Anti-HCV showed decreases in repeat and lapsed but slightly increased in first time blood donations. Anti-HCV prevalence in female was significantly lower than male donations, but the frequency of anti-HCV in age groups was similar.
Frequency of HIV in repeat donations was significantly less than first time blood donations.
Conclusion: The trends of transfusion transmissible infections prevalence in Iranian blood donations suggest that more of the safety measures employed in recent years in Iran.

P-051

EVALUATION OF HEMOGLOBINOPATHIES BY HIGH PERFORMANCE LIQUID METHOD (HPLC) IN IRANIAN POPULATION

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Objective: The aim of this study was to verify the conventional methods for screening and identification of carriers of hemoglobinopathies in Iranian population.

Methods: Altogether 932 blood samples from patients referred to the Iranian blood transfusion research center in Tehran were collected. Whole blood samples were collected from each subject in EDTA tubes. An aliquot (3 mL) was used for cell blood counting (CBC) and the other part was used for HPLC. Red blood cell indices were determined by Sismex K-800 cell counter and Hb analysis was performed on a HPLC system (Drew scientific). The Hb control AFSC (Helena, co) was used as an authentic Hb sample.
Result: In this study, 36 cases (3.9%) were diagnosed as different variants of haemoglobinopathy and 22 cases (2.39 %) were HbAD (12 male with 22±7 years of age and Hb value of 15.4±1 gr/dl and 10 females, 22±3 years of age with Hb levels of 13.5±2 gr/dl); nine cases (0.98%) HbAS (five male with 20±5 years old with Hb value of 14.4±1 gr/dl, four female with 20±5 years of age and Hb levels of 13.3±0.9 gr/dl); three cases (0.33%) HbAG (two male and one female); two cases (0.2%) HbAE comparising of one male and one female.
Conclusion: Given the importance of haemoglobinopathies and their prevention in this society, it is imperative to use precise and rapid diagnostic methods. Thus, the application of HPLC method is recommended to discriminate of abnormal hemoglobin.

P-052

VOLUNTARY NON REMUNERATED BLOOD DONORS AND HOSPITAL EMPLOYEES; A STUDY AT TEACHING HOSPITAL KURUNEGALA IN SRI LANKA

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Background: The ultimate goal of every transfusion service in the world is to establish a donor pool consisting of 100% voluntary non remunerated blood donors (VNRBD). Recruitment and retention of voluntary non remunerated blood donors is the first step towards this goal. The objective of the National Transfusion Service in Sri Lanka is to have a 100% voluntary non remunerated donor pool by year 2010. Health professionals whichever their level of employment, will have a major role educating as well as motivating and specially changing the attitudes of the general public regarding voluntary non remunerated blood donations.
Aim: To assist the policy makers to adopt strategies that will be used to get 100% contribution from all health care workers in achieving 100% voluntary non remunerated donor pool.
Method: A questionnaire was used as the investigation tool, which was distributed among 2414 employees of Teaching hospital Kurunegala.
Results: Employees consists of 24 Consultants, 276 Medical officers, 998 Nursing officers, 107 Paramedical staff, 101 Office staff and 908 of all other employment categories. Only 2306 questionnaires were returned. Gathered data were analyzed and it revealed that only 253 employees have ever donated blood. This is 10.97% of the employees. Only 68 (2.95%) employees are regular donors. 1292 employees know the meaning and the importance of the concept; voluntary non remunerated blood donor, which is 56.02% of the study group. 530 (22.98%) employees have educated or motivated the general public regarding VNRBD. Out of 2053 (89.03%) employees who have not donated blood, 1560 or 75.99% did not donate because of the fear to do so. 287 (14%) employees did not donate, because they were unable to satisfy the eligibility criteria.
Conclusion: According to the results 2.94% of the employees are regular voluntary non remunerated blood donors. This is a low percentage. Even though the concept and its' importance are known by 56.02% employees, only 22.98% of them have contributed for the message to be taken to the general public. Unnecessary fear regarding blood donations has prevented 75.98% employees from donating blood. This is a very high percentage. Hence the concept has not been well established within the health care system and its employees. Lack of knowledge, motivation and negative attitudes of the health care workers has hindered the concept been a reality.

P-053

TO IDENTIFY THE DONOR POPULATION FOR RED CELL APHAERESIS (ALYX SYSTEM) AND COMPARE IT WITH ALLOGENIC WHOLE BLOOD DONATIONS AT REGIONAL BLOOD CENTER, KURUNEGALA-SRI LANKA

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To identify the Donor population for Red Cell Aphaeresis (ALYX system) and compare it with allogenic Whole Blood Donations at Regional Blood Center, Kurunegala-Sri Lanka.
Background: National Blood transfusion service of Sri Lanka consists of 75 blood banks and it's centrally co-coordinated by National Blood Center, Colombo. Teaching hospital Kurunegala has 1318 bed Strength and National Thalessemia Center is attached to the hospital. The blood bank of Teaching Hospital Kurunegala is the regional blood center for north western province and it is controlled by National Blood Transfusion Service of Sri Lanka. During the year 2008 total blood collection was 19615. This is the only blood center in Sri Lanka which has Red Cell Apheresis, ALYX machine available. The Red Cell Apheresis, ALYX system is a machine designed for automated collection of red blood cells with the advantage of collecting 2 leuco depleted packed red cell units (LDB) during single donation.
Aim: Is to detect the frequency of red cell aphaeresis donors compared to the allogenic whole blood donors and to determine the basic demographic data such as Age, Weight, Haemoglobin concentration and haematocrit. Allogenic whole blood donors had to meet national eligibility criteria's and Red Cell Aphaeresis donors had to meet both manufacturer recommended criteria and the national eligibility criteria.
Method: The Data collected from January 1st to 31st December 2008.
Data collected from in-house donors presented to Blood Bank, Kurunegala
Includes: Donation no
Age
Weight Haemoglobin % Haematocrit
Donor age obtained from donors national identity card.
Donor weight checked with blood bank weighing scale (Salter)
Hemoglobin and Haematocrit was detected from a sample drawn prior to donation using Hematology analyzer.
Collected Data and Analysis: Total number of donors presented in studied period is 19615, with 5263 in-house donors and rest to mobile centers. Out of in-house donors only 132 were collected as red cell aphaeresis donors and none of them developed reactions and the process completed successfully. The age for ALYX donors rangers from 19 to 51 years with mean age of 32.6, weight rangers from 60Kg to 95Kg with mean weight of 72.64Kg, Hemoglobin concentration rangers from 13.2 %to 17.8 %with mean Hb of 15.42% and Haematocrit rangers from39.7 to 53.9 with mean of 46.05. Selected all were males.
Discussion: The presences of red cell aphaeresis donors were very low with percentage of 2.5%. The causes identified for the above includes the donor selected according to both national eligibility criteria and manufacture recommendation and the donor's enthusiasm on machinery. Females who met the criteria's were not confident on connecting to a machine.
Conclusion: The ALYX donor population can be described by mean age of 32.6 years, weight of 72.64 Kg, Hb of 15.42% and mean Haematocrit of 26.05 which are not higher as a normal individual cant achieve. As this machine able to collect two units of LDB, the advantages are much more as National Thelaesaemic center attached to this hospital. The low presence as 2.5% has to be increased so the propaganda on red cell aphaeresis is on demand.

P-054

PREVALENCE OF ABO AND RHESUS D BLOOD GROUP SYSTEMS AMONG BLOOD DONOR POPULATION IN SRI LANKA

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Background: Sri Lanka is an island situated in the Indian Ocean with an estimated population of 20926315. It has a centrally co-ordinated National

Blood Transfusion Service (NBTS), which caters the blood and blood component transfusion needs of it's population. During the year 2008, NBTS collected blood through 52 fixed donor clinics and numerous mobile blood collection campaigns, throughout the country.

There had been several studies done on ABO blood group distribution in Sri Lanka on samples of varying sizes, ranging from 938 to 50320 and the last study was done in 1984. Prevalence of ABO blood groups among donor population is important to plan out blood donation and donor call up programmes as well as to plan out strategies in blood component preparation, clinical transfusion practices and inventory management, so that this scarce resource can be utilized maximally.

Aim: To determine the prevalence of ABO groups and Rh D types among blood donor population in Sri Lanka.

Method: Retrospective analysis of ABO groups and RhD types of all the blood donors who donated blood during the period of one year from 01.01.2008–31.12.2008 throughout the country, was done. Data were reviewed via the computed records kept at the National Blood Centre. Prevalence of ABO groups and RhD types of them were calculated using computed records of their blood groups which had been recorded following donation testing.

Blood grouping was done using the tube technique, with monoclonal anti-A, anti-B, anti-AB & anti-D reagents in the forward grouping and group A1 and B reagent red cells in the reverse grouping. All Rh D negative groups were further tested with a blend of IgM and IgG anti-D reagent that detects weak D and D variant antigens by indirect anti-human globulin testing method.

Results: 2,55,793 donors had donated blood at fixed donor clinics as well as mobile blood collecting sessions, during the period of study. Prevalence of ABO groups and RhD types among them are given in table-01

Table-01: Prevalence of ABO and RhD groups among Sri Lankan blood donor population–2008

Blood Group	O+	B+	A+	AB+	O-	B-	A-	AB-
Number of donors	104792	66295	54968	15490	6212	3698	3348	990
Percentage	40·97%	25·92%	21·49%	6·06%	2·43%	1·45%	1·31%	0·39%
Results of blood group distribution study done in 1985	43·42%	25·78%	21%	5·13%	2·03%	1·19%	1·15%	0·3%

Conclusion: Prevalence of ABO and Rh D blood groups among the blood donors who donated during 2008, appear to be quite similar to the blood group distribution of the country, which was stated in the study published in 1985. But as this applies to the whole country, there may be area-wise discrepancies in donor blood group prevalence.

This data can be utilized in determining the minimal inventory levels of blood and blood components according to blood groups during stock management, as well as in decision making in blood component preparation, especially in the case of labile blood components such as platelet concentrates. This could also be used in clinical decision making as in the case of group switching of blood components.

P-055

THE IMPACT OF BLOOD DONOR RETENTION STRATEGIES IN IRAN

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Background: The goal of this study was to increase regular blood donors, by evaluating different donor strategies. Retaining regular blood donors is a step toward improving blood safety and sustaining the blood supply.

Design and method: 1362 first-time blood donors were randomly assigned to this intervention study. An educational booklet which included blood donation topics was mailed to 277 of the donors 3 months after their first donation. A sympathetic booklet which included thank you letters from thalassemic patients was mailed to 231 of the blood donors. 251 of first-time donors received a telephone recall 3 months after their first donation reminding them that they can donate again. 245 donors were educated face to face about the importance of regular blood donation. 244 donors were assigned to the control group. A second attempt to donate within 6 months was considered as the final outcome.

Results: A total of 362 (29%) first-time donors returned to donate blood within 6 months. Educational and sympathetic booklets were effective in increasing returns when compare to the control group. Telephone recalls were also effective in donor retention (31.5%). Face to face education was not an effective method to bring back first-time donors (22%) in comparison to the control group.

Conclusion: Mailing educational, sympathetic booklets and telephone recalls 3 months after the first donation were all significantly effective in increasing second donation. But face to face education at the first donation was not effective during this short-time period. It seemed intervention near to next donation is more effective than intervention used during the first donation. It's necessary to reevaluate the effectiveness of these strategies at longer intervals.

	Educational booklet group	Sympathetic booklet group	Telephone recall group	Face to face education group	Control group
Total number of donors	277	231	251	245	244
Returned donors	92	83	79	54	54
OR*	1·5	1·6	1·4	0·9	-
95% CI	1·1-2	1·2-2·1	1·0-1·9	0·7-1·4	-

* *Odds Ratio, compared to control group*

P-056

BLOOD SAFETY AND DEFERRED DONORS

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Background: Deferred donors play important rule in blood donation as there are will donate blood in other time if they are had temporarily causes and if they are permanent donors will be a kind of knowledge for other persons, so we should estimate these numbers of deferred donors to be useful in the future.

Aim:

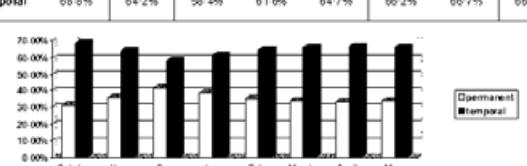
-To estimate the effect of self deferral education and culture of blood safety
-To quantify the ratio of deferred donors and classify the different deferral reasons

-subject:
Basic Requirements for Blood Donation:
-Must be at least 18 years of age.
-Weigh at least 50 Kg for whole blood, platelet and plasma donation.
-Be in good general health with no history of hepatitis or HIV/AIDS
-The donation interval after a whole blood donation is 3 months for males and 4 months for females.
This study done on persons who come to donate blood since 1st of Oct 2008 to 31st May 2009. These donors are classified according to previous reasons.
Results: Elements and activities in promoting voluntary non-remunerated blood donation include

1- National blood donor program for the education, recruitment and retention of low-risk blood donors, including community-based voluntary blood donor organizations and youth programs;
2- Appointment of an officer responsible for the national blood donor program to include donor education, motivation, recruitment and retention
3- Development of partnerships with nongovernmental organizations, such as national Red Cross and Red Crescent societies, voluntary blood donor organizations, national service organizations and the media
4- Identification of donor populations at low risk for transfusion-transmissible infections and development of strategies to promote positive attitudes towards voluntary blood donation;
5- Development of donor education and recruitment materials;
6- Educational and media campaigns in workplaces, communities and educational institutions;
7- Establishment and maintenance of a database/register of donor records;
8- Guidelines and protocols for donor selection and deferral, donor confidentiality and donor care;
9- Guidelines on the management of donor sessions and blood collection;
10- Monitoring of TTIs in donor population;
11- Training of staff in pre- and post-donation counseling;
12- Donor notification and referral for counseling;

	Oct.	Nov.	Dec.	Jun.	Feb.	Mar.	Apr.	May
Age	608	640	668	648	634	681	681	627
Anemia	795	795	795	795	795	795	795	795
History of jaundice	136	136	136	136	136	136	136	136
Drug addiction	201	201	201	201	201	201	201	201
Dental interference within 6 mon	644	644	644	644	644	644	644	644
Surgical interference within 1 yr	192	192	192	192	192	192	192	192
Chronic disease	235	235	235	235	235	255	235	235
Others	729	729	729	729	729	729	729	729
Total deferral	3643	3643	3643	3643	3643	3643	3643	3643
Total acceptance	7427	7427	7427	7427	7427	7427	7427	7427
% bet. Deferred and attendance	32%	32%	32%	32%	32%	32%	32%	32%
Total attendance	10960	10960	10960	10960	10960	10960	10960	10960

Months	October	Nov	Dec	Jun	Feb	March	April	May
permanent	31.2%	35.8%	41.6%	38.7%	35.3%	33.8%	33.3%	33.9%
temporal	68.8%	64.2%	58.4%	61.6%	64.7%	66.2%	66.7%	66.1%



Conclusion: Individuals disqualified from donating blood are known as 'deferred' donors. A prospective donor may be deferred at any point during the collection and testing process. Whether or not a person is deferred temporarily or permanently will depend on the specific reason for disqualification (e.g., a person may be deferred temporarily because of anemia, a condition that is usually reversible). If a person is to be deferred, his or her name is entered into a list of deferred donors maintained by the blood center, often known as the 'deferral registry.' If a deferred donor attempts to give blood before the end of the deferral period, the donor will not be accepted for donation. Once the reason for the deferral no longer exists and the temporary deferral period has lapsed, the donor may return to the blood bank and be re-entered into the system

P-057
EXPERIENCE OF NUCLEIC ACID TEST (NAT) BLOOD SCREENING IN BANDUNG BLOOD TRANSFUSION CENTER, INDONESIA

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Background: Blood screening for blood borne pathogens ensures transfusion and recipient safety. Until mid February 2009, we used HBsAg and anti-HBc serology to screening out HBV infectious blood donations. Since then we replaced the anti-HBc test with the HBV nucleic acid test (NAT).
Aims: The aim of this study was to evaluate NAT blood screening test and to analyze the demographics of NAT-positive donors at Bandung Blood Transfusion Center.
Methods: Descriptive retrospective research design was done in this study. Subjects were blood donors during period of one month. Characteristic of subject were collected and blood screening test was done for HBV DNA, HCV RNA, and HIV-1 RNA using the Procleix Ultrio NAT assay. Demographics of NAT positive donors were compared with our overall donor population.
Results: During the period of June 2009, 3,000 blood samples were collected and tested. The overall demographics of all donors tested were: male 2308 (77%) and female 692 (33%), aged 18–60 years, and repeat donors 2723 (90.7%). While for the 56 NAT positive donors, their demographic distributions were: male 45 (80.35 %) and female 11 (19.65%), aged 18–57 years with a mean of 33.1 years, and repeat donors 53 (94.6%).
Conclusions: Our data indicate that NAT positive donors tended to be more male, with younger age 18 year and repeat donors.

P-058
VOLUNTARY BLOOD DONATION-INFORMATION EFFORTS AND APPROACH IN STUDENTS OF ELEMENTARY SCHOOL

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Background and objectives: The aim of the project was to inform the students about the voluntary social contribution and the offering to the society. They need to know how blood donation works in our country Greece. Volunteer students today will become volunteer adults tomorrow who will eagerly participate in blood donation as well. (I learn and get prepared to act)
Materials and methods: Experiential approach by a project. In detailed this programme has to combine in a whole system knowledge, emotion and psychokinetic targets.

- To inform students about voluntarism and introduce them in the voluntary organizations that are active in their country and abroad.
- To be acquainted with blood donor organizations in their region and in Greece generally
- To be aware of blood donation and the need of blood that many people have in our planet
- To familiarize with the blood donation idea and promotion of this idea
- To encourage parents, brothers, sisters and friends to become voluntary blood donors

- Parents and local community to cooperate with school and education in order to support the collaboration of school-family and school-local community

Results: As a result from a questioner, students at the end of the school year knew enough about blood donation. They reported that they will become voluntary blood donors in their adult life. Also students materialize an information project about this subject to their school mates.

Conclusions: We consider that such kind of programmes are very useful to school students of primary education. They are very eager to accept information about blood donation and voluntarism so they will become active volunteers in their adult life.

P-059

MALARIA AND DONOR DEFERRAL

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Background: The transmission of malaria by blood transfusion was one of the first recorded incidents of transfusion-transmitted infection. On global scale malaria remains one of the most common transfusion-transmitted infections. Most part of Saudi Arabia is endemic for malaria, ensuring that the blood supply is free from malaria is problematical, especially as travel to malarias areas is increasing and there is some spread of the disease into new areas.

Aim: To review and update our practice in accepting donors with malarial history or visit to endemic malarial area and to compare our policy to other international policy for acceptance of donors visiting malarial endemic area or had history of malaria.

Method: Retrospective analysis of donor data during the year 2008, following our local policy which is to defer donors with history of malaria permanently and those visited to malarial endemic area for one year.

Result: During the year 2008 total number of donors were 11215, total accepted were 10213 (91.06% of total donors). Number of donors rejected were 1002 (8.93% of total donors), out of these total number of donor rejected on history of malaria permanently were 206(20.5% of total donors rejected and 1.80% of total donors) and total number of donor rejected on the basis of visit to endemic malaria area temporarily were 202 (20.1% of total donors rejected and 1.80% of total donor).

Conclusion: According to our result the rate of deferral for malaria were 40% of total donor deferral and around 3.6% of total donors accepted which is considered high. We conclude that we need to update our policy in accepting donors. If donor had malaria to defer them temporarily for 3 years after becoming asymptomatic and through performing validated test for malarial antibody which will add to safety, if the test is negative we can return them in to the donor pool.

P-060

MARKETING RESEARCH TO OBTAIN THE BEST MARKETING STRATEGY FOR RECRUITING VOLUNTRY BLOOD DONORS (NBTS OF EGYPT)

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Background: One of The National Blood Transfusion Services of Egypt strategic goals is to build a strong brand name among both the donors and the patients. Understanding that brands are more than just names and symbols. They are a key element in the organization's relationship with our both types of customers.

Aim: The main obstacle that we face in achieving our goals is the limited community awareness of the blood donation concept, this is why we have to promote for the increase of community culture orientation about blood donation together with promoting for the NBTS.

Methods:

-Products and services marketshare analysis The BCG growth- share matrix.
-Customer behavior analysis Customer relationship Groups.
-Competitor analysis Bench marketing.
- SWOT analysis

Results:

- When analyzing our products and Services we found that:
- In Blood donation we have a unique customer oriented strategy, Donor Care programs, Donor recall and retention programs, all of these criteria promotes us to further growing in our market share of blood donors.(Star)
- In our Screening and Confirmatory laboratories our customer's needs and wants are met to the highest quality standards, though we expect to expand our product line. (Star)
- In the field of Blood Transfusion we provide this service to almost all of the patients in our region, this is the most profitable business unit in our organization. (Cash cow)
- When studying the factors influencing our consumer behavior we found:
- Cultural factors: We are still lacking the regular voluntary blood donation value in our culture which is unfortunately due to lack of knowledge in almost all of the social classes in our community.
- Social factors: in promoting for our service we target the opinion leaders in every social group that have social influence on others.
- Personal factors: In blood donors we target both genders between the age 18 till the age of 65. Regardless of their occupation or economic situation. But we focus on people with more committed type life style because they are the safest group to obtain blood units from.
- Psychological factors: we are making efforts for changing the people beliefs and attitudes toward blood donation this could only be achieved through learning more about it.

Table 1: BCG Matrix

The BCG growth- share matrix

Star	Question mark
-Blood donation -Screening and Confirmatory laboratories	-Training and research
Cash cow	**Dog**
-Blood transfusion department	

Table 2: Bench marketing

	NBTS	University blood banks	MOHP blood banks	Private blood banks
Prices	++	++	+++	+
Quality	+++	++	+	+
Customer satisfaction	+++	+	+	++
Personnel training	+++	+	+	+
Safety	+++	+	+	++
Current profitability	++	++	+	+++
Market share growth	+++	+	+	+
Cash flow	++	++	+	+++
Technological leadership	+++	+	+	+
Service leadership	+++	+	+	+
Style & Image	+++	++	+	++

Conclusion:

- Though we had varied success with our current marketing approaches, but we struggles to both retain existing donors and connect with new

donor populations. So, its challenge was simple - reverse the trend by pumping new life back into its blood donation efforts
- We suggest working from a donor database, creating custom segments; segments were organized based upon their similarities in terms of their propensity and commitment level to donation. Armed with this information, we can:
- Identify optimal locations for fixed and mobile site growth
- Identify and quantify growth opportunities by segment
- Identify and quantify needs of donors to determine priorities for development
- Optimally target messages and services to attract donors

P-061

BLOOD DONOR CHARACTERISTICS EVALUATED IN REGULAR VERSUS NOVEL BLOOD DONOR DRIVE

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Background: Establishment of 100% volunteer non remunerated regular blood donor pool is the prime aim of any transfusion service. Organization of mobile blood donor drives is an important aspect in the process of developing such a donor pool. Mobile blood donor drives provide the opportunity for majority of individuals to donate blood at a convenient location and time frame in their busy schedules. Some mobile blood donor drives are organized at regular intervals at the same location.
Aim: Evaluate the characteristics of blood donors participate at mobile drives done in regular basis and to compare them with donors participating in a blood donation drive organized for the first time.
Method: The data was collected at two mobile blood donor drives conducted by the blood bank of Colombo North Teaching Hospital. The blood bank is one of the regional centers of the National Blood Transfusion Service (NBTS) of Sri Lanka. The regular blood donor drive was conducted annually for the 15th time in the same location in contrast to the other program organized for the first time in a different location. Both were done in relation to religious festival one in a church and the next in a temple. Donors were selected according to the donor selection criteria adopted by the NBTS including pre donation counseling and a medical check done by a medical officer of the transfusion service along with standard haemoglobin estimation. Donor characteristics were evaluated calculating the age and sex distribution of the donors and compared the number of first time donors versus regular donors in the two groups.
Results: There were 128 donors in the regular blood donor drive and a total of 76 individuals donated blood in the novel mobile. Out of the donors 63.28% had donated blood more than once in the regular blood donor drive in contrast to the 44.7% seen in the mobile organized for the first time. Percentage of female donors was 8.5% and 18.4% in the two groups. The age distribution was comparatively similar in both groups as represented in the table

	Age distribution in years (Percentage)			
	18 - 25	26 - 35	36 - 45	46 - 55
Regular blood donor drive	33 (25·7)	54 (42·1)	30 (23·4)	11 (8·5)
Novel blood donor drive	24 (31·5)	29 (38·1)	23 (30·2)	3 (3·9)

Conclusion: Majority of the donors were first time donors in the novel blood donation drive whereas there were more regular donors observed in the other group. Overall participation of the female donors were poor in both groups which is also the trend seen in the national statistics as well. In conclusion organization of regular blood donor drives in the same location at regular intervals encourage donors to donate blood regularly enabling the transfusion service to reach the target of 100% volunteer non remunerated regular blood donors with safe blood.

P-062

INCIDENCE OF HBSAG, ANTI HCV ANTIBODIES & ANTI HIV ANTIBODIES AMONG THE BLOOD DONORS IN NORTH CENTRAL PROVINCE; SRI LANKA

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Background: Transfusion transmitted infections spread through the blood & blood component & therefore it is mandatory to screen all the donor blood units for HIV, HCV, & HBV markers. The North Central Regional Blood Centre test for above markers on each & every blood unit collected from approximately 1.2 million populations, by Anuradhapura, Polonnaruwa & Vavuniya blood banks.
Aim: To determine the incidences of HBsAg, Anti HCV & Anti HIV antibodies among the blood donors in north central province of Sri Lanka during 2007 & 2008.
Method: 50,203 units of blood received by the Regional Blood Centre of North Central Province were tested for HBsAg, Anti HCV antibodies & Anti HIV antibodies using EIA from Ortho Clinical Diagnostic Vitros ECI.
Results: From the total of 50,203 units screened, Positive HBsAg found in 135 sample (0.26%), Positive Anti HCV Antibodies found in 180 (0.35%) & Positive anti HIV antibodies found in 85 (0.16%). Majority of the positives found in first visit male donors (87%).
Conclusion: Incidences of positive viral screening markers for Hepatitis B, Hepatitis C & HIV were higher in first visit donors than the regular donors. Correct donor selection through proper counselling & education along with compulsory screening of donor units for HBsAg, Anti HCV antibodies & anti HIV antibodies reduce the risk of transfusion transmitted infections.

P-063

DONOR RECRUITMENT - MOTIVASION

Wijenayake APH
National Blood Transfusion Service - Sri Lanka, Narahenpita, Sri Lanka

National Blood Transfusion Service of Sri Lanka is one of the best transfusion services in south East Asia Region. 92%of the total collection is donated by voluntary, non-remunerated (VNR) donors. There are two ways of collecting Blood. From walk-in donors to blood banks and from the donors of mobile blood donation drives. Total collection of blood is gradually increased over the past years and in 2008 it has reached 320,000 units. Surveys conducted by NBTS revealed that most of our mobile donation drive organizers are well educated about the importance of safe blood donations.
Maintaining ample blood stocks at a reasonable level throughout the year is one of the most important factors. Fluctuations of stock levels are noticeable as during Buddhist religious festival season, collection is very high because most Buddhists donate blood during these seasons. During Sinhala and Hindu New Year period it sharply drops as many people engaged with family & social activities.
Apart from other promotional activities of NBTS to maintain adequate blood stocks, we invite the volunteer organizers of blood donation drives and assign mutually agreed targets within their capacity. And one gentleman who supports us wholeheartedly since late 1990s, Mr. Dudley Ashoka Liyanage of Asarana Sarana Foundation was assign with the highest target to arrange 50 mobile drives during the year 2008. Unbelievably he succeeded with organizing 60 drives and collected blood amounting to 5811 pints of blood from VNR donors. Majority of his

collection sites were temples and of religious Importance and organizes on days where many devotees are gathering. He has scheduled donation drives every week or even twice a week. During mobile collection drives he does not forget to provide refreshments to donors. Apart from Banners & Posters for publicity, he sends personal invitations or makes personal phone calls to regular donors to invite. Priests and leaders too pass the message of importance of blood donation.
Another fact of significant importance is that majority of these donors are regular donors and the safety level of the blood is very high and walk-in donors too become regular. He is now self motivated and his own target for 2009 is to collect over 7500 pints of blood by increasing mobile drives to 75. One might question why he is doing this. His answer was that he is from a wealthy family and does not want to earn anything more to live the rest of his life and he wants to do something remarkable to his fellow citizens. In Sri Lanka it is not so easy to find sufficient funds for organizing such events. However people who have adequate money but finds difficult to organize such events are routed to him. He is now well known in the community for his silent commitment.
In appreciation of his activities NBTS felicitated him few times at WBDD celebrations. In our news bulletin last year his program was highly commended.
By publishing these real stories if we can encourage and inspire another, it is an achievement''

P-064

A REAL LIFE STRATEGIES IN DONOR RECRUITMENT

Wijenayake APH
National Blood Transfusion Service - Sri Lanka, Narahenpita, Sri Lanka

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P-065

WHOLE BLOOD DONOR DEFERRAL PATTERN IN A SRI LANKAN POPULATION

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Background: Whole blood donors are deferred due to various grounds on temporary & permanent basis. North Central Regional Blood Bank collects blood from the northern part of the country. The collection was increased remarkably during last 2yrs with the presence of civil war in the country. A total of 37,713 units were collected in & around Anuradhapura district area from 1st of January to 31st of December 2008.
Aim: To identify the donor deferral pattern in this part of the country.
Method: In this retrospective study 3,525 (8.55%) were found to be deferred out of 41,238 donor declaration forms. The deferrals were categorized as temporary (< 365 days) & permanent (>365 days), on age intervals (< 30 years, 30–50 years, >50 years), first time & repeated donors.
Results: Of the 3,525 deferrals 65.08% were males & 34.92% were females (respective non deferred donor rates were 80.05% & 19.95%) & 59% were first time donors (50.1% males & 75.8% females). Most of the deferrals were on temporary basis (89.59%; 3,158 cases). Majority of the deferral donors belong to 30–50 years (50.04%) which is in parallel with the same finding in the non deferral donor age group. The order of the type of deferrals for male & females were different. Top five reasons for male deferrals were; on medications 18.13% (416), having infected wounds 14.99% (344), elevated blood pressure 12.9% (296), elevated temperature or sore throat 10.07% (231) & returning to donate blood in less than 4 months 9.41% (216). For females the low Hb was the main reason (25.59%;315) & other main reasons were, on medications (19%), elevated blood pressure (17.62%), having infected wounds (6.17%)& elevated temperature or sore throat (5.11%). Commonest causes for permanent deferral were the presence of chronic illnesses (30.11%;111) & history of hepatitis (28.4%;104). The homosexuality found at a low percentage of male donors only (0.34%; 8 cases) & high risk sexual behavior other than homosexuality in 1.74% males. A significant number of males deferred due to the age less than 18 years (4.4%)
Conclusion: the donor deferral pattern varies across the world probably due to different geographical, cultural & economic factors.

P-065a

DEVELOPING A PANDEMIC COMMUNICATIONS PLAN TEMPLATE FOR THE NATIONAL BLOOD PROGRAMME IN SINGAPORE

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Background: The biggest threat during a pandemic is the drop in blood supply due to additional donor deferral criteria, decreased donor attendance and cancellation of mobile blood drives. In Singapore, donor attendance fell by nearly 50% during the peak of the Severe Acute Respiratory Syndrome (SARS) epidemic in 2003.
The current global Influenza A(H1N1-2009) pandemic outbreak presents a myriad of blood safety and supply issues, including donor deferral and potential risk of H1N1 transmission through blood. As part of emergency planning, communications strategies with pre-scripted messages must be

developed ahead of time for such potential pandemic scenarios to reassure public of the safety of donating blood, as well as imparting donor deferral information.

Aims: This poster presentation outlines the pandemic preparedness communications plan that has been developed by Singapore's Health Sciences Authority (HSA) with inputs from the Singapore Red Cross (SRC) to address the impact and risks of the ongoing Influenza A(H1N1-2009) pandemic on the National Blood Programme in Singapore and its blood supply.

Methods: A detailed communications plan template for different scenarios complete with key messages, holding statements, releases, updates and frequently asked questions, based on different alert levels of Singapore's Disease Outbreak Response System [DORSCON]. The communications plan template was developed by the HSA Corporate Communications Department in consultation with HSA Blood Services Group. Inputs were sought from SRC through a joint HSA-SRC communications workgroup committee, which was set up since 2006.

Results: A detailed communications plan template was first drawn up in 2004 for various emergency scenarios of different blood supply needs. One scenario is a pandemic outbreak, and in 2006, the template was reviewed and updated in anticipation of the possible avian flu pandemic. This template for avian flu pandemic serves as a critical time-saving tool that enables the quick revisit, review and revision of the various messages for the rapidly evolving H1N1 situation. It provides a ready base to work out specific messages that can effectively convey desired information at different stages of the anticipated pandemic.

Summary/conclusions: While the potential impact of pandemic influenza on the safety of the blood supply is likely to be minimal because of its limited viraemia, there is more concern of its potential impact on the availability of blood supply. Hence, a key element of a response and business continuity plan must include a communication strategy which pre-defines, as far as possible, a consistent pandemic communication plan, complete with tailored communications to specific target audiences to address different issues in anticipated scenarios.

This template will continue to serve as a long-term communications tool for any emerging infectious disease outbreak.

2.2. Blood Donation Blood collection

P-066

PLATELET APHERESIS WITH A SINGLE NEEDLE FROM FEMALE DONORS WITH 3200ML OR LESS TBV – USING THE TRIMA ACCEL AUTOMATED BLOOD APHERESIS MACHINE

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Background: In Japan, Female blood donors tend to have low body weight and to have lower total circulating blood volume. And the intermittent flow apheresis centrifugation system with a single needle is widely available for platelet collection, However, high ECV per cycle (400–500 mL) is a restricting factor. Plasma apheresis collection has been considered to be preferable choice for the donors whose TBV are less than 3200 mL. In this study, PC-10 (platelet count:2–10e11 /bag or more) from these donors with 3200 mL or less TBV has been collected successfully.
Method: Apheresis device was Trima Accel version 5.1. This is the single needle platelet collection system with the continuous flow centrifuge, AVF 17 gauge needle and 196 mL ECV.
Target donors are 46 Female volunteers with 3200 mL or less TBV. At pre-counting, Hct is 36% or more, PLT is more than 26–104./μL, platelet collection target is 2.3~2.5–10e11/bag.
Results: The hematological data of target donors were was 3023±96 mL TBV. 39.5±1.5% Hct, 29.2±3.3–104/μL PLT. The blood processed volume was 1581±124 mL, the procedure time was 60±9min, the draw speed was 36±2 mL, ACD-A consumption was 197±15 mL, and platelet efficiency was 56.5±7.9%. In this study, PC-15 (3.0–10e11/bag) was collected at three cases, PC-10 (2.5±0.3–10e11/bag) was at 34 cases, and PC-5 (1.6±0.2–10e11 / bag) was at nine cases.
Discussion: In this study, PC-10 was able to obtained at the case of 80%. However, it was resulted as PC-5 products at nine cases. Reviewing these nine cases, four cases of PLT pre-count were ranged in (22.4 ~ 24.4)- 104/μL that was lower than the set criteria. Other five cases, the draw flow rate was maintained constantly during the procedure, but draw speed was slowed down and procedure time is prolonged. That is, draw rate became slow in the range of 30 to 38 mL/min, and no anticipated blood collection related side-effect incident was reported.
Conclusion: Apheresis blood collection with single arm becomes to an only and a standard procedure in Japan today. It is now possible to collect PC-10 from low body weight female donor using single arm continuous apheresis device of Trima Accel by maintaining maximum blood flow and by selecting optimum blood vessel and right vein-puncture procedure.

P-067

THE PREVALENCE OF LIPAEMIA AMONG DONORS IN KAOHSIUNG BLOOD CENTER IN 2007

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Background: The Kaohsiung Blood Center is located in the southern Taiwan, with jurisdiction areas covering Kaohsiung City, Kaohsiung County, Pingtung County, and Penghu County. According to the 2007 database, the whole population in these areas was 3,743,227 and the donation counts were 311,133 (inclusive of aphaeresis donors and whole blood donors), and the donation rate was 8.31%. A diverse workforce constitutes the occupational structure - the urban areas being commercial or service-industry oriented and the rural areas focused on agriculture and fishery. Due to the westernization of the Taiwanese diet (as a result of rapid internationalization), delicate foods high in saturated fat and low in dietary fiber prevail and people lack for exercise, so health issues concerning metabolic problems have been increasing. The most notable case for Kaohsiung Blood Center is lipaemia (the presence of lipid in the blood). The purpose of this research is to analyze the occurrence of lipaemia among blood donors, and to seek and find probable causes. The findings shall help develop related public health education, provide guidelines for governing the practice of blood collection, and raise the overall quality of blood donation.
Material and method: To gain a grasp of the prevalence of lipaemia among donors at the Kaohsiung Blood Center, we took a visual inspection of the entire blood bags in the year 2007 by placing each blood bag's plasma portion against the Lipaemia Grid, which is roughly Triglyceride 500mg/dl. The data were performed with non-paired *t*-test, frequency analysis, and Chi-Square Test by SPSS 14.0.
Result: The prevalence of lipaemia of men is four times as much as that of women, and the more potential incidence rises among those who are (1) overweight, (2) over the age of 30, and (3) with higher ALT. With respect to occupation, the highest prevalence belongs to people in agriculture and fishery up to 3.7%, the secondary laborers 2.3% and the lowest students 0.4%.
Discussion: Contributing to the prevalence of lipaemia among male adults over 30, and obese, the probable causes are these groups of people are considered financially stable, much less moving about physically and with slowing metabolism (all lead to the deposit of fat in their body). Besides the shifting lifestyle, diet is also an important factor. Those in agriculture and hard labor industry tend to acquire food high in fat content and those in commerce may not labor more than the former, but dine out and acquire delicate food more often. The students, on the other hand, have the least chance to have lipaemia, owing to their higher physical activity level and relatively simple diet.

P-068

COMPARISON OF TWO METHODS OF MEASUREMENT HEMOGLOBIN LEVEL BETWEEN CAPILLARY AND VENOUS PUNCTURE

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Background: Hemoglobin level is one of indicator for selection of blood donation. To improve quality of blood, several method is needed to get a good performance of hemoglobin level. This research is conducted to compare between measurement of hemoglobin level from capillary and venous puncture.
Aim: To compare and analyze the measurement of hemoglobin level from capillary and venous puncture.
Method: This research was conducted in Central Blood Center Indonesian Red Cross with 136 samples on May 2009. Each sample will get the examination and take the blood sample from capillary and venous puncture. Sample from capillary puncture analyzed by photometer method and venous puncture analyzed by SLS method. Results of hemoglobin level will be analyzed with comparing means using SPSS 11.5.
Results and conclusions: From 136 samples, there is no significant difference between measurement of hemoglobin level from capillary and venous puncture. Mean of hemoglobin level from capillary puncture is 13 864 g/dL and venous puncture is 13 421 g/dL. This result correlate with the previous research, which is hemoglobin level from capillary and venous puncture have difference range between 0. g/dL and 1 g/dL(1,2,3).
From this research, the conclusion is measurement of hemoglobin level from capillary puncture can represent hemoglobin level on the blood bag which is collected from the donor, even though the result can be higher or lower than venous puncture. After that, on selection of blood donation can use photometer method from capillary puncture to check the hemoglobin level of blood donor.

P-069

COMPARISON STUDY ON THE YIELD OF GRANULOCYTE COLLECTIONS WITH AND WITHOUT THE USE OF HYDROXYETHYL STARCH

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Background: Granulocyte apheresis from healthy donors are used as therapeutic means of treatment to prevent fungal and bacterial infections for severely neutropenic patients. A significant dose of at least >1 x 10^10 cells is required to establish an immune response towards an infection [1, 2]. Growth factors such as granulocyte colony stimulating factor (G-CSF) are often administered to donors to obtain a greater yield of granulocyte collection. In addition, high molecular weight hydroxyethyl starch (HES) is often used as a sedimenting agent to coat red blood cells in order to obtain good separation of granulocyte and hence a better yield. HES use and the return of HES coated red blood to the donor however may be associated with side effects such as hypocoagulation, headache, peripheral edema and allergy reaction. In addition, HES are volume expander hence might result in potential contraindications and adverse effects on donor's cardiac and renal function.
Aims: We look at our granulocyte collection results with and without the use of HES, and demonstrate that a granulocyte yield of >1 x 10^10 cells can be achieved even without the use of this agent.
Methods: A total of 29 harvests had been performed for two recipients (25 and 4 respectively) between December 2008 and June 2009. All donors were given 8 mg oral Dexamethasone and 300 mcg S/C GCSF injection 12 hours before collection. All harvests were performed using COBE® Spectra' apheresis system. Granulocyte cells were collected at a hematocrit of 7.5%. 25 harvests intended for the first recipient were performed without the use of HES while the 4 harvests for the second recipient used an anticoagulant preparation that contains 30 mL of triCitrasol to 500 mL of the 6% solution of HES (AC ratio 13:1). Cell counts were measured using ADVIA cell counter.
Results: Successful collection of >1 x 10^10 absolute neutrophil count were achieved for 22 out of 25 harvests intended for the first recipient and all 4 harvests for the second recipient.
The median granulocyte yield without the use of HES was 4.54 x 10^10 (range: 0.53 – 11.96 x 10^10), as compared to the median collection of 9.01 x 1010 (range: 2.78 – 12.06 x 10^10) with the use of HES.
Conclusions: Our results show that although the use of HES allows a higher median granulocyte yield, however a good collection is still achievable without its use. Given the concerns of the potential side effects of HES, the collection of granulocytes without this agent can be considered with good feasibility.
References:

1. Vamvakas EC, Pineda AA. Meta-analysis of clinical studies of the efficacy of granulocyte transfusions in the treatment of bacterial sepsis. J Clin Apheresis. 1996;11:1–9.
2. Engelfriet CO, Reesink HW. Granulocyte transfusions. Vox Sanguinis. 2000;79:59–96.

P-070

PLATELET INVENTORY MANAGEMENT

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Background: Maintaining a stable inventory level is a challenging task for a Blood Center, especially for platelets because of their short 5-day shelf-life. It is therefore crucial to improve collection strategies to better respond to hospital demands.
Aims: We have developed a tool to optimize platelet collection and inventory management.
Methods: At our Center, the platelet supply strategy is based on a proportion of 80% collected by apheresis (single and double) and 20 % prepared from PRP (platelet-rich-plasma) from whole blood donation. This strategy gives us increased flexibility in the choice of collection sites since the 8 hour delay for platelet production from whole blood becomes less of an issue. The tool was developed using Microsoft Excel. It is based on the analysis of the total number of platelets (from whole blood and by apheresis) shipped to hospitals over a 52 week period (from 04/01/2007 to 03/31/2008). From this analysis, we were able to create collection menus indicating the number of donations required per product, by ABO and CMV, per day of the week. Ten (10) menus were constructed, ranging from 2700 to 3600 total platelets shipped weekly, to account for seasonality and/or sudden fluctuations in hospital demand.
Results: The tool enables better planning for the collection of platelets and allows for adjustments resulting from changes in hospital demand - either in daily requirements or in the ABO CMV distribution. In terms of efficiency, the outdate rate for total platelets dropped by 67% in two years and the platelet inventory is managed on the FIFO (first-in, first-out) principle.
Conclusion: By using the tool described, platelet collection can be calculated more accurately and the Blood Centre's inventory can be managed more effectively. Although the tool is updated on a regular basis, collections and shipments need to be closely monitored in order to maintain stable inventory levels.

P-071

WILL BLOOD DRIVE VOLUNTEERS BE AVAILABLE IN THE FUTURE?

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Background: The Blood Centre, a non-profit organization, is responsible for managing the blood supply to service 90 hospitals and needs to collect some 1 000 donations a day to meet their demands. The collection strategy is organized in such a way as to maintain an inventory of at least 6 days in each blood group. This collective blood supply is obtained from three fixed sites, a mobile unit, and nearly 2 000 blood drives the latter providing 85 % of the total blood collected. Historically, volunteers have played a crucial role in the organization of the blood drives and 16 000 volunteers participate in these drives every year. They come from various backgrounds: 67% from local groups, 11% from municipalities, 10% from the educational sector, 7% from the business sector and 5% by government organizations. There are currently concerns about the sustainability of volunteer resources over the medium and long terms: the volunteers' average age is continuing to rise, the local organizations are finding it difficult to attract enough new members, and, because members may participate in various causes in their communities, they are often prone to exhaustion.
Aims: The objectives of this research project are to document the organizational structure of the blood drives, understand the role that volunteers play, and reflect on ways of fostering the recruitment and retention of new volunteers.
Methods: Sixty five semi-structured interviews were performed with staff and volunteers involved in organizing blood drives,each interview lasting an average of two hours. The main themes covered were: the history of the blood drives' organizational structure, the reasons why the volunteer plays a key role in these drives, the evolution of the volunteer's involvement, and the volunteer's motivations for associating with the cause of blood donation.
Results: Preliminary data show that new volunteers are mainly recruited from within existing volunteers' personal social networks. Newly recruited volunteers initially feel obliged to meet this demand due to their personal relationships with the volunteers who have recruited them. Other motivations soon also emerge to ensure the retention of these volunteers: developing a new circle of friends, using their skills and participating in a cause that they believe in. Volunteers thus play a key role in renewing the resources needed to organize blood drives, but the fact that they are only recruiting people within their personal network limits the Blood Centre's capacity to draw upon a larger circle of resources (younger people, less traditional organizations, and people from other ethnic backgrounds in

urban areas). Moreover, volunteers are not aware of their importance in the recruitment process and are given little training in this area.
Conclusion: The first results already indicate that to ensure volunteer sustainability, the Blood Centre will have to improve the training of volunteers as ''recruiters''of others. It will also have to consider alternate recruiting methods to ensure a diversity of volunteer resources: partnerships with new local organizations and an increased emphasis on blood drives in the institutional and corporate sectors.

P-072

COMPARISON OF THE EFFICIENCY OF THE THREE SEPARATORS IN COLLECTING DOUBLE-UNIT APHERESIS PLATELET

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Background: Apheresis platelet is often preferred then pooled random platelets as it reduces the risk of platelet refractoriness and alloimmunisation. This is especially true in patients with haematological malignancy who require frequent platelet transfusion.
Aims: The aim of this study is to assess the efficiency of our three separators.
Methods: This is a retrospective study, which looks into the apheresis platelet collected by our separators, namely Amicus, MCS+ and Trima. Double-unit apheresis platelet were considered when the actual platelet yield was more than 6–10^11 cells/ul in one apheresis session. By default, the predicted platelet yield of 6.5×10^11 /ul was set for the donors with pre-platelet count of greater than 300 ×10^3 cells/ul and weight of greater than 80 kg. The yield estimator will then calculate the amount of whole blood to be processed based on the donor's weight, height, hematocrit and pre-platelet count. All pre-procedure and post-procedure platelet counts and actual platelet yields were performed with the same automated cell counter. The processing time (mins) is the time taken for double unit collection from the start of the procedure until reinfusion. Coefficient of linearity (R^2) was calculated based on actual platelet yield versus the processing time. The efficiency of the separators were assessed based on R^2, mean ratio of actual platelet yield over the predicted platelet yield (A) and ratio of actual platelet yield over processing time (B).
Results: A total of 467 apheresis donations were collected between January 2008 and April 2009. The results are summarized in the table below.

Table 1: Summarized Table of Apheresis Platelets Collection

16 months (Jan 2008- Apr 2009)			
Platelet Separator	Amicus	MCS+	Trima
Total no of collections (n)	266	101	100
Total no of double units apheresis platelet	65 (24%)	6 (6%)	7 (7%)
Processing time (mins)			
Mean ± SD	76 ±13	84 ±15	61 ±15
Median	76	89	57
Range	50-100	62-100	43-84
Actual Platelet Yield ($x10^{11}$ cells/ul)			
Mean ± SD	7·16 ± 0·66	7·50 ±1·72	6·36 ± 0·17
Median	7·07	6·73	6·4
Mean Ratio (Actual Platelet Yield / Predicted Yield)	1·10	1·15	0·98
Actual Platelet Yield / Processing Time ($x10^9$ cells/ul/min)	9·4	8·9	10·4
Coefficient of Linearity (R^2)	0·0157	0·0227	0·2252

Conclusion: The findings suggest that Trima is probably the more efficient separator as it gave the value of R^2 and A that is closer to 1.0 with a higher value of B. These indicate that Trima gives a more accurate predicted platelet yield and higher platelet yield in a shorter processing time. However, the number of double units apheresis platelet collected from Trima was only 7% of the total apheresis performed. These were mainly due to the donors' data which disqualified them from double unit collection. The above findings will need further validation by doing a randomized prospective study on the three separators.

2.3. Blood Donation Donor adverse events

P-073

DIFFERENT METABOLIC EFFECTS OF CITRATE EXPOSURE IN HEALTHY VOLUNTEERS

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Background: Current data about metabolic effect of citrate anticoagulation are mostly based on western population. Better understanding the possible difference between races and even genders would be very helpful to instruct daily work domestically or to cooperate internationally.

Aims: To investigate the difference of metabolic effects induced by citrate exposure between genders and races in healthy volunteers.

Methods: A cross over, placebo-controlled study was conducted. A total of 22 age-matched Chinese (11 males and 11 females) and 10 male Caucasian volunteers were enrolled after informed consents were obtained. Volunteers received either a standardized infusion of citrate at 1.5 mg/kg body weight/min or the equal volume of placebo, separated by a wash-out period of two to three weeks. Serial blood and urine samples were collected during observation period for the determination of electrolytes and albumin.

Results: (1) Citrate intervention led to more pronounced decrease of ionized calcium level in Chinese females compared to Chinese males [(28.65Â ± 4.46)% Vs. (23.03Â ± 3.78)%, P<0.005], which was independent of serum basal level of albumin and ionized calcium. Excretions of urine calcium were increased among Chinese volunteers due to citrate load (before 0.39Â ± 0.32 Vs. after 0.92Â ± 0.45ï¼ŒP<0.0001)ï¼Œthe percentage increases of urine calcium excretion were correlated to the percentage decreases of serum ionized calcium (P = 0.037ï¼Œr = 0.457). There were not difference in the changes of serum phosphate, magnesium and albumin between Chinese males and females under the citrate intervention. (2) No difference was detected in the change pattern and extent of serum ionized calcium, phosphate, magnesium, albumin and urine calcium excretion between Chinese males and Caucasian males due to citrate infusion, except Chinese males had a higher basal level of serum ionized calcium [ï¼1.27Â ± 0.04ï¼‰ mmol/L Vsï¼1.22Â ± 0.02ï¼‰mmol/Lï¼ŒP = 0.0015] and higher amplitude of biological rhythm in serum level of albumin (P<0.0001).

Conclusions: Citrate anticoagulant exhibits a gender-favor effect on calcium metabolism, with a steeper decline of serum ionized calcium level in female than male when introduce. This effect might basically be responsible for the higher rate of citrate-related side effects occurred in female donors during plateletapheresis.

P-074

EFFECTS OF CALCIUM SUPPLEMENTS ON IMPROVING ACUTE CITRATE-RELATED SYMPTOMS

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Background: Citrate toxicity side effects occurred frequently during plateletapheresis. However, the donor-dependent characteristic and the efficacy of calcium supplement in reducing the frequency of citrate-related symptoms have not been fully discussed although calcium administration has been used as a prevention measure in some collection centers.

Aims: To evaluate the therapeutic effects of calcium supplement on improving the acute citrate-related symptoms.

Methods: A cross over, placebo-controlled study was conducted.A total of 22 volunteers with age-matched were enrolled after informed consents were obtained. Volunteers received four standardized interventions containing: A, placebo (saline solution) infusion only; B, citrate infusion only; C, citrate infusion plus 1.2 g oral calcium administration 10 minutes before citrate infusion; D, citrate infusion plus simultaneous intravenous infusion of calcium at the rate of 100 mg/h. Citrate infusions were performed in the fixed dose of 1.5 mg/k.w/min and the fixed duration of 80 minutes. During the observation period, Citrate toxicity symptoms were registered and the serial blood samples were collected for the determination of ionized calcium level.

Results: The most frequent symptoms due to citrate intervention were anaesthesia of labia oris, prosopo and limbs, chest distress, dizziness and nausea etc. At the end of intervention B (80 mins), symptomized volunteers had lower ionized calcium level compared to non-symptomized volunteers [t=2.767ï¼ŒP<0.05].

Women experienced more frequent symptoms than men ï¼x2 = 6.6, P<0.05ï¼‰associated with lower ionized calcium level in the females than the males at the end of intervention B and two hours after ï¼t=3.47ï¼ŒP<0.01ï¼'t=2.63ï¼ŒP<0.05 respectivelyï¼‰ï¼'â'

- Both calcium administrations obviously relieved the citrate toxicity symptoms ; Intervention D had a better effect than intervention C, and intervention C had a better effect than intervention B ï¼x2 = 17.2-ï¼ŒP<0.05ï¼‰. Intervention D resulted in a higher ionized calcium level in whole intervention period when compared to intervention C and Bï¼F = 10.03 and F = 14.852ï¼ŒP<0.01 respectivelyï¼‰, while the difference of ionized calcium level was less significant between intervention C and B during the intervention period except intervention C showing more rapid recovery of ionized calcium level to the basal and higher ionized calcium level than intervention B two hours after end of citrate infusionï¼F = 14.277ï¼ŒP<0.01ï¼‰.

No correlation were observed between the frequency of symptoms occurred and the body weight.

Conclusions: Citrate-related symptoms significantly correlate with the decreases of ionized calcium level. Female experienced more frequent symptoms and steeper decline of serum ionized calcium than male due to citrate exposure. Both calcium supplement, either by oral or intravenous infusion, had the effects to lessen the citrate toxicity symptoms. The effects by intravenous infusion are better than that of oral administration.

P-075

CALCIUM SUPPLEMENTS ATTENUATE THE ELEVATED BONE TURNOVER INDUCED BY ACUTE CITRATE LOAD

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Background: Short-term increase of bone turnover in healthy volunteers has been noticed when citrate was administrated in the model as routine plateletapheresis. Whether calcium supplement can serve as a prevention measure to attenuate this change by means of increasing the serum ionized level remains unclear.

Aims: To investigate the effect of calcium supplements on the elevated bone turnover induced by acute citrate load.

Methods: A crossover, controlled study was conducted in 22 volunteers after informed consents were signed. Volunteers received four standardized interventions with a interval two to three to weeks in random order, containing: treat A, placebo (saline solution) infusion only; treat B, citrate infusion only; treat C, citrate infusion plus 1.2 gram oral calcium administration 10 minutes before citrate infusion; treat D, citrate infusion plus simultaneous intravenous (i.v.) infusion of calcium at the rate of 100 mg/h. Citrate infusions were performed in the fixed dose of 1.5 mg/k.w/min, fixed duration of 80 minutes and the fixed starting time of 8:30 am. Serial blood samples were collected for the determination of ionized calcium, intact parathyroid hormone and bone markers C-telopeptide of type 1 collagen (CTX) and osteocalcin (OC) at the start of infusion, in the middle and at the end of the infusion, and 120 minutes after completion of infusion and 24 hours later.

Results: Treat B resulted in a continuous increase in serum levels of the bone formation marker OC [(39.8 ± 16.8)% for peak] and bone resorption marker CTX[(61.9 ± 34.4)% for peak], followed by a slow recovery after the end of intervention. Both calcium administrations depressed the increment of CTX at the endpoint of citrate infusion (i.v calcium) or afterward (oral calcium), and rapidly normalized the serum level of CTX to the basal two hours after end of citrate infusion, which was still retained higher level in treat B [(36.0 ± 30.4)%]. While calcium administrations have no effects on the changes of elevated serum bone formation marker OC induced by citrate exposure. Changes of CTX were correlated to the changes of ionized calcium in treat C and D ($P < 0.005$). Further analysis showed that i.v. supplement of calcium significantly enhanced the ratio of OC/CTX during the period from starting infusion to two hours after end of infusion when compared to treat B, while similar effect for treat C was only detected after end of citrate infusion. No differences were detected for all determinants after 24 hours among four interventions.
Conclusions: Calcium supplement has effect to attenuate the short-term elevated activity of bone resorption induced by citrate infusion. Routine taking calcium supplement before plateletapheresis for donors may be a good option not only to reduce the citrate toxicity symptom, but also to alleviate the disturbance of bone turnover induced by citrate anticoagulant.

P-076
EFFECT OF CALCIUM SUPPLEMENT ON THE CHANGES OF SERUM ANALYTES INDUCED BY ACUTE CITRATE LOAD

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Background: Calcium supplement is considered to be a possible prevention measure to counteract the hypocalcemia induced by citrate anticoagulation during apheresis processes. However, the characteristic of this practice have not been fully analyzed under controlled study.
Aims: The metabolic effects of oral and intravenous infusion calcium (Ca) supplementation during citrate load in the model of plateletapheresis were evaluated in the purpose to provide a logical option on applying calcium supplement in routine plateletapheresis.
Methods: A crossover, placebo-controlled study was conducted in 22 volunteers after informed consents were obtained. Volunteers received four standardized interventions with an interval of two to three weeks in random order, containing: A, placebo (saline solution) infusion only; B, citrate infusion only; C, citrate infusion plus 1.2 gram oral calcium administration 10 minutes before citrate infusion; D, citrate infusion plus simultaneous intravenous infusion (I.V.) of calcium at the rate of 100 mg/h. Citrate infusions were performed in the fixed dose of 1.5 mg/k.w/min and the fixed duration of 80 minutes. Serial blood samples were collected for the determination of serum analytes at the start of infusion, in the middle and at the end of the infusion, and 120 minutes after completion of infusion and 24 hours later.
Results: Treat B resulted in a continuous decrease of serum levels of ionized calcium (iCa), inorganic phosphate, potassium and chloride, followed by a recovery after end of citrate infusion. I.V. calcium supplement, but not oral calcium, alleviated the declined of serum iCa level induced by citrate intervention throughout the infusion period ($t = 12.37$, $P < 0.001$), while oral calcium supplement only elevated the iCa level at the endpoint of infusion ($t = 2.30$, $P = 0.03$). Both calcium supplements have no effects on the changes of inorganic phosphate during the period of infusion but expedited the recovery process of serum iCa and inorganic phosphate to the baseline within two hours after end of infusion and led to increased serum level of inorganic phosphate at 24 hours later as compared to that of before intervention ($P < 0.05$). However, calcium supplements have no effects on the changes of potassium and chloride induced by citrate throughout the whole observation period. Changes of serum iCa and phosphate were correlated to each other ($P < 0.001$), while potassium was correlated to the chloride ($P < 0.005$). Treat B, C and D also led to increased urine excretion of calcium ($P < 0.001$), with no effects on the excretion of phosphate. No changes on serum levels of sodium, magnesium and albumin were observed in all interventions.
Conclusions: Calcium supplements exert an impact on the serum levels of ionized calcium and inorganic phosphate induced by citrate intervention. Dose and timing of administration should be taken into account to achieve the best effect when oral calcium supplement is chose to counteract the hypocalcemia during plateletapheresis.

2.4. Blood Donation Rare donor programme

P-077

AN ITALIAN FAMILY WITH THE BM BLOOD GROUP

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Background: In Western Countries, the weak B-variants are very rare, probably due to the low frequency of the B gene. Only two single cases of Bm variant have been described in Italy so far. Here we report an Italian family with the Bm blood group.
Aims: To study the characteristics of the weak Bm variant.
Methods: A discrepancy in the ABO blood group determination by direct and indirect test was detected in a 25-year-old man. His blood group had been previously defined as a probable B3 at another center. Fourteen members of his family (six men, eight women; four generations), all born in southern Italy, were studied. Direct test was carried out by column agglutination (gel test) using monoclonal and polyclonal antisera (A, B, AB, D, CDE card). Tube test was performed using different monoclonal (3) and polyclonal (3) antisera, and sera anti-A (10), anti-B (10), anti-A,B (10) of normal blood donors. Anti-A1 (Dolichos biflorus) and anti-H (Ulex europaeus) were also used. The indirect test was performed with a NaCl-card and red cell testing (A1, A2, B, O) at 22 and 4°C. Tube test was also carried out using red cells (A1, A2, B, O) of 10 normal blood donors. The search for irregular antibodies, performed on a Liss-Coombs card against test red cells, was negative. Adsorption/elution test was performed to demonstrate a weak B on the erythrocytes. Polyclonal and monoclonal anti-B and anti-A,B were used for the adsorption of anti-B antibodies on the red cells of the examined subjects. The eluate, obtained by Glycine-HCl/EDTA method, was tested against B and O control cells on a NaCl-card and a Coombs card. The Lewis phenotype was defined in order to identify the secretor subjects. The heath inactivated saliva of the secretors was used to inhibit the reaction between anti-B and anti-H sera with B and O control cells.
Results: A discrepancy between direct and indirect test was observed in 7/14 examined subjects, suggesting the hypothesis of a weak B. Direct test on column agglutination (gel test) showed the absence of agglutination in 5/7 subjects. In the same individuals the indirect test showed only A1 and A2 control RBCs agglutination. The remaining 2/7 subjects, were positive with anti-A and anti-AB sera, while indirect test was negative with A1, A2, B and O control RBCs (Table 1). These results were confirmed by tube test. A total of 4/7 were secretors and showed positivity for H and B substances at saliva inhibition test.

Pt (sex)	Direct test Anti- A	B	AB	Indirect test A1	A2	B	O	Secretor status	Adsorption/elution	Saliva substances A	H	ABO phenotype
1 (M)	+	-	+	-	-	-	-	No	+			ABm
2 (F)	-	-	-	+	+	-	-	No	+			Bm
3 (M)	-	-	-	+	+	-	-	Yes	+	+	+	Bm
4 (F)	-	-	-	+	+	-	-	No	+			Bm
5 (M)	-	-	-	+	+	-	-	Yes	+	+	+	Bm
6 (F)	-	-	-	+	+	-	-	Yes	+	+	+	Bm
7 (F)	+	-	+	-	-	-	-	Yes	+	+	+	ABm

Conclusions: The absolute absence of agglutination at direct test excluded the possibility of B3 or Bx phenotype. Bel variant was excluded because of B substance in the saliva of the secretors. Based on Salmon et al. criteria, the weak B variant of 7/14 members of the described Italian family was Bm. Weak A or B phenotypes have been correlated with a single base substitution, usually in the exons 6 and 7 and the interposing intron of the gene on the chromosome 9. At the moment, ABO genotype determination for this family is in progress.

P-078

IDENTIFICATION OF DONORS LACKING A COMBINATION OF CLINICALLY SIGNIFICANT COMMON BLOOD GROUP ANTIGENS

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Background: ABO and Rh are the two most important blood group systems in transfusion medicine. The antigens of the other blood group systems are not routinely investigated for, but the serum of patients are screened for detection of clinically significant irregular antibodies so that compatible and antigen negative blood can be provided to the patient when antibodies are present. For patients who develop antibodies against common antigens or against a combination of common antigens, selection of blood requires screening with a large number of donor units. Donors lacking a combination of common antigens are also considered rare donors.
Aim: To screen donors for ABO, Rh, Kell, Kidd, Duffy, MNSs, P1 blood group systems and identify donors lacking common antigens or a combination of common antigens to prepare a registry of these donors - a pilot study.
Methods: Donor blood samples collected in a blood bank in Mumbai, India were tested for different blood group antigens using the respective blood group antisera following the manufacturer's instructions by standard tube technology.
Results: This pilot study included 358 donors who were tested for different blood group antigens. Seventeen donors (4.74%) lacked a combination of common antigens of Duffy and Kidd blood group systems, i.e they lacked Fya and Jka antigens which are common antigens. None of these 17 donors lacked the common antigen "s"of the MNSs blood group system. One donor out of these lacked Fya, Jka and e antigen and two donors lacked Fya, Jka and C antigen which make them rare donors.
In addition to the usual Rh phenotypes only one donor was found to show the R2R2 phenotype, one R2Rz and four were R1Rz which are all uncommon phenotypes.
Conclusion: A registry of donors who lack a single common antigen or a combination of common antigens can be prepared from repeat regular donors which will prove useful for patients with multiple antibodies against common antigens so that these donors can be contacted in emergencies.

P-079

PREVALENCE OF BLOOD GROUP IN BLOOD DONOR POPULATION OF TOTAL 98012 DONORS AT HUSAINI HAEMATOLOGY AND ONCOLOGY TRUST KHI, PAKISTAN, IN THE YEAR 2008

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Introduction: In Pakistan, the resource limitation is not the only contributing factor towards the poor state of the Blood Transfusion Services. An estimated 1.5 million units of blood are transfused annually in Pakistan, although the actual demand is likely to be much higher.
Because of fragmented blood transfusion services there is no proper registry of blood group prevalence's, no of voluntary blood donors, prevalence of rare blood groups etc. maintained by the authorities.
Husaini Haematology and Oncology Trust is the largest blood bank and transfusion center of Pakistan working since last more than three decades.

Background: Blood group serology plays a vital role in transfusion medicine. The Bombay (Oh) phenotype is characterized by the absence of A, B, and H antigens on red cells and occurs rarely. The study reports thirteen cases of the rare Bombay phenotype with identification of one patient of thalassaemia major with Bombay blood group.

Aims and objectives: The prevalence of blood groups frequencies including Bombay in blood donor population who donated blood between Jan and Dec 2008 in Husaini Haematology and Oncology Trust. (All branches) were evaluated. The aim was to find out the blood group frequencies in the population of blood donors in Pakistan and percentage of blood groups.

Patients who test as type O may have the Bombay phenotype if they have inherited two recessive alleles of the H gene, (their blood group is Oh and their genotype is 'hh'), and so do not produce the 'H' carbohydrate (fucose) that is the precursor to the 'A' and 'B' antigens. Individuals with Bombay phenotype blood group can only be transfused with blood from other Bombay phenotype individuals. Given that this condition is very rare, any person with this blood group who needs an urgent blood transfusion will probably be unable to get it, as no blood bank would have any in stock. So it is necessary for the blood banks to maintain a register of the rare blood types such as bombay.

Materials and methods: Red Blood Cells were tested with three antisera, i.e., anti-A, anti-B, and anti-H (lectin) for forward reaction. Agglutinations of plasma with A, B, and O (H) red cells (reverse reaction) were also tested for the presence or absence of antibodies in the serum performed at all branches of Husaini Blood Bank, a division of Husaini Haematology And Oncology Trust.

Results: Total no of 98 173 donors who donated blood between Jan and Dec 2008 at Husaini Haematology And Oncology Trust were analyzed. We found the following results.

1. B+ 32,331 33.0%
2. A+ 20,512, 21.0%%
3. A- 981 1.0008%
4. B- 1960 2.0%
5. O+ 30,003 30.611%
6. O- 1962 2.001%
7. AB+ 6840 7.0%
8. AB- 309 0.0031%
9. A2+ 1955 0.0199%
10. A2- 98 0.0009%
11. A2B+ 981 1.0008%
12. A2B- 67 0.0006%
13. BOMBAY 13 0.0001%

(A thalassaemia major female child, resident of Karachi, age three years is identified to have bombay blood group).

Table 1: Blood group prevalence statistics.

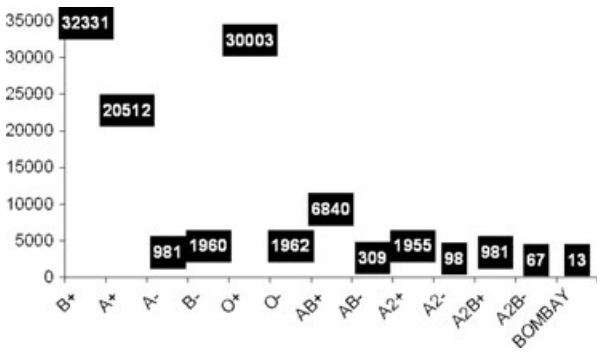
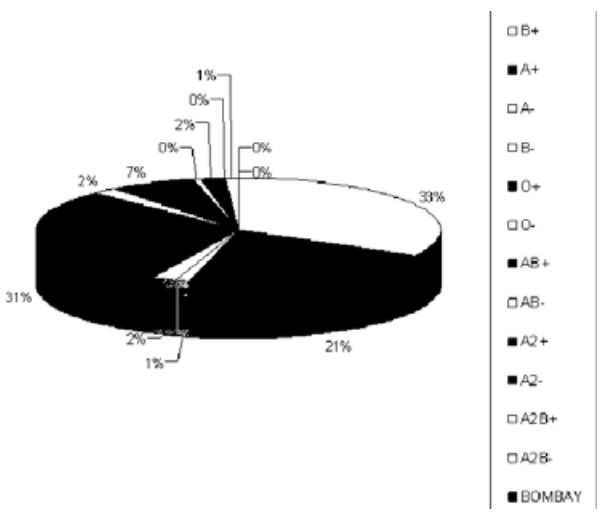


Table 2: Blood group data of blood donors



3.1. Blood Products Blood components

P-081

EVALUATION OF DEGLYCEROLIZED RBCS IN ACP 215 AND STORAGE IN 0.9-PERCENT NACL IN 24 HOURS

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Background: The Chinese Society of Blood Transfusion (CSBT) had approved the tactic of long term storage of glycerolized rare blood at lower than -65' and deglycerolization in ACP215, and postwash RBCs' storage at 4°C in no more than 24 hours. As washing solution of 0.2%HES-0.9%Nacl had not been producted in China, and such foreign reagent had not been registered in mainland area, there was not valid solution of 0.2%HES-0.9%Nacl to be applied in the procedure of deglycerolization. In addition, any of preservation solution in China was permitted to be added into postwash RBCs except 0.9% Nacl, compared to AS-3 as one of addition in postwash according to the SOP of deglycerolized RBCs preparation of AABB.
Aims: Of which two solutions including 0.2%HES-0.9%Nacl and preservation solution were not used to prepare deglycerolized RBCs from ACP 215 in China, changes of deglycerolization procedures were researched in this paper, and qualities of deglycerolized RBCs and effects of storage at 4' in 0.9% Nacl in 24 hours were evaluated.
Methods: Two units of whole bloods were collected from each of seven healthy male volunteers, the RBCs were collected in CP2D by the CSBT-approved whole blood collection protocol, plasma was separated after centrifuged, and suspending RBCs were stored at 4°C in AS-3 for six days. Each bag of RBCs were transferred to a 1000 mL PVC plastic bag, a procedure was used to glycerolize RBCs to a concentration of 40% (wt/vol) glycerol with a single disposable glycerolization set in ACP 215(Haemonetics), and frozen at -80°C. Then another modified deglycerolization procedure with 9-percent Nacl and 0.9-percent Nacl were used to deglycerolize from the same RBCs with single disposable deglycerolization set in the ACP 215. The deglycerolized RBCs were stored at 4°C in 0.9-percent Nacl for no more than twenty four hours. Corresponding indexes of deglycerolization effects were tested during storage.
Results: The mean +/− SD freeze-thaw recovery value was 93.6 +/− 2 percent, the mean +/− SD freeze-thaw-wash recovery value was 78.2 +/− 6 percent, the mean +/− SD residual glycerol was 6 +/− 2 gram per L. During stored at 4°C in 0.9-percent Nacl within twenty four hours the mean +/− SD supernatant hemoglobin was 0.2 +/− 0.1 percent, the supernatant potassium level was 3.75 +/− 2.0 mmol per L, and the units were negative for both aerobic and anaerobic bacteria.
Conclusions: The postwash of deglycerolized RBC had a acceptable FTW recovery value, and storage at 4°C in 0.9-percent Nacl for twenty four hours had a acceptable hemolysis. Abandoned washing solution of 0.2%HES-0.9%Nacl and preservation solution had not affected the quality of deglycerolized RBCs prepared in ACP 215 and postwash storage at 4°C within 24 hours.

P-082

IMPROVE PLATELET'S PRESERVATION QUALITY BY NITRIC OXIDE

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Background: The platelet could keep liquid status and biologic function within five days when stored at 22'. In the process of centrifuge and storage, platelets were easily activated and resulted in platelet storage lesion (PSL). Increasing the concentration of cyclic guanosine monophosphate (cGMP) could restrain calcium current transported into/out the membrane of platelet, leading to a lower platelet's action level. Protoheme connected with nitric oxide (NO) in platelet was able to active soluble guanylyl cyclase (sGC), which then raised concentration of cGMP. The Nitric Oxide Synthase (NOS) isolated from platelet and the discovery of L-arginine channel on platelet's membrane, provided the substance background to the theory that NO had a effect on inhibition of platelet's action.
Aims: This paper was to observe effects of NO on platelet's adhesiveness during WBC filter process, and indications of platelet storage in five days influenced by optical dose of NO.
Methods: Platelet-rich plasma (PRP) was separated into aliquots from fresh whole blood, copper and diluted hydrochloric acid were used to produce NO in the environment of argon air, different diluted NO solutions were then infused into PRPs before WBC filtration, an optimal dose of NO solution could decrease platelet's adhesiveness and increase platelet's recovery rate. The optimal dilution of NO solution was obtained from dose-response measurements. Under optimal NO concentration, some marks of platelet associated with quality were detected in the following researches. CD62p labeled with anti-CD62p-PE was analyzed by FCM, NO and cGMP concentration were by EIA, PH value was by PH counter, MPV and PDW were by Coulter machine, micro lesion observation was by electronic microscope photos. Significant differences in the mean values between treatment group and blank group were analyzed by paired/matched *t*-test for dependent samples.
Results: The optimal dose of NO was a rate of infusion that diluted saturated NO by about 10–2 to achieve a transient concentration of 0.028 mmol/L. There was significant difference between blank group and NO-treated group at marks of cGMP and CD62p re-expressing rate ($P <= 0.01$). Indications like PH, MPV, PDW showed little difference between groups. Electronic microscope film showed that there was little lesion of platelet in NO-treated group.
Conclusions: Various studies had shown that the addition of authentic NO into PRPs was potentially an efficacious method to generate anti-platelet action compounds, giving a rise to the level of cGMP and CD62p re-expressing rate.

P-083

PLATELETAPHERESIS IN YOGYAKARTA BLOOD TRANSFUSION SERVICE, INDONESIA: TWO YEARS EXPERIENCE

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Background: In Indonesia, plateletapheresis is a relatively new technology in the blood transfusion service. It was recognized that apheresis derived platelet products are a substitute for platelet concentrates (PCs) derived from whole blood. The average requirement of PCs in the Yogyakarta blood transfusion service are 923 PCs/month. In patient care, the platelet transfusion dose for an adult patient is equivalent to transfusion of 5–6 PCs. The cost per PC is Rp 140.000 00 (US$ 14). On the other hand, the cost of a plateletapheresis derived product is 20 times of that. Therefore, the price of one unit of apheresis platelets is equivalent to four times of PCs. Since these additional costs are not covered by insurance companies, not all of the patients can afford this product. Because of these reasons, in our situation plateletapheresis is very helpful in periods of relative shortage of whole blood donations e.g. the Dengue season. With this study, we would like to present the Yogyakarta blood transfusion service starting-up experiences on plateletapheresis.
Methods: Between November 2006 and March 2009, in total 126 plateletapheresis procedures were performed using MCS3p (Haemonetics®). The obtained data from these procedures were analyzed.
Results: In 29 months, on average 4 (range 0–16) plateletapheresis procedures per month were performed without any technical problems. Based on the patients needs, from 126 donors, 126 products were achieved, 19 (15%) from blood group A donors, 24 (19%) from B donors, 63 (50%) from O donors, and 20 (16%) from donors with blood group AB. In 91 minutes (range 66–135) a product of 357 mL (range 192–519) with 3.1x1011 (range 2.5–3.9x1011) platelet was collected. The apheresis derived platelet components were transfused to pediatric patients (n = 30 (23.8%)), mainly with

Dengue Hemorrhagic Fever (DHF) and to adult patients in internal medicine ward (n = 96 (76,2%)) mainly hemato-oncologic patients without side effect reported in the patients.
Conclusions: In two years plateletapheresis experience in the Yogyakarta blood transfusion service, no technical difficulties were observed. Apheresis derived platelet components products are mainly transfused to adult hemato-oncologic patients and pediatric patients with DHF. No side effect in the patients were reported. Plateletapheresis gives the opportunity to achieve platelet components especially in periods of limited availability of whole blood donors e.g. the Dengue season. Therefore, increase of the knowledge of clinicians regarding plateletapheresis is needed.

P-084
BUFFYCOAT DERIVED GRANULOCYTE TRANSFUSION PRODUCT: A BRIDGING PRODUCT?

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Objective and design: There is an indication for granulocyte transfusions in patients with an acquired severe neutropenia or neutrophil dysfunctions in combination with a life threatening infection without adequate response to antibiotic/antimycotic therapy. Because of donor selection, testing, and G–CSF/dexamethasone stimulation 12 hours prior to the apheresis procedure, patients with life threatening infections have to wait for 24–48 hours before the availability of apheresis derived granulocyte components. This delay can be lethal. Because of that reason, we developed a buffycoat (BC)-derived transfusion product complying with the European and national guidelines for granulocyte transfusion products (e.g. volume ≤ 500 mL, ≥ 1x1010 granulocytes/unit, preferably > 0.8x108 granulocytes/kg of patient's body weight).
Methods: By pooling 10 overnight stored AB0 and rhesus D compatible whole blood donation derived BCs, a BC pool was made (n = 11). This pool was centrifuged (1,800 g, 5 min) in a Top-and-Bottom bag. The red blood cell and the plasma layers were removed manually through the top and bottom outlet, using a plasma clamp. The remaining white blood cell rich product was irradiated with at least 25 Gy. The composition of products was determined on an automated impedance counter (Sysmex XT 2000i).
Results: The composition of the achieved products is shown in the table

Table 1: Composition of achieved products

N=11	Mean± SD	Range
Product volume (mL)	104± 18·4	85 - 140
Haematocrit (L/L)	0·42± 0·04	0·36 - 0·50
Erythrocytes (x 10^{12}/unit)	0·47± 0·08	0·36 - 0·60
Platelets (x 10^{9}/unit)	337± 78·5	195 - 497
Leukocytes (x 10^{9}/unit)	22·5± 2·8	18·2 - 28·7
Granulocytes (x 10^{10}/unit)	1·13± 0·17	0·95 - 1·47

Conclusions: We are able to produce BC derived granulocyte transfusion components, complying with the European guidelines and with the national guidelines. This BC derived product can be of value to bridge the period until availability of apheresis derived granulocyte components.

P-085
GRANULOCYTE APHERESIS WITH MCS+: A NEW PROCEDURE

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Objective and design: There is an increasing demand for granulocyte components in children. In practice, all procedures to collect granulocytes are performed with COBE® SpectraTM. However, this machine is not widely available in the blood donor centers. MCS + (Haemonetics) equipment is available on most of the centers, but no collection protocol for granulocyte apheresis is available. Therefore, a granulocyte apheresis procedure with MCS+ was written. To achieve basic figures, we evaluated the outcome of 10 granulocyte apheresis procedures in voluntary donors not stimulated with G-CSF or dexamethasone. Based on these results, granulocytapheresis procedures were performed on G-CSF and dexamethasone stimulated donors to achieve a granulocyte component for patient use.
Methods: To achieve final parameter settings, after informed consent of voluntary blood donors (n = 10), a 4-cycle leukapheresis procedure was performed with the MCS+, applying an adapted PBSC protocol using the LN970E disposable set. To avoid donor reactions, no sedimentation agents such as hydroxyethylstarch (HES) were used. After evaluation of these results, 8-cycle procedures to collect granulocytes for clinical use (n = 5) were performed on G-CSF and dexamethasone stimulated donors. To increase sedimentation in the centrifuge chamber, HES was added to the apheresis machine during the procedure. Donor full pre-donation blood counts and composition of products were determined on an automated impedance counter (Sysmex, XT2000i).
Results: The results are shown in the table.
In donors, no adverse reactions were observed.

Table 1: Donor blood counts and product composition

Donor / procedure	Non-stimulated	G-CSF and dexamethasone stimulated
	Mean±SD	Mean±SD
WBC count (x10^{9}/L)	5·4± 1·2	47± 17
Granulocyte count (x10^{9}/L)	3·2± 0·8	43± 15
Volume processed (mL)	2,068± 105	4,716± 180
Procedure time (min)	67±4	161± 23
Efficiency to collect granulocytes (%)	14±4	28± 10
Product	**Mean± SD**	**Mean± SD**
Volume (mL)	70±1	144± 9
WBC count (x10^{9}/unit)	3·9± 1·3	56± 18
Granulocyte count (x10^{9}/unit)	0·9± 0·4	51± 18
Ht (L/L)	0·25± 0·02	0·15± 0·06

Conclusions: By performing procedures on donors not stimulated with G–CSF, we were able to write a granulocytapheresis procedure on MCS+. In G–CSF/dexamethasone stimulated donors a product of 140 mL with 5×1010 granulocytes/unit conforming to European guidelines was collected using this program. It is likely that the increase of the collection efficiency is caused by the use of G–CSF and dexamethasone stimulated donors and the use of sedimentation agents such as HES. In conclusion, we succeeded to make a procedure to collect a granulocyte component with a limited volume with MCS+.

P-086
THE COMPARISON LEUKOCYTE CONTENT IN WASHED RED CELLS, PACKED RED CELL (PRC) AND PACKED RED CELL-LEUKOCYTE REDUCED

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Patients with recurrent febrile nonhemolytic (FNH) transfusion reactions should be transfused with appropriate blood component, e.g. leukocyte-reduced blood or washed red cell. It takes a specific bag to produce leukocyte-reduced blood or a bed side filter to reduce leukocyte. When processing leukocyte-reduced blood, there are additive solution to support red cell survival and function up to 42 days. Washed red cell is an alternative component that 99% of plasma proteins, electrolytes, and antibodies were removed. Washed red cells must be used within 24 hours because preparation is usually acomplished in an open sytem. Removal of the anticoagulant-preservative solution compromises long-term preservation of cell viability and function in washed red cell. Afterall, we want to compare the leukocyte content of blood component per unit.
We collect 180 component bags from Agustus 2005 until Agustus 2006 and we count the leukocyte using Sysmex KX-21. AABB standards define a leukocyte-reduced blood as one with <5x106 residual donor leukocytes per final product. By comparison, European guidelines define leukocyte reduced components as those with < 1 x 106 residual leukocytes per unit. The leukocyte content in both washed red cell and PRC leukocyte-reduced meet the AABB standards. Only 3,3 % of washed red cells and 21,7% of PRC leukocyte reduced have more than 1 x 106 residual leukocytes per unit leukocyte. There are 2 PRC (3,3%) which exceed the AABB standards

and 13,3% which meet the European guidelines. There is no significant difference leukocyte content of blood component per unit. Each Blood Transfusion Service must consider the cost-effectiveness of each component, the contamination risk, the cell viability and function.

P-087

IN VITRO ASSESSMENT OF PLATELET CONCENTRATES FILTERED BY HOME-MADE PLATELET FILTERS

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Background: Removal of leukocytes in platelet concentrates(PCs) to sufficiently low levels can prevent the undesired reactions and protect patients from immunological and infectious side effects. Many studies have demonstrated that it can be done by leukofiltration. An ideal filter should have such characteristics as high degree of platelet recovery, low amount of residual leukocytes without causing platelet activation, which decreases ability of pletelet to function and to survive in vivo. The use of filter technology for leukoreduction of PCs has been widely practiced in many countries, but it is just at begining in China.
Objective: The aim of this study was to evaluate two kinds of home-made leukodepletion filters in terms of the in vitro quality of platelet concentrates.
Methods: Whole blood units (400ml) were collected in Chengdu Red Cross Blood Center. Platelet rich plasma derived PCs were produced by two centrifugal steps. Twenty pools of 6–8 ABO matched PCs were prepared and randomly divided into two groups. The leukofiltration of each group was performed with one kind of home-made filters(named A and B respectively). Samples were taken from the PC container before and after filtration. Standard quality measures were performed: volume and concentration of platelet, residual leukocytes number and hypotonic shock response (HSR).
Results: There were no statistically significant differences in the two groups. The PC pools averaged (2.92 ± 0.41)-1011 vs (2.80 ± 0.39)-1011 platelets and (9.79 ± 3.42)-107 vs (8.96 ± 2.63)-107WBCs before filtration. The volume was (293.2 ± 19.7) vs (284.4 ± 14.5) ml while HSR was (82.27 ± 4.38)% vs (80.48± 2.94)%. After filtering, final volume was (281.8 ± 18.8) vs (275.2 ± 9.58) ml. Number of platelets averaged (2.57 ± 0.32)–1011 vs (2.52 ± 0. 12)-1011 and the amount of residual leukocytes was (0.47 ± 0.22)-106 vs (0.32 ± 0.28)-106. The values of HSR were still in normal range'(80.06 ± 3.77)% vs (78.44 ± 5.47)%?and relative change was (4.73 ± 2.15)% vs (4.52 ± 2.04)%. The platelet recovery was (88.55 ± 4.46)% vs (90.04 ± 3.45)% and leukocyte depletion rate were 99.49% and 99.78% respectively. There were no significant differences in MPV and PH after the process.
Conclusions: The in vitro quality results for leukoreduced PCs meet our internal specifications. The homemade leukodepletion filtesr for PCs are validated to have the characteristics of high degree of platelet recovery and low amount of residual leukocytes. They are safe and effective and enable us to prepare high quality leukoreduced PCs. However, more precisely estimating of platelet activation and function are needed to ensure the clinical benefit of platelets, which may better predict clinical efficacy of PCs.

P-088

THE DIFFERENT CAUSES OF BLOOD COMPONENTS DISCARD IN AHWAZ BLOOD TRANSFUSION SERVICE DURING MARCH-SEP 2008

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With an ageing population, advances in medical treatments and procedures requiring blood transfusions the demand for blood continues to increase in wealthy countries. The national blood service of England and Wales says that in 2004 blood donors saved or improved approximately one million lives. In many countries, however, people still die due to an inadequate supply of blood and blood products. According to American national statistics, 4.5 million Americans would die each year without blood transfusions. Blood is a rich product which can be broken down into many parts. Study of discard causes of blood components is important step to manage blood supply loss and prevent cost and energy loss.
In this descriptive (cross sectional) survey we studied the discard causes in all blood productions during six months (March-Sep 2008) in Ahwaz blood transfusion service. The reactive units in HBV, HCV and HIV tests not included in this study. The data analyzed by using SPSS14.
In this period 53078 Units(19589 Packed RBC,412 Whole blood, 13676 random Platelet,1532 FFP,13515 PPP, 1334 CP, 1542 Washed RBC and 1478 Leuco reduced FFP) produced from 20394 donations. Total discarded Unites was 4551(8.57 %). The main discard causes was icteric (yellow or jaundice appearance) unites (2.82 %), bloody Plasma or platelet (2.53 %),lipemic unites (1.16 %). The number of waste or discard in PPP was higher than other products.(6.87 % of PPP discarded as bilirubinemic unites, and 4.39 % bloody unites).
Our data showed high discard rate. The main cause billirubinemic unites, because judgment of that unites was based on only visual experience, many unites may vain discarded. Other cause of discard great numbers of discarded unites was bloody (pinkish) unites that occurred because the technical error (instrument or personal errors). With periodical service and training we can prevent to vain blood supply loss. Establishment of reliable and accurate criteria for discard the blood components to avoid vain blood and cost recommended.

P-089

CORRELATION BETWEEN LACTATE DEHYDROGENASE (LD) LEVEL AND PLATELET INDEXES IN PLATELET CONCENTRATES DURING STORAGE

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Background: Platelet concentrates were used for transfusion as an important supportive therapy in thrombocytopenic patients. During storage, in vitro quality parameters may be changed, called as Platelet Storage Lesion (PSL). Changes of metabolic parameters, cellular, and biochemical components were already reported in many publications. The correlation between parameters was still poorly explained.
Aims: To analyze correlation of LD level and platelet indexes i.e.mean platelet volume (MPV), platelet distribution width (PDW) in platelet concentrates during one, five, and seven days of storage.
Methods: The PCs were derived using PRP method, and stored for 7 days with standard method. Supernatant LD level were measured enzymaticly by automatic chemistry analyzer, the MPV and PDW were measured by hematology analyzer at day 1,5 and 7 of storage. Pearson test was used to analyze the correlation between parameters.
Results: At day-7 of storage, there were increased mean of LD level, MPV and PDW i.e. 1251.6 ± 295.57 IU/L, 8.31 ± 0.45 and 8.47 ± 0.74 compared to 647.1 ± 184.6 IU/L, 6.7 ± 0.35 fL and 6.58 ± 0.43 respectively at day-1 ($P < 0.05$). Positive correlation were found between LD and MPV ($r = 0.65$;$p<0.01$), LD and PDW ($r = 0.57$;$P < 0.01$).
Conclusions: During seven days storage of PCs, there were significant increased LD level, MPV, and PDW. Significant positive correlation were found between supernatant LD level with both MPV and PDW.

P-090

TRALI: MANAGING AB PLASMA : A CONSTANT CHALLENGE

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Background: Since April 2008, all plasma for transfusion has been prepared from blood donated by male donors only, after implementing criteria to reduce TRALI risks.
Aims: The impacts on inventory management of AB plasma at the Blood Center and at the hospitals is presented.
Methods: All ABO plasma shipments to each hospital were analyzed over a 52 week period (from 04/01/06 to 03/31/07). While 3,5% of our donor population is AB, it represented 8.3 % of total plasma shipped to hospitals. Nonetheless, after evaluating the proportion of AB male donors, it was determined that the demand for AB plasma could probably be met but with the active collaboration of hospitals. A communication plan was developed and presented at a special meeting of the hospital blood bank personnel in June 2007. Items discussed were statistics on AB plasma produced and shipped, the hospital's role in the revision of their AB plasma stock and their transfusion protocols. Hospitals were also asked to review AB cryoprecipitates and supernatant requests since these products are in direct competition with plasma AB production. Daily reports via the information system were created to monitor the quantities of AB plasma issued per week per hospital. This facilitated follow-ups with the hospitals and allowed fpr the effective adjustment of the AB plasma production. Finally, post-TRALI AB plasma management was addressed at every meeting with hospital blood bank personnel.
Results: After one year, the proportion of AB plasma issued dropped by 8% and inventory levels at the Center became sufficient to supply hospital demands without compensatory measures. As well, hospitals reviewed their transfusion protocols for AB cryoprecipitates and this product is now only requested for exceptional needs.
Conclusion: After implementation of our male only plasma protocol and by monitoring AB plasma production and shipments we were able to adequately supply hospitals who, in parallel, responded positively by implementing follow-ups on plasma ABO inventory management and on plasma AB transfusions. However, soon after implementation, this plan was rapidly challenged by the introduction of new protocols for massive transfusion requiring a red cell to plasma ratio of 1:1. As of March 2009, we have had to configure the information system to be able to process plasma from female donors with no history of pregnancy.

P-091

EVALUATION OF THE HAEMONETICS? MCS+? C-SDP PROTOCOL: CONCENTRATED PLATELETS IN ADDITIVE SOLUTION (M-SOL)

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Background: For prevention of non-hemolytic transfusion reactions, washed and/or replaced plateles (W/R-PC), concentrated platelets suspended in additive solution (C-PC) are believed to be effective. A preparation of C-PC at the time of blood collection introduces the option of additional concurrent plasma collection which can be used for source plasma. Additionally C-PC products meet the specific requirements for platelet pathogen inactivation methods. This study evaluated in vitro function of C-PC collected and prepared on the MCS+ device (Haemonetics Corp, USA) and examined operational performance.
Methods: More than $2x10^{11}$of C-PC per bag was collected using a C-SDP protocol on the MCS+ from 10 healthy donors and M-sol was used as the platelet additive solution. Collection time and collection efficiency of platelets were evaluated, as well as in vitro properties, including pH, aggregation, % Hypotonic Shock Response (HSR), CD62P and sCD40L. The data were analyzed during seven days storage in KBP1000FPN Polyolefin bag (Kawasumi Corp. Japan) continuously agitated at 22 °C. As a control, a standard apheresis platelet concentrate (PC) suspended in 100% plasma was collected from the same donor one month later, and the two groups were compared.
Results: There was no statistical difference between C-PC collection efficacy (64.8 ± 10.1%) and standard PC collection (67.4 ± 5.1%). Mean collection time for the same donor for C-PC (67 ± 9min) and standard PC (62 ± 8min) was statistically not different and the C-SDP protocol collected a mean of 120 ml extra plasma. Residual plasma ratio was 34.3 ± 3% (n = 5) for 10 units (1unit is about $2x10^{10}$ platelets) C-PC, 37 ± 2% (n = 3) for 15 units C-PC and 41 ± 5% (n = 2) for 20 units of C-PC. pH of C-PC on day seven was in between 7.3–7.6, for standard PC in between 7.0–7.2. sCD40L at day seven was 2,980 ± 310 pg/ml, which was lower value than standard PC average of 5,094 ± 642 pg/ml. There were no other statistical differences.
Conclusion: MCS+ collects C-PC in fully automated process, which results in a final product with plasma content of approximately 35% level, which according to published literature is sufficient to reduce plasma-related adverse effects. Additionally, C-PC introduction will provide additional source plasma.

P-092

A COMBINATION FILTER FOR PRION AND LEUKOCYTE REDUCTION, ITS PRION REDUCTION PERFORMANCE ASSESSED BY EXOGENOUS BIOASSAY TEST

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Background: Filtration is recognized as the most promising measure to reduce transfusion-transmitted vCJD risk. It has been reported that the ASAHI combination filter has sufficient leukocyte reduction capability from red cell concentrate (RCC) with filtered RCC quality in storage, and also prion reduction capability both from leukocyte-reduced (LR-) RCC and non-leukocyte reduced (NLR-) RCC with a wide range of controlled plasma protein concentration. The applicability of the combination filter for integration within a blood bag collection system, which could minimize impact on current SOPs, has been also reported regarding its sterilization stability with e-beam, steam and even double steam (excess exposure for test purpose) and its biological safety test results in compliance with ISO 10993.
Aims: To assess the prion reduction capability, the exogenous bioassay test was conducted in addition to Western Blot measurement method.
Methods: A human LR-RCC unit was spiked with 9.5 mL of microsomal fraction (10 w/v%) prepared from brain homogenate derived from scrapie-infected hamster with the 263K hamster adapted scrapie strain, and then filtered with the combination filter sterilized with steam. The prion levels were measured with Western Blot method and the Log10 reduction factor was calculated. For bioassay study, the serial tenfold dilutions of test materials were prepared with samples taken from the RCC before and after filtration. The serially diluted materials were injected intracerebrally to Golden Syrian hamsters, with 0.05 mL for each. In total, 36 hamsters were injected with samples before filtration, 48 hamsters after filtration, 36 hamsters with 263k microsomal post nuclear fraction as positive control, 12 hamsters with Hank's balanced salt solution as sham injected negative control and 6 hamsters were not injected as uninjected control. These hamsters were randomized into cages of 6 hamsters per cage prior to injection, and observed every working day up to 12 months. The brain slides from all the hamsters were microscopically evaluated for histopathological examination. The infectious titer was calculated according to the Spearman - Kaerber method.
Results: The Log10 reduction factor was higher than 3.0 by Western Blot method. The bioassay test gave the Prion reduction factor higher than 4 Log10 calculated from the titer (Log10 LD50/mL) of samples before and after filtration, 6.63 +/− 0.33 and 2.52 +/− 0.22, respectively.
Any abnormal or unexpected observation was not confirmed in the positive control, sham injected and uninjected groups.

Conclusions: The high Prion reduction performance of the ASAHI combination filter was confirmed by both Western blot method and the exogenous bioassay test.

P-093

EVALUATION OF PLATELET ACTIVATION AS QUALITY MARKERS OF PLATELET CONCENTRATE DURING STORAGE

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Background and objectves: The process of platelet concentrate storage by platelet rich plasma method could activate the platalet, which contributes to decreased ability of stored platelet to function and to survive in vivo after transfusion compared with that seen with freshly prepared platelets. Measuring platelet activation andices are useful mean to evaluate the quality control platelet concentrate.
Materials and methods: In this study, 24 concentrates were prepared via PRP method. This products stored for five days. The platelet count and CD62p,CD63,CD61 expression were evaluated. Special monoclonal antibodies that conjugated with flourecence dye in flocytometric method were used for CD62p,CD63,CD61.
Results: The average platelet count, CD61 expression in platelet rich plasma method showed no significant difference up to five days, but CD62p and CD63 expression increase during storage.
Conclusions: It is concluded that there is a close relaionship between CD62p,CD63 expression and platelet storage. The platelet count and CD61 may be less sensitve assays for evaluate platelet activation. The measurent of CD62P, CD63 expressions markers can act as a useful in vitro mean to determine the quality of platelet components.

P-094

THE DECREASE OF GLUCOSE LEVEL CORRELATES TO PLATELET YIELD IN PLATELET CONCENTRATES (PCS) DURING STORAGE

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Background: During storage, platelet earned the energy mostly via glucose metabolism from the plasma and preservative solution in PCs. Changes of metabolic parameters e.g. glucose and cellular components have been reported in many publications. The correlation between parameters was still poorly explained.
Aims: To analyze correlation of the decrease of glucose level and the platelet yield in platelet concentrates during seven days of storage.
Methods: Thirty PRP-derived PCs were stored for seven days with standard method. Supernatant glucose level were measured by GOD-PAP method using Beckman chemistry analyzer, the platelet count was measured by Coulter hematology analyzer at day one and seven of storage. Pearson test was used to analyze the correlation between parameters.
Results: At day-7 of storage, mean of glucose level was 351.63 ± 29.95 mg/dL, compared to 421.17 ± 19.80 mg/dL at day-1, with mean of its decrease 69.53 ± 27.02 mg/dL. The mean yield and platelet count were 27,179.39 ± 8,533.63/ unit and 455.9 ± 137,08/uL respectively.
Positive correlation were found between the decrease of glucose level and platelet yield ($r = 0.5$;$P < 0.01$).
Conclusions: Significant positive correlation was found between the decrease of glucose level and platelet yield in PCs during storage.

3.2. Blood Products Plasma products

P-095

A SIMPLE AND EFFICIENT METHOD FOR PREPARATION FIBRIN SEALANT FROM SINGLE DONOR PLASMA IN BLOOD BANK

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Introduction: Fibrin sealant (FS) is a plasma derived product and has hemostatic, sealing and healing properties. It is made by mixing fibrinogen and thrombin, which mimics the last step in the blood coagulation cascade. Fibrin sealant are frequently used to reduce blood loss during and after surgery.

The component of fibrin sealant can be prepared from large pools of plasma or from single donor plasma donations (autologous or homologous) often described as commercial and blood bank products, respectively. Commercial forms carry the risk of contamination or have been extensively processed to reduce that risk, which adds to the cost of them. Blood bank autologous fibrin sealant has no risk of transfusion transmitted disease and its production has a lower cost than commercials and that,s way it is frequently used by developing countries.

Materials and methods: In this study fibrinogen was precipitate by use of protamin sulfate from cryoprecipitate. Fibrinogen concentration was assayed with an enzyme-linked immunosorbent assay and clotting clauss method, also the effect of temperature on fibrinogen precipitation was evaluated.

Thrombin prepared by adding glass bead, kaolin, CaCl2 and ethanol to citrated plasma and its activity was determined using specific chromogenic substrate by spectrophotometry. Thrombin stability in different temperature evaluated and clotting time was measured. Tensile strength and adhesion strength were evaluated with tensiometery device. Clot lysis time test was done by solubility in 5 molar urea.

Results: Fibrinogen concentration precipitated with protamin sulfate measured by ElISA and clotting clauss method were 73 ± 8 and 71 ± 7 mg/ml respectively. The recovery of fibrinogen from cryoprecipitate was %93. The highest precipitation occurred at 24Ċ. Thrombin mixed with fibrinogen had clot time of less than five seconds.

Tensile strength and adhesion strength of fibrin sealant were 60 ± 8.9 g/cm^2 and 55 g respectively. The mean activity of produced thrombin was 59/6 NIH and it was stable until 6 hr at 4, 2hr at room temperature and more than three month at -28. Adding anti-fibrinolytic agent to fibrinogen concentrate has no effect on clotting time and tensile strength but it caused to improve the stability of fibrin clot.

Conclusions: Fibrinogen and thrombin prepared in this experiment have appropriate properties for production of fibrin sealant.

P-099

COMPARATIVE STUDIES ON CRYOPROTECTANTS OF HUMAN COAGULATION FACTOR?

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Background: Human Coagulation Factor is primarily used for the cure of hemophilia A patients with bleeding symptoms, which is an unstable protein and easily loses activity. The freeze-dried preparations are convenient for storage and transport, and the application of cryoprotectants is one of the key factors for the quality assurance of preparations treated by freeze-drying and dry heat (for the second virus inactivation), thus, the application of cryoprotectants determines the quality of final preparations. However, the studies on cryoprotectants of factor are normally confidential or proprietary, and only a few studies were reported.

Aims: To guarantee the excellent appearance of factor ? preparation and the less activity loss after being treated by freeze-drying and dry heat (100',30 min), and ensure the final preparation comply with the national standards.

Methods: Based on the same freeze-drying condition and certain cryoprotectants, five types of cryoprotectants were added with glycine, sucrose, albumin, xylitol and arginine, respectively. These cryoprotectants were then compared by analyzing the index related.

Results: After adding 3% of glycine, the preparations treated by freeze-drying and dry heat had good appearance, but a small amount of floccule existed after being redissovled. Using 5% of sucrose, the preparations shrank, and a part of water was remained after the process of freeze-drying. Two percent of albumin is helpful to guarantee the excellent appearance of the preparations, and the activity recovery of factor ? is relatively high, but the floccule also existed after being redissovled. Using 5% of xylitol, high clarity appeared in the reparations treated with freeze-drying, but the floccule and low activity recovery of factor ? remained after the dry-heat treatment. 1.2% arginine made the preparations shrink, but high clarity was observed after being redissolved.

Conclusion: The results indicated that there are significant differences about the effects of different cryoprotectants. In the process of freeze-drying and dry heat, glycine has a fine effect on the shape formation, while arginine mainly benefits to the stability of the proteins in preparations, and albumin plays a better protective role on the activity of factor ?. High concentration of sucrose hampers the removal of water from the preparations treated by freeze-drying. Xylitol is not suitable as a cryoprotectant for the human factor ? preparation, which is further dry heated at 100'.

P-101

AUTOLOGOUS FIBRIN SEALANT (FS) IN PLASTIC SURGERY - REVIEW

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Background: Fibrin sealant also known as fibrin glue (FG) is used in many surgical fields because of its' functional properties and unique physical advantages. The fields of surgical application of FS adhesives are rapidly developing. FS is prepared from the patient's own blood. Autologous use has the advantage of avoiding the risk of transfusion transmitted disease (TTD).

Aim: By using dermolipectomy and different techniques of hernioplasty we want to underline that the combining of these two techniques and the use of fibrin sealant at the same patient is a benefit.

Material and methods: In the period from October 2007 to October 2008, 11 female patients, at the age of 32 to 48 years, were treated. All of them were submitted to infraumbilical dermolipectomy. Eight of them, having incissional hernia after ''sectio cesarea'', were treated with implantation of polypropylene mesh-graft, and the remaining three of them, having incisional hernia after dehiscence of local peritonitis as a consequence of appendectomy, were treated with local hernioplasty. Nine of the patients were submitted to a long drain therapy in order to eliminate the lymphatic fluid. These drains produce additional reactive fluid and prolong the initial healing time. In the case of four patients we applied autologous FS and the drains were removed in the fourth post opp day with no seroma sequealae and preventing swelling. The autologous pre-donation red blood cells were also administrated the next post opp day.

Conclusion: These surgical techniques are well known procedures, but the use of pre-donatory autologous blood and FS which is physiologically compatible with human tissues, does not induce necrosis or other reactions and make this method safe. Autologous FS has the advantage of avoiding the risk of TTD and makes the operation easier, safer, cheaper and therefore more acceptable for the patients and the surgery teams. Trends in minimal invasive surgery also contribute to the increasing use of this biomaterial.

P-102

STUDY OF THE INCREASING THERMAL DENATURATION RESISTANCE OF HUMAN SERUM ALBUMIN BY ACETYLTRYPTOPHANATE AND CAPRYLATE

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Background: A comprehensive study of the thermal stabilization of human serum albumin was carried out by the aid of adding caprylate and acetyltryptophanate to human serum albumin solution.
As one of the step in human albumin production is pasteurization at 60-C for 10 hours, the increasing thermal resistance of the albumin is a key point.
Aims: In our study we investigate the increasing thermal denaturation resistance of human serum albumin.
Methods: Human seum albumin as a biological drug was prepared by cold ethanol fractionation of human plasma. By fractionation of human plasma under certain conditions by adjustment of temperature, concentration of ethanol, pH, and ionic strength different proteins as intermediate paste can be separated. One of these intermediates is fraction V, which albumin can be formulized out of it at the concentration of 5% and 20% and it should be pasteurized for 10 hours at 60°C. Sodium salt of caprylic acid and N-acetyl-DL-tryptophan with different concentration was added to the 5% solution of prepared albumin and then it was filtered. By observing visible clot formation during pasteurization, we studied the thermal denaturation resistance of human serum albumin.
Results: We found out that those solutions without stabilizer, after two hours heating in water bath at 60°C became opalescent and after 5 hours visible clot was formed, while those solutions with sodium caprylate and sodium acetyl tryptophanate could resist the temperature of 60°C for 10 hours and the amount of polymers based on HPLC protein profiles before heating of the albumin solution from less than 1% polymer were increased to about 4% polymer after heating the albumin solution for 10 hours at 60°C in water bath.
Summary/Conclusion: In our study we found out that sodium salt of caprylic acid and acetyltryptophanate are effective as stabilizer for increasing the heat resistance denaturation of human albumin during pasteurization.

P-103

THE PREPARATION METHOD IMPROVEMENT AND QUALITY EVALUATION OF FACTOR VIII DEFICIENT PLASMA

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Background: Factor VIII deficient plasma (FVIIIDP) is very important for diagnosing the patients with hemorrhage disorders and the quality control of FVIII concentrates. In China the FVIIIDP is difficult to obtain and mainly depends on import. Besides, there are some differences between imported and domestic FVIIIDP, thus it limits the detection of this factor.
Objectives: The method for the preparation of FVIIIDP was improved and quality was evaluated.
Methods: The conditions of preparation, including the EDTA concentration, incubation time and temperature, dialysis ionic strength and pH, and additional concentration of factor V, were optimized. And the stability, the linearity, and the comparison between domestic and imported products were evaluated.
Results: The FVIIIDP made by the improved method was evaluated: intra-batches variation coefficient (n = 10) were 0.64% (APTT), 1.7% (PT), 11.2% (FV: C) and 1.37% (FVIII: C), inter-batches variation coefficient (n = 3) were 3.34% (APTT), 3.72% (PT), 13.75% (FV: C) and 8.46% (FVIII: C), correlation coefficient of linearity curve exceed 0.99 (n = 3). Then comparing with the imported products, FVIII activity was less than 2% (of normal) and the activities of other factors were more than 50% in both of them.
Conclusions: The results suggested that the FVIIIDP which was made by the improved method had steady quality and good linearity. Compared with the imported products, the factor activity levels of FVIIIDP could meet the requirement in coagulation assays. Hence the FVIIIDP made by the improved method could be used for Hemophilia diagnosis and the quality control of FVIII concentrates.

P-104

ISOLATION AND PURIFICATION OF HUMAN COAGULATION FACTOR V

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Background: Human coagulation factor V (FV), found in plasma and platelets, an essential protein in the blood coagulation cascade, serves as a cofactor for the reaction of coagulation factor X to produce thrombin and serves as a cofactor for protein C to produce anti-thrombin. The study of FV has several decades, and much is known about the protein structures and functions and the relationships between FV gene mutations and diseases. To establish method for purifying FV and study the related reagents, will be useful for the study of biochemistry of blood coagulation, the evaluation of various disorders affecting the coagulation system and monitoring the quality of plasma products.
Objective: To establish a method for isolating FV from fresh frozen plasma. More over, the FV will be used as antigen to obtain hybridoma antibodies against FV.
Methods: Inhibitors of proteolytic enzyme were added to plasma for keeping FV stable during purification. FV was isolated and purified from plasma by using barium citrate adsorption, polyethylene glycol 6000, DEAE-Sepharose Fast Flow ion exchange chromatography and Sephacryl-S300 gel filtration chromatography. Characterization of FV by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) ;Test of protein concentration by Ultraviolet Spectrophotometry;Assay of FV activity by one-stage coagulation assays.
Results: There was a protein band on gels by SDS- PAGE,that the molecular weight was about 330KD. When FFP was thawed without DFP, the final product contained not only undegraded FV, but also several lower molecular weight forms representing partially degraded FV. FV was recovered with the rate of (11.21 ± 3.54)% yield rate, the specific activity of purified FV was (30.46 ± 0.31)units/mg, and the purification factor was (2538.33 ± 25.83) fold.
Conclusion: FV can be effectively purified by the purification method.

P-105

ISOLATION AND PURIFICATION OF HUMAN COAGULATION FACTOR?

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Background: Coagulation factor VII(FVII) with a molecular weight 50 kD, which is synthesized by hepatocyte depending on vitamin K, is a single-strand glycoprotein. FVII is composed of 406 amino acids with 13% glycan, and is a serine proteinase zymogen, which have similar prolease structure with others. Main physiological actions of FVII are combining with TF, and then activating FX which further starts extrinsic coagulation pathway.
Objectives: To investigate how to purify FVII from Cohn fraction III (CFIII) combining DEAE ion exchange chromatography with Heparin affinity chromatography, and to identify purification product.
Methods: Initially, FVII was isolated and purified from CFIII via dissolving CFIII, absorbing FVII onto barium citrate and eluting, and ammonium sulfate fractionation. Secondly, by the methods of DEAE-Sepharose Fast Flow ion exchange chromatography and Heparin Sepharose CL-4B affinity chromatography, FVII was purified further. And then purity of FVII was identified by SDS-PAGE. FVII coefficient (FVII: C) and concentration were measured by the methods of one-stage coagulation assays and ultraviolet spectrometry separately.

Results: With above approaches, the purification factor was (4954.22 ± 164.43) fold, the specific clotting activity of purified FVII was (147.19 ± 4.89) IU/mg, and the overall yield recovery rate of FVII was (21.09 ± 1.93)% (n = 5). There was only one clear protein band at about 50 kD site of Protein Molecular Weight Marker on the gel.
Conclusions: DEAE-Sepharose Fast Flow ion exchange chromatography and Heparin affinity chromatography are effective in concentrating FVII from CFIII, which will optimize FVII purification technology.

P-106

SCREENING STABILIZERS FOR THE ACTIVITY OF PROTEIN C TREATED WITH SOLVENT/DETERGENT

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Background: Protein C (PC), a vitamin-K-dependent serine protease zymogen, is the pivotal anticoagulant and antithrombotic in the human coagulation cascade. PC product is indicated for many lethal diseases, such as sepsis, severe congenital PC deficiency, purpura fulminans, etc. Cohn Fraction IV (CFIV) is a byproduct (usually discarded) of a plasma fractionation process. It is quite an inexpensive protein C source that retains approximately 90% of PC of plasma. Inactivation of virus is essential for the safety of PC product during its production. Solvent/detergent [S/D,0.3% tri(n-butyl) phosphate and 1% Tween 80] is quite effective to inactivate lipid-enveloped viruses, while the activity of PC may be affected.
Aims: To screen efficient stabilizers for the activity of PC when CFIV is treated with S/D to inactivate lipid-enveloped viruses.
Methods: CFIV was dissolved with trisodium citrate buffer in a certain ratio for 3 hours at 4',and then centrifuged at 5000 rpm for 40 minutes at 4'. The supernatant was treated with a 0.65 μm filter. Each a certain concentration of either glycine, arginine, histidine, tyrosine, glutamic acid, glutamine, lysine, alanine, aspartic acid, or a combination was added into the solution. Subsequently, it was treated with S/D for 6 hours at 24'. The activity of PC was determined using chromogenic substrate method referenced against the World Health Organization (WHO) International Standard. It was assumed that the activity of PC in CFIV without S/D treatment was 100%.
Results: The results(mean values ± SD; n = 3) were as follows: the activity of PC treated with S/D without any stabilizer was 33 ± 4.60%; the activity of PC protected by glycine, arginine, histidine, tyrosine, glutamic acid, glutamine, lysine, alanine or aspartic acid were 67.03 ± 10.70%, 64.1 ± 1.39%, 66.23 ± 6.34%, 50.53 ± 10.81%, 42.03 ± 2.51%, 43.29 ± 4.67%, 34.89 ± 4.02%,42.13 ± 4.05% and 23.42 ± 2.79%, respectively; the effect of the combination of glycine, arginine and histidine was much better than independent action.
Conclusions: The combination of glycine, arginine and histidine could be an efficient stabilizer for PC during S/D incubation to inactivate lipid-enveloped viruses.

3.3. Blood Products Pathogen inactivation

P-107

PATHOGEN INACTIVATION AND IN VITRO FUNCTION OF PLATELET COMPONENTS COLLECTED IN 100% PLASMA TREATED WITH INTERCEPT BLOOD SYSTEM™

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Background: The INTERCEPT Blood System was initially developed to inactivate pathogens and leukocytes in platelet components (PCs) suspended in a combination of approximately 35% plasma and 65% platelet additive solution (InterSol'). This system was CE Mark registered in 2002. To date >350,000 INTERCEPT PCs have been transfused in routine clinical use.

Aims: Here we present results of pathogen inactivation and maintenance of in vitro platelet function following INTERCEPT' treatment of PCs suspended in 100% plasma.

Methods: Either apheresis or pooled buffy-coat-derived PCs containing 2.5–7.0 x10E11 platelets in 255–390 mL of plasma were used in a series of experiments. Inactivation experiments were performed by inoculating PCs with 10E5–10E6 cfu/mL of bacteria or 10E5–10E6 infectious units (TCID50)/mL of virus, adding 150 μM amotosalen, and illuminating with 3.0 J/cm^2 UVA (320–400 nm). Samples taken before and after treatment were assayed for viable organisms and results used to calculate the level of inactivation (log-reduction). Four to eleven replicate experiments using independent PCs for each organism were performed. For in vitro function experiments, seven double-dose apheresis platelet collections in plasma were each split evenly into two identical units. The CONTROL was not treated. The TEST was treated with amotosalen and UVA. After six hours of incubation in a compound adsorption device (CAD) to reduce the amotosalen concentration, the treated PCs were stored under blood bank conditions and evaluated for platelet count, volume, pH (37°C), pO2, pCO2, bicarbonate, glucose, lactate, LDH, swirling, MPV, and soluble p-selectin.

Results: The results showed significant inactivation of viral and bacterial pathogens in PCs suspended in 100% plasma following treatment with INTERCEPT (Table 1) and are consistent with product claims for INTERCEPT platelets in InterSol'. After treatment and storage for up to nine days, there were no statistically significant differences between the CONTROL and TEST PC units for lactate, LDH, MPV, and p-selectin. Swirling was observed for all units up to nine days of storage with the exception of one CONTROL and one TEST unit with no swirling on Day nine. Differences were observed for measurements of platelet content, pH, blood gases, and glucose levels for most of the storage days. Although there was glucose remaining on Day nine to provide energy, bicarbonate was depleted in some of the TEST units for continued maintenance of pH. All units had pH values greater than 6.4 on Day five meeting Council of Europe requirements. On Day seven all in vitro parameters indicate metabolically viable platelets with a mean pH of 6.91 (SD 0.3), however, pH of one TEST unit fell below 6.4 (pH = 6.36).

Table 1: Results of Pathogen Inactivation.

Organism	N	Mean Log_{10} Reduction
Staphylococcus aureus	4	>7·6 ±0·1
Staphylococcus epidermidis	4	>7·4 ±0·1
Escherichia coli	4	≥7·3 ±0·2
Klebsiella pneumoniae	4	≥6·7 ±0·6
HIV-1 (cell-free)	11	≥5·0 ±0·3
Pseudorabies virus (PRV)	7	≥4·7 ±0·4
Bovine viral diarrhea virus (BVDV)	8	≥5·4 ±0·2

Conclusion: These results provided the basis for CE Mark approval to expand the INTERCEPT treatment guardband range to include PCs suspended in 100% plasma without additive solution. INTERCEPT PCs in 100% plasma can be stored for up to five days.

P-108

THE STUDY OF FACTORS INFLUENCING ON THE QUALITY OF PLASMA WITH VIRUS INACTIVATION BY METHYLENE BLUE

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Background: Virus inactivation of fresh-frozen plasma can be achieved by photodynamic methods in the presence of methylene blue (MB), which was first used clinically by Paul Ehrlich in the 1890s and had been used to kill viruses in the 1950s. Though the MB concentration in MB-treated plasma is much lower than clinical dosage, there exist concerns about the toxicity of MB in plasma. Thus the removal of MB from the plasma before transfusion may be Advantageous. However, the treatment may result in changes in the functional activation of coagulation factors and fibrinogen.

Aims: The aim of the study is to evaluate the factors influencing on the quality of plasma by photodynamic virus inactivation step, including MB, light, filters with different sterilizing methods et cl. Therefore, prothrombin time (PT), activated partial thromboplastin time (APTT), coagulation factors (FVIII), fibrinogen and total protein are examined.

Methods: The plastic disposable set contained the MB in the form of a dry pill, which dissolved when the 200 ml plasma flowed into the illumination bag, and the concentration of MB was controlled at 1μmol/l ± 0.2. Then, the plasma was illuminated for 30 minutes by red light(illumination device produced by IBT). In the end, the residual MB was removed when the treated plasma passed through the filter.

Results: Compared to the untreated plasma, after addition of MB to plasma, there was a significant loss of FVIII level (8.14% ± 0.28). This was associated with an increase in both PT ratio (6.2% ± 0.71) and APTT ratio (2.87% ± 0.12). Next, after illumination by red light and fluorescence respectively, PT and APTT was both significantly prolonged (PT:7.6% ± 0.45vs 9.2% ± 0.76, APTT: 15.3 % ± 1.13vs 18.4 ± 1.02). Accordingly, activity of FVIII had decreased more than untreated plasma (26.96% ± 0.63vs 29.32% ± 0.93). The results suggested that fluorescence had more effect on coagulation system than red light. Finally, MB was removed by filters with different sterilizing methods, and loss of activity of FVIII had significant contrast between the two sterilizing methods such as ethylene oxide and radiation (22.6% ± 0.47vs 36.4% ± 1.83), which showed that filter sterilized by radiation affected the activity of FVIII more heavily due to the change of the structure of filtering material during process of radiation. In addition, the degree of fibrinogen activity and total protein recovery were also examined after filtration, which were 72.6% ± 0.02 and 93.94% ± 0.078 respectively.

Conclusions: The loss of activity observed in MB-treated plasma in our study was similar to that previously reported, moreover, the result showed that illumination of fluorescence caused the most loss of activity of FVIII. In addition, suitable sterilization is also important for filters, and the ways such as radiation may affect the coagulation system seriously. In conclusion, if the condition of MB photodynamic method of virus inactivation is suitable, the levels of these parameters would remain within the normal ranges and the effect of the quality of plasma would be acceptable.

P-109

CELLULAR AND MOLECULAR BIOLOGICAL ANALYSIS BASED EVALUATION OF METHYLENE BLUE PHOTOCHEMISTRY VIRUS INACTIVATION

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Background: Risk of virus and other pathogen infection through transfusion of whole blood and blood components still exists in spite of the blood donor screening. This may partly caused by false negative results due to the defect of the screening methods or the pathogens are not included in screening. That is the reason why virus inactivation is important. Methylene Blue(MB) with visible light treatment of plasma started to be used clinically to inactivate virus in China. It is necessary to moniter the

effectiveness of virus inactivation. Since lots of medically important viruses including HBV and HCV are still unable to culture directly in vitro, Cytopathic effect(CPE), a traditionally used method is not suitable for virus inactivation monitor. On the other side, Sindbis virus is widely used as a model virus in virology study and could be used in virus inactivation monitor while CPE be used to study cell growth status. Since CPE analysis is complicated and difficult to be used as a routine method clinical, powerful and proper methods need to be developed and evaluated to monitor the process of virus inactivation. In this study, to determine whether gene amplification method could be taken as a tool to evaluate the effectiveness of virus inactivation in addition to CPE analysis, sindbis virus was cultured pre and post Methylene Blue Photochemistry(MB-P) treatment in vitro while CPE were detected at the same time.

Objective: To evaluate the effectiveness of virus inactivation by Methylene blue Photochemistry using cellular and molecular biological methods.

Methods: Cytopathic effect analysis and SYBR Green I based real-time quantitative RT-PCR were used to study the changes of virus infection activity and its genome during the process of virus inactivation by photochemistry with methelene blue of different concentration.

Results: MB-P treatment could inactivate Sindbis virus effectively. With the increase of MB concentration, the titer of virus activity decreased significantly from 6.5l gTCID50 to undetectable level. On the other hand, virus gene quantity showed significant decrease of different contentï¼P€lt;0.05ï¼‰. Combined analysis of the changes from both the virus titer and virus genome quantity provided significant relativity with R2ï¼ž0.97.

Conclusions: MB-P could destroy the Sindbis virus gene. The higher the MB concentration, the more severe the content of destroy. The destroy of virus genome is relative to the lost of virus activity. Our study showed that SYBR Green I-based real-time RT-PCR could be used to evaluate the process of MB-P virus inactivation.

4.1. TTID
Updates on hepatitis (HBV)

P-110

SHIMLA BLOOD BANK REGISTERS SHARP DECLINES IN HEPATITIS-B SEROPOSITIVITY AMONG BLOOD DONORS-ANALYSIS OF ELEVEN YEAR BLOOD BANK DATA, SHIMLA, INDIA

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Background: India, has a Hepatitis-B carrier rate of 4%, and nearly one million HBV infections are added to the HBV pool yearly. Of this 3.7% is transfusion related. In Shimla blood bank, all donors are screened for HbSAg, however no analysis of trends in Hepatitis-B Seropositivity is available, therefore this study was undertaken.
Objectives: To know the trends in Seropositivity and draw appropriate lessons for corrective measures in the interest of blood safety.
Methods: Secondary data from Shimla blood bank records were abstracted and analysed from,1997–2007. Using Microsoft excel and epi-info software 3.3.2. Hepatitis-B Seropositivity rate among blood donors was calculated. Seropositivity was confirmed using highly sensitive rapid kits for detection of Hepatitis B surface antigen (HbSAg)
Results: Of the total 10 212 blood donors, 65% were between 25–44 years of age and 87.5% males. Cumulative seropositivity rate was 0.94%. While the voluntary blood donation increased from 44% in 1997 to 57% in 2007, seropositivity decreased from 1.5% in 1997 to 0.7% in 2007. Trends in Seropositivity in voluntary and replacement donors showed wide fluctuations across years. Seropositivity in voluntary donors significantly decreased from 1.65% in 1997 to 0.3% in 2007(Chi-Square = 4.77, Df = 1, P = 0.02) Among replacement donors only slight decrease was observed from 1.97% in 1997 to 1.21% in 2007. Seropositivity significantly declined among females from 8.34% in 1997, to 0.5.1% in 2007 (Chi-Square = 9.73, Df = 1, P = 0.001), while in males it decreased from 1.53% in 1997 to 0.74% in 2007. Overall Seropositivity among blood donors was all times less in the eleven year period than in general population of Shimla, which as per studies done by Ganju et al was 3.5%.Voluntary blood donor retention is only 27%.
Conclusion: Hepatitis B Seropositivity more than halved during the eleven year period and may be possibly due to the increases in voluntary donation and effective pre-donation medical screening of donors. Therefore there is need to increase voluntary donation and retain voluntary donors to assure blood safety as the replacement donors have more risk of high Seropositivity than voluntary donors. Based on this study, the scholar undertook a study to identify factors associated with high drop out of voluntary donors to suggest measures to retain them.

P-111

RESOLVED HEPATITIS B VIRUS INFECTION IN BLOOD DONOR: CASE REPORT

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Background: According to the National recommendations in Serbia serological screening of the hepatitis B virus (HBV) infection in the blood bank has been based only on HBsAg testing. However, HBsAg seronegative donations could be able to transmit the HBV during a very recent phase of HBV infection (window period) or the late stage of infection.
Aims: This paper presents a case report of repeated blood donor who was seropositive for HBsAg and become seronegative during acute resolving of HBV infection.
Methods: A 18 years old male volunteer come in our institution on October 2008 to donate his blood for the second time. Six months earlier his first donation found to be negative for HBsAg, whence this donation was found to be highly reactive for HBsAg with a signal to cut-off (S/CO) ratio of 48.39. The method of testing was EIA (BioRad Monolisa HBsAg Ultra). The same sample was subsequently tested on the other EIA (Biomerieux Hepanostica HBsAg Ultra). It was highly reactive for HBsAg (S/CO ratio was 52.63).
Results: One week later donor recalled for counseling and retest. He reported risk sexually behavior (numerous sex partners in the past six months). In the second sample S/CO ratio of HBsAg was significantly decreased (BioRad 2.14; Biomerieux 1.68). Two weeks later third sample was HBsAg negative with increased levels of alanine aminotransferase (48 U/L) and total bilirubin (25.4 μmol/l). Anti-HBc IgM antibody was positive (101 IU/ml).
Conclusions: This situation did not impose a risk of transfusion transmitted HBV infection because HBsAg positive donation was discarded, than indicated the possibility of the occurrence of HBV transmission from blood donors to recipient in condition where HBsAg is solely a marker of HBV infection. Implementation of anti-HBc antibody test in routine screening would be reduced risk of transfusion transmitted HBV infection.

P-112

DONOR COUNSELING AND SELF EXCLUSION OF HBV AND HCV POSITIVE BLOOD DONORS

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Background: Since 2005 an infection surveillance programme was introduced at the Institute of Transfusion Medicine in Skopje, Macedonia. The programme consists of following up the infections in blood donors that are monitored through the collection of information about donations tested and infected donors identified.
Aims: To improve the donor's attitude to give right and honest information about their health status, and to make self exclusion after their positive HBV or HCV notification. Safe blood is as important to HBV and HCV prevention as VCT and PMTCT. Pre-donation and post-donation counseling lower the rate of HBV and HCV positive blood donors.
Methods: During 2005 – 2008, 755 have been identified positive on HBV (594) and HCV (161) markers. 755 have been contacted by phone and/or certified letter informing them of the risk of donating and requesting them to contact the blood center for further testing and counseling.A total of 152 (20%) came for further testing and counseling. They were retested after three to six months and 124 (82%) of them got positive result and 27 (18%) got negative result. When they came for further counseling they were interwove for behavior changes toward blood donation.
Results: Their attitude toward blood donation show improvement in their readiness to give right and honest information about their health status. Eighty two percent of blood donors positive on HBV and HCV markers, self excluded on the donation following notification and agree to have therapy at the Clinic for Infection diseases in Skopje. And 18% with negative result, made self exclusion for some period.
Conclusion: HBV and HCV positive donors after being notified and counseled made self exclusion from blood donation. We realize that in the process of pre and post donation it is of big importance to include donor counseling, especially for blood donors who are HBV and HCV positive and who are at risks continuing to donate HBV and HCV infected blood.

P-113

AN EFFICIENT METHOD OF EXTRACTING HBV DNA FROM PLASMA WITH LOW VIRAL LOAD: APPLIED TO THE DETECTION OF OCCULT HBV INFECTION IN HIV INFECTED DONORS

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Background: Occult hepatitis B virus (HBV) infection (OBI), defined as the presence of HBV DNA without detectable HBV surface antigen (HBsAg),

remains a risk of post-transfusion hepatitis B virus (HBV) infection. This is in part due to the HBV DNA load being too low (< 500 IU/mL; mostly <100 IU/mL) to detect in occult HBV infections. Thus, it is important to establish a sensitive method to detect occult HBV infection in donors.
Aims: To develop a simple, rapid and efficient method to extract HBV DNA from plasma with low viral load to increase the sensitivity of detection of OBI.
Methods: The HBV DNA level of 10 plasma samples from donors were tested by real-time fluorimetry PCR with TaqMan probe using the Diagnostic Kit for Quantification of Hepatitis B Virus DNA (Daan Gene, China) and then the samples were diluted to the concentration of 103, 102, 80, 40, 20, 10 and 5 copies/mL, respectively. HBV DNA were extracted from these diluted samples using boiling lysis method (Daan Gene, China),spin-column chromatography (TIANGEN, China) and magnetic bead method (Bioeasy, China) at the same time. Nested PCR was performed using specific primers of the X region of the HBV genome according to the method previously reported to determine the detection limit by the three methods above for HBV DNA extraction. The HBV DNA of Ninety-seven HBsAg-negative and anti-HIV-positive plasma from donors were extracted by the method with lowest detection limit and detected by Nested PCR.
Results: The detection limit of nested PCR using the HBV DNA extracted by magnetic bead method as template was approximately 10 copies/mL, lower than by the other two extraction methods. Using this method, 4 of the 99 HBsAg-negative and anti-HIV-positive samples were HBV DNA-positive by nested-PCR.
Conclusion: The magnetic bead method is a simple, rapid and efficient method to extract HBV DNA from plasma with low viral load and the nested-PCR with the HBV DNA extracted by magnetic beads as template is very specific and sensitive for the detection of OBI in HIV infected donors.

P-114
WINDOW PERIOD AND OCCULT HBV INFECTIONS AMONG BLOOD DONOR POPULATION IN HONG KONG

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Background: HBV infection is endemic in Hong Kong despite two decades of vaccination program to the newborns. However, the prevalence of confirmed HBsAg prevalence in new donors has been on a decreasing trend from 8.6% in 1989 down to 1.8% in 2008. Routine blood screening for HBsAg was done by the most sensitive serologic assay using the Prism platform (Abbott, Abbott Park, IL) since 1997. Surprisingly, transfusion-transmitted HBV infections were rarely reported. To enhance blood safety, direct detection of HBV DNA using nucleic acid amplification technology (NAT) can screen out HBV DNA-containing donations collected during the early infectious window period (WPI) and also donations with occult HBV infection (OBI) where blood contains low-level HBV DNA with anti-HBc and/or anti-HBs but not HBsAg.
Aims: To analyze the NAT yield rates of HBV WPI and OBI among blood donor population in Hong Kong.
Methods: In April 2007, the HKRCBTS implemented in-house routine NAT screening using the HIV-1/HCV/HBV triplex Procleix Ultrio Assay and automated Tigris platform (Chiron-Novartis, Emeryville, CA) for individual donation testing (ID-NAT). NAT reactive samples were retested with discriminatory assays to determine viral specific reactivity. Samples with discordant results between NAT and HBsAg assay (Prism, Abbott) were further tested with alternative NAT at Cambridge Blood Centre, UK, and for other HBV serologic markers, i.e. anti-HBc and anti-HBs.
Results: From April 2007 to December 2008, a total of 357 440 donations were collected and tested. Of the 1 325 (0.37%) HBsAg confirmed positive samples, 1 201 (90.6%) were NAT positive. For the 356 115 HBsAg negative samples, 93 were Ultrio assay reactive and discriminated for HBV. However, three of these were unconfirmed after further testing. Four of the 90 confirmed NAT yield samples had insufficient volume for additional serology. For the remaining 86 NAT yield cases, 6 (7%) were WPI: all were male (aged 20 – 56) with two first time donors and four repeat donors. Genotyping could be done on three of these six and all three were type B. For the remaining 80 NAT yield cases, all contained anti-HBc and were therefore considered OBI cases. Of these, 45 (56%) were also anti-HBs positive. For these 80 cases, one had history of past HBV infection on further inquiry, 16 had received HBV vaccination, 48 had not, and the remaining 15 had unknown vaccination status. Of these 16 OBI cases with past vaccination history, 11 (68.8%) were anti-HBs positive suggesting past breakthrough infections. Although genotype C is dominant in Hong Kong general population, 67% of OBIs in the blood donors with HBV gene sequencing results available were genotype B.
Conclusions: Combining serology and NAT, the overall HBV positive rate among donor population in Hong Kong was 0.40%. Of these, 6.4% could only be detected with ID-NAT and therefore, the NAT yield rate was 1 in 3 970. The majority (93%) of NAT yield cases belonged to OBI and many (25%) had record of HBV vaccination in the past.

P-115
SENSITIVITY OF EIGHT HEPATITIS B SURFACE ANTIGEN ASSAYS

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Background: HBV surface antigen (HBsAg) is the established serological marker routinely used for the diagnosis of acute or chronic HBV infections and the screening of blood or organ donors. Natural variation and mutations in HBV S gene can induce HBsAg conformational changes that may affect the performance of HBsAg assays. In this study, we analysed HBsAg mutant detection capability of eight HBsAg assays.
Study design: The studied HBsAg assays: ARCHITECT HBsAg, Abbott (A); Bioelisa HBsAg 3.0 (B); Enzygnost HBsAg 6.0, Siemens (C); ETI-MAK 4 HBsAg, Dia Sorin (D); Hepanostika HBsAg Ultra, bioMérieux (E); Monolisa HBsAg Ultra, Bio Rad (F); Murex HBsAg v.3, Abbott (G) and PRISM HBsAg, Abbott, (H) were evaluated for (i) analytical sensitivity performance with French national reference HBsAg panel and WHO international standard (00/588), (ii) the detection of HBsAg mutants with a panel of 25 recombinant mutants at 3 different dilutions (N = 73).
Results: The following table shows the results. Assays F and H showed the best analytical sensitivity. All eight assays detected HBsAg mutants at the highest concentration except D and H which failed to detect mutant 145 and 123 respectively. Assays A and F had the highest score for mutants detection.

Assay	A	B	C	D	E	F	G	H
Limit of detection								
French national panel (ng/ml)	0·11	0·32	0·14	0·07	0·09	0·02	0·09	0·04
WHO 00/588 (IU/ml)	0·04	0·09	0·08	0·04	0·04	0·01	0·05	0·01
Recombinant samples (n=73)								
Number positive								
Concentration A (n=23)	23	23	23	22	23	23	23	21
Concentration B (n=25)	24	20	23	24	25	25	25	23
Concentration C (n=25)	23	5	9	14	16	23	14	20
Total number positive	70	48	55	60	64	71	62	64
Score (%)	95·9	65·7	75·3	82·2	87·7	97·2	84·9	87·7

Conclusion: Assays with a high analytical sensitivity had a high score of detection of HBs mutants with low concentrations, but factors as the use of mixtures of monoclonal antibodies likely warrant a better recognition of such viruses.

P-116
HBV VIRAL LOAD IN HBV NAT POSITIVE DONORS: EXPERIENCE FROM THAILAND

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Background: Thailand has a high prevalence of hepatitis B viral (HBV) infection as average prevalence in recently survey of 4.5 %. Minipool NAT

using multiplex Polymerase Chain Reaction (Roche cobas TaqScreen MPX) was implemented to prevent transfusion transmitted hepatitis B infection since April 2007. The positive screening samples were discriminated by using Roche Cobas Ampliscreen HIV,HBV, and HCV. All HBV Nucleic Acid Test positive donation was subjected to HBV viral load determination.
Aims: We report here the HBV viral load detected in blood donors from April 2007 to September 2008.
Methods: All donation which had positive HBV discrimination test was sent to department of Immunology for HBV viral load. The viral load was done using Roche Cobas Taqman which has limit of detection of 6 IU/mL.
Results: We tested cobas TaqScreen MPX in 69 570 donations. 45 donations gave positive result and discrimination tested revealed HBV in 38 donations. In 38 HBV NAT positive donations the viral load was detected in 22 donations and one sample was not available for testing HBV viral load. Viral load were less than 6 IU/ml in 15 donations. In HBV viral load detected group the median viral load was 45.5 IU/mL. The lowest detected viral load was 7 IU/mL whereas the highest HBV viral load was 2590 IU/mL. The serological marker of four cases with highest HBV viral load in tested group correlated with acute HBV infection.
Summary/conclusions: Eighty four percent of positive infectious screening NAT tested in donated blood were HBV infection. The HBV vial load can be detected in 58 % of cases. The median HBV viral load was low. Clinical data, HBV serological profile and donor follow up in addition to HBV viral load are essential for interpretation of HBV infection.

P-117

INDIVIDUAL DONOR NAT TESTING USING THE COBAS S201 SYSTEM: 6 MONTHS EXPERIENCE IN A MOLECULAR BLOOD TRANSFUSION CENTER

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Background: Nucleic Acid Technology (NAT) for HIV, HCV and HBV has been widely implemented to ensure blood safety. Molecular testing of blood units has been centralized in Greece and henceforth is performed in individual format in Molecular Blood Testing Centers.
Aim: To evaluate the performance of the Cobas s201 system during routine single donor blood screening in a large setting.
Methods: Blood donations from 11 blood transfusion services are collected and screened daily in our Center using the Cobas s201 system/ Cobas TaqScreen MPX assay (Roche Molecular Systems). Three Cobas s201 systems, each consisting of three Cobas AmpliPrep instruments and two CobasTaqMan Analyzers in addition to two Hamilton Star pipettors (1:3:2 configuration), were used.
Initial reactive samples were retested at least once using either the same sample or sample from the plasma bag and were additionally subjected to discriminatory assay using the Cobas AmpliScreen HCV, HBV and HIV assay (Roche Molecular Systems). All samples were serologically tested for anti-HIV, anti-HCV and HBsAg using MEIA or ChLIA. Additional serology tests including anti-HBc, anti-HBs, HBeAg and anti-HBe were performed depending on sample availability.
Results: During the period January 2009 through to June 2009, a total of 43.872 blood donations were individually screened for HIV-1, HIV-2, HCV and HBV using the Cobas TaqScreen MPX assay. Throughout the screening period, 1183 samples (2.7%) were invalid due to instrument failure, invalid controls or user errors. The higher rate of invalid tests was observed during the first months (3.2–4.3%) and was significantly decreased over the time (1.28%), most probably due to the acquiring skills by the operators.A total of 220 blood donors (0.50%) out of 43.872 were initial reactive and blood donations were discarded. 148 donations of these were repeatedly reactive (67.3%). Following discrimination, 123 donor samples were found positive, of which 17 were HCV positive, 103 were HBV positive and three were HIV positive. No HCV or HIV NAT yield case was identified. Nonetheless, 20 HBV NAT yield cases were intercepted. The majority of them (90%) were anti-HBc positive, while only 2 out of 20 (10%) were anti-HBc negative. These cases most probably represent occult HBV infections and further characterization, including complete serology status, viral load and follow up samples is ongoing. Most importantly, 1 out of the 20 HBV NAT yield cases was IgM anti-HBc positive, revealing that it most likely represents an HBV infection in the window period (WP NAT yield case). The donor is currently followed up to evaluate the putative window period.
Conclusion: Over a six months screening period in our Molecular Blood Testing Center, an increased number of potential infectious blood units have been intercepted through the identification of 1 WP HBV NAT yield case and 19 occult HBV cases using Cobas TaqScreen MPX assay. The Cobas s201 system is an automated system which has demonstrated ample operational performance for routine blood donor screening in single unit format.

P-118

CORRELATION OF ID-NAT RESULTS FROM TWO NAT ASSAYS WITH HBSAG SEROPOSITIVE BLOOD DONORS

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Background: The overall benefit of the use of Nucleic Acid Technology (NAT) in the improvement of blood safety is unambiguous. Implementation of NAT in routine screening in addition to more sensitive serology assays has proven an efficient tool for reducing the risk of transfusion - transmissible viruses.
Aim: The present study was undertaken to compare NAT results in a number of seropositive blood units using the two commercially available NAT assays and an in house real time PCR method.
Methods: All blood donations from our transfusion center were routinely screened for serology markers using ChLIA (PRISM, Abbott) and were individually tested using Multiplex Procleix Ultrio Assay (Chiron Blood Testing). Discrimination was performed using Procleix dHBV assay (Chiron Blood Testing). When necessary, some samples were screened for additional serological markers, namely anti-HBc, anti-HBs, HBeAg, anti-HBe (Ax-SYM, Abbott). A number of blood samples were individually tested using the Cobas s201 system/Cobas TaqScreen MPX assay (Roche Molecular Systems). Quantification of viral load was performed with an in house Real Time PCR (LC II HBV DNA sensitive v1.3).
Results: Over a two year period (2006–2008) a total of 56.630 blood units were routinely screened both serologically and with Procleix Ultrio NAT assay. Although 173 donations (0.31%) tested positive for the presence of HBsAg, only 155 (89.6%) were NAT reactive. An increased number of blood units were NAT negative, though HBV seropositive. More specifically, 18 out of the 173 HBsAg positive samples (10.4%) showed no presence of viral DNA during initial routine screening. All of these samples were total anti-HBc positive and IgM anti-HBc negative. Following repetition of NAT screening with either Procleix Ultrio assay or Procleix Discriminatory Assay for HBV, the percentage of HBV seropositive/NAT negative samples decreased to 7.5%, as five more blood samples proved NAT positive. Of the 18 original donor samples, 11 were available in adequate volume and were also screened individually using the Cobas TaqScreen assay on a Cobas s201 platform in order to compare the specificity of the two systems. Interestingly, 8 out of these 11 samples were identified NAT positive, indicating that Cobas TaqScreen MPX assay is most likely more sensitive in detecting low viral load HBV infections. The use of an alternate in house real time PCR for quantification, when volume permitted, corroborated that the majority of these samples contained low viral load HBV DNA.
Conclusion: Comparison from serology screening and NAT testing with Ultrio revealed an elevated incidence of non viremic, seropositive blood units. The rate of HBsAg positive/ NAT negative donor samples was reduced when Cobas TaqScreen MPX assay was used, indicating a possible superiority of Cobas TaqScreen in detection of HBV low viral loads.

P-119

DETECTION OF HEPATITIS B VIRUS (HBV) USING NUCLEIC ACID TEST (NAT) AND ANTI-HBC SEROLOGY IN BANDUNG BLOOD CENTER INDONESIA

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Background: Blood screening test for transfusion transmitted pathogens in donor blood is important to ensure the recipient safety. Screening test should have the capability to identified infectious donations at the earliest stage after infection. Donations contaminated with hepatitis B virus (HBV) are traditionally detected with serologic methods for the presence of HBsAg and/or anti-HBc. These two methods have its advantages and disadvantages.
Aims: The aim of this study was to detect HBV DNA by using the Procleix Ultrio assay with the fully automated Tigris system (Chiron-Novartis, Emeryville, CA), and the results were compared with anti-HBc results.
Method: Study using this NAT system on our donor samples was conducted in June 2009 at Bandung Blood Center Indonesia. During a period of one month, 3 000 blood samples were tested in individual donation format (ID-NAT). Reactive samples were further tested with the discriminatory assays using the same platform. Samples tested reactive with the HBV discriminatory assay were further tested for the presence of anti-HBc.
Results: Of these 3 000 samples tested, 56 (1.87%) were reactive in the Ultrio NAT assay. Following discriminatory test, 48 (85.7%) samples were reactive for HBV. Of these 48 samples, 41 (85.4%) showed HBsAg and anti-HBc reactivity, while the remaining seven samples were nonreactive for both HBsAg and anti-HBc. Since the Ultrio NAT assay detects viral nucleic acids directly while serologic markers appear later, this discrepancy could be interpreted as infectious donations donated during the seronegative window period.
Conclusion: Based to our study, NAT method could detect HBV infection earlier than HBsAg and anti-HBc serology. Further testing is ongoing.

P-120

ANALYSIS OF QUASISPECIES OF HEPATITIS B VIRUS GENOME IN THE PRECORE REGION BY COLD PCR

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Background: When considering strategies to prevent transfusion-transmitted viral infection, it is important to determine viral characteristics or infection stages. As the duration of infection increases, the number of viral quasispecies tends to increase. Analysis of viral quasispecies may help distinguish chronic infection from resent infection among virus positive blood donors.
Aims: We developed a method of analyzing quasispecies of the hepatitis B virus (HBV) genome in the precore region, using COLD PCR (Nature medicine, 14, 579–584, 2008). COLD PCR enables the selective amplification of mutations in a wild-type background, based on the fact that wild-type and mutant hetero duplex DNA can be denatured at a temperature lower than the melting temperature of wild-type homo duplex DNA. Priority amplification of mutant sequences can occur using the lower temperature for denaturing.
Methods: Three types of HBV DNA, containing the precore region, were cloned into plasmid DNA, named #1, #2, and #3. The sequence of #1 was wild type, #2 differed from wild type in nt 1896 that results in a stop codon of HBe antigen, and #3 differred from wild type in three nucleotide positions including nt 1896. A region of 123 bp, including the precore region, was amplified by COLD PCR and subjected to sequencing (COLD PCR direct sequencing). COLD PCR started with construction of hetero duplex DNA of mutant and wild-type sequences, that is, denaturation of DNA at 94' for one minute and annealing at 70' for three minutes were carried out prior to amplification cycles. As #1 was hardly amplified by regular PCR in which DNA was denatured at 82', amplification by COLD PCR was performed at 81.5' for 20 seconds (the denature step), 61' for 30 seconds, and 72' for 30 seconds for 35 cylces. COLD PCR direct sequencing was performed using four HBV positive, HBc Ab-negative samples donated. We had found in the four samples that HBV DNA sequence had been determined as wild type at nt 1896 and that mixed sequences had been observed in other regions by conventional PCR direct sequencing.
Results: The sequence of #3 was detected significantly even in a starting DNA mixture that contained 4000 equivalents (eq) of #1 and 40 eq of #3, and it was considered that sequence #3 was very selectively amplified. The sequence of #2 was detected in a starting DNA mixture that contained 4000 eq of #1 and 400 eq of #2, and it was considered that sequence #2 was moderately selectively amplified. The four HBV-positive plasma samples were found to have not only the wild-type sequence but also the mutant sequence at nt 1896.
Conclusion: COLD PCR is an efficient method to analyze viral quasispecies. In HBV DNA-positive and HBc antibody-negative plasma samples donated, minor quasispecies of the HBV genome at a stop codon of the HBe antigen were detected by COLD PCR.

P-121

EPIDEMIOLOGICAL STUDY OF HEPATITIS B VIRUS GENOTYPE A IN JAPANESE BLOOD DONORS

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Background: Hepatitis B virus (HBV) genotypes B and C are predominant in Japan. Despite the distinct geographical localizations of HBV genotypes, the distribution rate of genotype A has increased in Japanese blood donors and acute HBV patients. It is suggested that subgenotype Ae might have been brought to Japan from the US. To provide an overview of the current state of HBV genotype A infection in Japanese blood donors, a phylogenetic tree analysis of HBV genotype A was carried out.
Study design and Methods: Data on Japanese blood donors from October 2006 to September 2007 were obtained from the Japanese Red Cross (JRC) database. The total number of blood donors was 4 974 911, and the number of HBsAg-positive donors was 2 043 (0.041%). The number of available samples was 1 979, and the HBV genotypes (A~F) were determined in 1 887 samples using enzyme immunoassay (EIA). The subgenotypes Aa (Asian type) and Ae (US and European type) were determined on the basis of the α region of 1 556 bases (nt2,333–3,221&1–667). The presence of the immunoglobulin-M antibody against the HBV core antigen was determined by EIA among all the HBsAg-positive donors.
Results: The rate of HBV-positive donors has declined yearly in Japan. However, recently, the distribution of genotype A has increased in blood donors and acute HBV patients. There were 106 HBV-genotype-A-infected donors. These donors might have been infected with HBV subgenotype Ae horizontally, because genotype A was not reported in Japan twenty years ago. The male/female ratio of those infected with subgenotype Ae is very high compared with the ratio of those infected with other genotypes. Particularly, IgM-HBc- or NAT-positive donors are found only in males. This is reminiscent of the fact that the HBV subgenotype Ae is predominant among HBV-HIV dually infected Japanese men who had sex with other men (MSM). Although donation places are located nationwide, the sequences of subgenotype Ae donated by 20-29- year-old donors were highly homologous. On the other hand, the distribution of subgenotype Aa was restricted to the Sanyo district (mainly Hiroshima prefecture).
Conclusions: The gender-specific distribution of HBV subgenotype Ae, which originated from the US or Western countries, was observed in male Japanese donors. HBV-subgenotype-Ae-infected donors tend to become HBV carriers. To reveal the epidemiology of viral genotype distribution and identify the donor population with possible high risk for infectious disease will help establish the strategies to decrease the risk for transfusion-tar-ansmitted viral infection.

4.2. TTID
Updates on hepatitis (HCV)

P-122

SERIAL SEROLOGICAL FOLLOWUP OF REPEAT VOLUNTARY BLOOD DONORS (RVBD) FOR HEPATITIS C VIRUS (HCV) INFECTIONS IN AN ENDEMIC COUNTRY

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Background: Incidence of HCV infection in repeat voluntary blood donor is usually very low because RVBD are tested after each blood donations. However, it was observed in endemic countries that some RVBD became HCV ELISA reactive after multiple non reactive blood donations. It was thought to be due to occult infections in RVBD. After few occasions, these donors again become non-reactive for ELISA. These types of erratic serological ELISA results lead to loss of eligible blood donors. Biological false reactive (BFR) results also lead to component loss. There was no scientific study about occult HCV infection from endemic countries on RVBD that becomes positive after multiple negative donations. This study was planned to carry out prospective follow-up of more than 50 000 voluntary donors and a group of committed RVBD for HCV ELISA reactive results who come repeatedly for donation. It was aimed to correlate ELSA reactivity and infectious status of RVBD with sensitive techniques.
Materials and methods: A total of 51 023 donors were included for anti-HCV by ELISA for a period of 14 months. Once blood door was reactive with one ELISA kit (kit:A) it was confirmed with another kit (kit:B). All samples reactive were tested by Nucleic Acid Test (NAT) and RIBA. The previous anti-HCV results of RVBD were looked back from the software database and blood donor testing registers.
Results: Out of 51 023 voluntary donors, 140 (0.3%) were anti-HCV positive. Thirty five (25%) of them were RVBD who showed ELISA reactivity. Sixteen (11.4%) were reactive by single ELISA kit and 19 (13.4%) were reactive by two ELISA kits. All 35 were tested by NAT and RIBA and none was positive. These RVBD were donating from 2–17 times for 8–65 months duration. From database, it was observed that 35 RVBD were negative by a particular ELISA kit (either kit: A/B). When the kit is changed due to logistic reasons in the blood bank, the donor became reactive to ELISA. However, if the test is done with the previous one, the donor again showed non-reactive results. Donors, who were reactive even once with two ELISA kits, were consistently reactive by the same two ELISA kits in their next donations also.
Conclusions: All RVBD ELISA reactive samples were false positive and it was kit specific probably due to cross reactivity. RVBD sample reactive in single ELISA kit should be tested by a second ELISA kit. Only if it is reactive in the second kit, the blood unit may be discarded. Donors who are reactive in two kits should be tested by NAT and RIBA. If they are positive by NAT and RIBA, they should be permanently deferred. If they are negative by NAT and RIBA, these donors should be followed up for 'reasonable period' and may be included in the donor pool in future.

P-123

SCREENING OF HCV ANTIBODIES AMONG BLOOD DONORS IN SOUTH BACKA REGION

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Background: Blood Transfusion Institute Vojvodina (BTIV) collects blood units in the South Backa Region of Province Vojvodina (north part of Serbia). Screening of HCV antibodies in blood donors has been mandatory since 1994.
Aims: The aim of this study was to report results of screening HCV antibodies among blood donors in the South Backa Region in observing time.
Methods: This study analyzed routine screening results of HCV antibodies during the period from January 1, 2004 to December 31, 2005. In BTIV samples were tested using comercial EIA anti-HCV antibody test (Ortho, BioRad, Biomerieux). Initialy reactive samples were retested in duplicate using the same test. Repeate reactive samples were tested by EIA test from diferent manufacturer and by confirmatory tests INNO-LIA tm* HCV Score test or CHIRON®RIBA®HCV 3.0 SIA. Blood units were discarded from clinical use.
Results: During monitoring period had been tested total of 58916 samples/donations: 515 (0.87%) were initialy reactive samples, 118 (0.2%) were repeatedly reactive samples. 36.07% of repeatedly reactive results were confirmed to be positive by confirmatory test.
Of the tested donations 5367 (9.11%) were from first-time donors: 0.71% were repeatedly reactive; 0.29% found to be positive for HCV antibodies by confirmatory test.
Prevalence of HCV antibodies among repeat donors was 0.149%.
Conclusions: During the investigated period a prevalence of HCV antibodies among blood donors in the region of South Backa was low. Despite of a low prevalence of HCV infection among blood donors during the monitoring period we suggest implementation of NAT assays in routine screening in the order to increase safety of transfused blood/components.

P-124

DEVELOPMENT OF A NOVEL NON-PCR NAT FOR THE DETECTION OF VIRAL NUCLEIC ACIDS IN DONOR SERA

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Background: Nucleic acid amplification testing (NAT) has been implemented in the blood services of many countries (including Japan) to detect viral DNA and/or RNA. Currently, the most widely used technique to amplify nucleic acids is polymerase chain reaction (PCR), which was developed in the 1980's and has both high sensitivity and specificity. However, only a few authorized commercial PCR tests are routinely used.
Aims: We developed a different NAT system with an isothermal amplification technique in order to (1) settle PCR-undetermined cases in the current NAT system, (2) serve as an alternative NAT from the viewpoint of the risk management, and (3) reduce the cost for NAT in the future.
Methods: We utilized Isothermal and Chimeric primer-initiated Amplification of Nucleic acids (ICAN®, TaKaRa Bio, Japan) technology. To establish ICAN® prototypes for HBV, HCV, and HIV-1, plasmids that encode a part of each viral genome were used as a template for safety. To determine the primers/probe sequences, we examined the performance of ICAN® using different sets of primers/probe candidates selected by a homology search of all deposited sequences of HBV, HCV and HIV-1 in the GenBank. We thereafter improved the performance of the each ICAN® test by modifying the condition and concentration of the included reagents. Finally, the sensitivity and specificity of the current version of HBV and HCV ICAN® was evaluated using positive sera. The sensitivity of HIV-1 ICAN® was examined using normal sera containing a different amount of recombinant HIV-1 clone (non-infectious pNL4-3 mutant, clade B).
Results: All tests were performed in a 100μL scale for 60 to 120 minutes. The concentrations of viruses detected at 100% in the current version of HBV, HCV and HIV-1 ICAN® were 100 IU, 100 IU and 100 vge, respectively. With regard to specificity, all of the ICAN® established in this study tested negative for HBV, HCV or HIV-1 in the 100 negative sera samples determined by our routine test.
Summary/conclusions: The novel non-PCR NAT based on ICAN® technology is promising, although we must improve the sensitivity and examine the efficacy of the test on a wide variety of isolates of HBV, HCV and HIV.

P-125

GOOD AND EFFECTIVE STRATEGIES IN THE SIGNIFCANT REDUCTION IN HCV PREVALENCE AMONG BEHBAHAN BLOOD DONORS DURING 2006-MARCH 2009

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Blood transfusion saved the life of millions people in the entire world. Occurrence of blood borne diseases such as hepatitis C threatens the blood safety and blood recipient's life. It is estimated that 180 million people in the world infected HCV and 3–4 million are newly infected each year. HCV is responsible for 50–76% of all liver cancer cases, and two thirds of all liver transplants in the developed world. It is predicted that Complications of liver disease related to hepatitis C virus infection are expected to increase over the next 10 to 20 years. Because the high number of multi transfused patients in our region, increase the transfusion demand and the importance of blood transfusion in HCV transmission study of prevalence helps us in evaluate the efficacy of preventive program.

In this descriptive cross sectional study all blood donors referred to Behbahan blood transfusion centers during March2006–2009 were checked for HCV Ab depend on Enzyme immuno assay and reactive samples retested in duplicate manner. Repeatedly Reactive (R.R) samples were confirmed with Recombinant Immuno Blot Assay (RIBA). The data were analyzed with SPSS14.

We found that the 1.267 %, 0.615 % and 0.329 % of our blood donors were (R.R) and 0.336 %, 0.305 % and 0.170 % confirmed by RIBA respectively during three years of our study. In the last year of our study the comprehensive software implemented (from registration to dispatch) for that center. The HCV prevalence in confidential exclusion unites was 1.36 % and 2.07 % respectively in two last years of study.

Our study shows the significant decrease in repeatedly reactive and HCV prevalence in blood donors at the end year of study. Use the comprehensive software for blood center helped us in improvement of good blood donor's recruitment and properly donors' selection program. Improvement of procedures and educational programs for blood donors and our physicians also had great role in this reduction. Monitoring of this prevalence to reduction of HCV prevalence continuesly improvement of donor selection and establishment of information technology to increase the blood safety and recommended.

P-126

DYNAMIC CHANGES OF MULTI-MARKER IN HCV-INFECTED BLOOD DONORS –FOLLOW-UP OBSERVATION ON 27 SUBJECTS FOR 15 YEARS

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Background: In the early part of 1990s, the amount of plasma collected by plasmapheresis had been increasing rapidly in China. At the same time, some plasma donors were infected with HCV. In order to understand their status of the health and disease course, some HCV-infected plasma donors were selected as subjects of following-up observation.

Aims: To track the dynamic changes of HCV- infected multi-marker and prognosis in blood donors during 15 years' HCV infection.

Methods: Blood specimens from 27 HCV-infected subjects were collected periodically, and multi-markers including serum (plasma) HCV-RNA, anti-HCV, ALT and thrombinogen time were detected. Liver biopsies were performed on nine volunteered donors after 15 years' infection. General physical examination and B-ultrasounds on liver and spleen were also performed on all subjects.

Results: In 15 years, 400 serial serum samples and 18 liver biopsy samples from 1993–2009 were collected. According to HCV RNA and anti-HCV positive rate, ALT abnormal rate, as well as the dynamic change tendency, 27 subjects can be divided into two groups: eight subjects (the first group) tend to resolve their HCV-infections, in which HCV RNA were lower than the detection limit (< 1000 cops/ml) in the most recent detections (2006 and 2009); The HCV RNA positive rate and the ALT abnormal rate in 15 years is 42.6%ï¼58/136ï¼‰and 11.1%ï¼16/144ï¼‰ respectively. The liver biopsies of 3 subjects showed one case was G1S0, and two Cases were G1S1 according to the national standard.19 subjects (the second group) remain chronically infected and were clearly HCV RNA positive in the most recent detections. HCV RNA positive rate and the ALT abnormal rate during 15-year infection is 88.5ï¼…ï¼293/331ï¼‰and 49.4%ï¼172/348ï¼‰ respectively. The liver biopsies in six subjects showed three cases were G2S1, and three cases were G1S0ï¼ŒG1-S1ï¼ŒG2S0. All 27 subjects were generally in good health condition in the passed 15 years. According to the liver biopsy result, all 9 volunteer donors were diagnosed as mild chronic hepatitis. Some of subjects were detected to be hepatolienomegaly by B-ultrasound detection and physical examination.

Conclusion: The 27 HCV-infected subjects have different disease progression rate during 15-year HCV infection. eight subjects were in HCV-infected convalesce stage, and 19 persons in the second group might be in progress stage. The different progress rate after HCV infection might be correlated to different HCV subtype. It is suggested to treat the 19 disease progressing subjects.

P-127

SCREENING OF SEROLOGICALLY NEGATIVE PLASMA POOLS FOR HEPATITIS C VIRUS BY NAT

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Background and objective: A residual risk of HCV infection by different blood products exists due to blood donations collected during the serological window period in the early stages of infection. The aim of this study is nucleic acid amplification technique (NAT)-based screening of the anti-HCV negative plasma pools obtained from Ahwaz blood transfusion center, in Iran for HCV RNA.

Materials and methods: This study was descriptive and cross-sectional. 20000 samples which were negative for anti HCV (EIA, third generation) screened for HCV RNA in 25 mini-pools. A total of 800 mini-pools were tested using RT- PCR method for qualitative detection of HCV RNA, with a lower limit of detection of 200 IU/ml.

Results: All of samples were negative for HCV RNA by RT-PCR method. On initial testing, two false positive results were defined as positive but on repeat single testing were negative.

Conclusion: The residual risk of transmission can be reduced by identification of early infection, which can lead to an improved safety of blood components.

P-128

DETECTION OF HCV RNA AMONG IRANIAN HCV-SEROPOSITIVE BLOOD DONORS

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Background: Only 15–25% of persons infected with hepatitis C virus (HCV) clear viremia. The factors and mechanisms of apparent viral clearance after seroconversion are not well understood but may be related to host and viral factors. Since introduction of nucleic acid amplification testing (NAT), the viremia status of HCV seropositive donors is available.

Aim: This study assessed the prevalence of HCV RNA negativity among anti-HCV (EIA) and HCV RIBA positive samples and investigated correlation between HCV RNA resolution and donation status, education level and demographic data.

Methods: A tital of 681 anti-HCV (EIA) and HCV RIBA positive samples collected from blood donors across the country, 2006 through 2008.A total of 371 (55%) samples from first time, 255 (37%) samples from lapsed and 55 (8%) samples from repeat blood donors were obtained.

All of samples were evaluated for HCV RNA by reverse transcriptase-polymerase chain reaction (Rt-PCR) assay. The sensitivity of the assay was 380 Copies/ml.

Results: Twenty eight percent of of confirmed-seropositive samples were HCV RNA negative. 33% (18 out of 55 samples) of repeat blood donors, 28% of first time and lapsed blood donors were tested negative for presence of HCV RNA. The prevalence of HCV RNA negativity in repeat blood donors is less than first time and lapsed blood donors but there was no significant difference in the frequency of HCV RNA negativity and donation status. There was no association between HCV RNA status and age, sex and education level of blood donors.

Conclusion: These results suggest that the frequency of HCV RNA negativity among seropositive blood donors is not related to donation status, age, sex and educational level as a marker of socioeconomic status. Follow-up studies of HCV RNA among negative blood donors are recommended for evaluation the viral clearance in this group blood donor.

P-129

THE INCIDENCE OF HEPATITIS C IN THALASSEMIC PATIENTS FOLLOWING SCREENING IN BLOOD TRANSFUSION CENTERS: A TWELVE-YEAR STUDY

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Introduction: Blood safety has always been and is the Iranian Blood Transfusion Organization's (IBTO) concern. In Iran the Hepatitis C screening test(HCVAb)became obligatory for blood donations from 1996. We therefore decided to determine the incidence of new cases of HCV in our thalassemia patients, who are regular consumers of blood donations and their blood is directly supplied by the IBTO.

Methodology: We studied patients of adult thalassemia Clinic. A cross-sectional examination was carried out on patients who had complete files with authentic information. Basic information and HCV test results were extracted from their files. Only cases that had a positive HCV Ab result following a negative result in the clinic file were considered as new cases. Cases that had no test results prior to 1996, and whose first test available in the file was positive were not considered as new cases. Patients' files were created in different years and followed up; therefore the incidence was calculated on the basis of person-year. Also, in order to increase accuracy and compare incidence in recent years, the incidence rate was calculated at five and seven year intervals (1996–2000, and 2001–2007).

Result: A total of 395 files were studied. There were 229(58%) male 166(42%) female. 274(69.4%) thalassemia major, 110(27.8%) intermedia, 10(2.5%) sickle thalassemia, and 10.3% HbH. The mean age was 27.5 years. In 395 patients, 109(27.5%) were infected with Hepatitis C, out of which 21 (19.2% of infected cases) had contracted the disease after 1996; meaning they had been screened for Hepatitis C prior to that date and had shown negative test results.

The estimated incidence of HCV cases in 12 years(1996–2007) was 5.1/1000. The estimated incidence in the first five-year period(1996–2001)was 6.8/1000, and 4.2/1000 in the second seven-year period(2001–2007).

Conclusion: The prevalence pattern of Hepatitis C is on the decline in Iran, both in blood donors and recipients. We owe this to the improved blood safety in the IBTO that has taken up better strategies. The thalassemic population has also benefited from this decline in prevalence. Even though the residual risk will never reach zero and we may still see new cases of Hepatitis C arise, but it will definitely be with a lower rate. The fact that we have had no new cases among our thalassemic patients bears witness to this matter. Regarding Hepatitis C in multi-transfused patients, a complete information system on donors should be available in the blood transfusion system, or at least the donor's blood sample should be frozen (Retention Sample) and made available for examination whenever necessary. On the other hand, medical transfusion centers should examine their blood transfusion hygiene system,(e.g. changing gloves from one patient to the other during blood transfusions, or not using common cotton swabs)to control intra-hospital infection transmission. Eventually, all routes of transmission of infection should be carefully observed and controlled.

4.3. TTID Update on HIV

P-130

REFERRAL HUMAN IMMUNODEFICIENCY VIRUS (HIV) SCREENING FOR BLOOD DONORS IN INDONESIA YEARS 2005–2007

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Background: In 2001, global approximately 14.000 new cases of HIV were found every day. 95% it was found in developing country. In Indonesia the first HIV/AIDS case was found in April 1987 and until March 2008 there were 17998 people with HIV/AIDS whom have died were 2486.* HIV infection could be transmitted through blood transfusion, therefore all donor's blood should be screened for HIV before transfusion. Central Blood Transfusion (CBTS) Indonesian Red Cross is a referral centre of HIV blood screening and help the Ministry of Health (MOH) to run surveillance of HIV infection among blood donors.
Aim: The study was run to see the repeated reactive of anti HIV blood screening result on donors blood that will reported as a surveillance data of HIV positive among blood donors.
Method: The Initial Reactive samples from the Branch Blood Transfusion Units in Indonesia was sent to the CBTS for re-testing using similar reagent that was used by the Branch BTUs and another Elisa reagent. If one or both the test showed reactive results, we identified the samples as Repeated Reactive samples.
Result: In the period of 2005–2007, there were 1797, 1683 and 1418 Initial reactive samples consecutively sent to the CBTS. Twenty four percent of these samples was tested using Rapid test, 70% using ELISA, 1,5% using both Rapid test and ELISA and 4,5% using Chemiluminesence. The repeated non reactive result was found in 32% of the samples, while 41% gave repeated reactive result. The remain 23% of the samples were indeterminate, and 4% were not able to be tested due to poor quality samples.
Conclusion: Thirty two percent non reactive results showed that false reactive rate in the Branch BTUs is very high that will impact to cost inefficiency of blood service. The false reactive results was suspected caused by high percentage of rapid test being used. Centralizing the Transfusion Transmissible Infection (TTI) blood screening by using the standardized ELISA is believe could increase the safety of blood. Meanwhile, the 41% repeated reactive results showed that donor selection criteria and method need to be improved in order to defer high risk blood donors.
*: Data from Ministry of Health until March 2008

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TRENDS IN THE FREQUENCY OF BLOOD DONORS WHO GAVE BLOOD TO BE TESTED FOR HIV IN SHIRAZ FROM 2004 TO 2006

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Introduction: Some blood donors may donate blood to obtain an HIV test; these donors can endanger blood safety. We surveyed donors to investigate whether education about the risk of HIV transmission through donated blood and the availability of free, anonymous HIV testing were effective in reducing the frequency of donation to discover HIV status.
Material and methods: This cross-sectional study involved 14 752 volunteer blood donors seen during a three year period from 2004 to 2007. Participants completed a specially-designed questionnaire with items covering demographic characteristics and their motivation for donation.
Results: In each year, 14.2%, 11.2% and 9.3% of all donors gave blood in order to be tested for HIV. The frequency decreased significantly over the time ($P < 0.05$). Donating for an HIV test was more frequent in men, first-time, single, younger and less educated donors ($P < 0.05$).
Discussion: The frequency of blood donation to obtain an HIV test decreased during the study period. This is evidence of the success of our center's educational efforts to inform people about the risk of HIV transmission via blood transfusion, and to encourage them to use centers that provide anonymous, free HIV testing instead of blood donation centers to discover their HIV status.

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IDENTIFICATION OF TWO HIV-1 SERONEGATIVE WINDOW PERIOD DONATIONS IN MALAYSIA

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Background: It has been shown that implementation of routine blood screening using the nucleic acid amplification testing (NAT) technology for HIV-1, HCV and HBV can significantly shorten the seronegative infectious window periods and hence, enhance the safety of the blood supply.
Aims: To report two HIV-1 seronegative window period cases identified in our laboratory since implementation of routine NAT blood screening.
Methods: After significant finding from the pilot studies on automated Tigris system (Chiron-Novartis, Emeryville, CA), the National Blood Center of Malaysia located in Kuala Lumpur, had implemented routine NAT blood screening using the Procleix Ultrio assay in individual donations testing (ID-NAT) format since November 2007. Ultrio-reactive samples were tested with the discriminatory assays on the same platform. NAT results were compared with serologic results. Samples with discrepant results between NAT and serology were further tested and corresponding donor were called back for follow-up testing.
Results: For the first 14 months of NAT screening, a total of 208 134 donations were tested, and 68 of them were found positive for HIV-1. Of these, 66 were also seropositive, while the remaining 2 were seronegative and later confirmed as window period infections upon follow-up testing. The first case was a 20 years old male who donated a seronegative unit 11 months earlier; this donation was not NAT tested. The index donation was positive for NAT and p24 antigen but was borderline negative in antibody tests. Three components were produced but intercepted. A five month follow-up sample was confirmed seropositive but with insufficient volume for NAT. The second case was also a male of 21 years old who donated a seronegative donation 13 months prior, which was not NAT tested. The index donation was negative in all anti-HIV tests but positive for p24 and NAT. Three components were prepared from this donation and intercepted. A sample collected 2 months later was antibody positive and NAT positive. Our results show an HIV positive rate of 1 in 3 061 and NAT yield rate of 1 in 104 067 among our donor population.
Conclusions: Since implementation of routine NAT screening, in 14 months 2 HIV-1 window period donations were identified. As a result, six components were intercepted and therefore, potentially six transfusion transmitted infections were prevented. Our data demonstrate the importance of NAT screening in protecting our blood supply and patients.

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COMPARISON OF TWO COMMERCIAL REAL-TIME PCR ASSAYS FOR MEASUREMENT OF HIV VIRAL LOAD

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Introduction: Viral load measurement of HIV is an essential and standard tool for diagnosis and treatment of HIV infections, i.e., to monitor treatment, determine prognosis and risk of disease progression and to identify treatment failure. Thus, accurate measurement of viral load is important. Currently, some commercial assays are available in quantification of HIV RNA. In the present study, we compared the performance of two kinds of

widely used commercial real-time PCR assays; COBAS TaqMan HIV-1 (CTM; Roche Diagnostics, Mannheim, Germany) and Abbott RealTime HIV-1 (ABB; Abbott, Wiesbaden, Germany).

Materials and methods: Samples were obtained from 181 HIV-infected patients whose HIV-1 viral load tests were requested to the laboratory in a university hospital from November 2008 to March 2009. HIV-1 viral loads were measured using both CTM and ABB following manufacturer's instructions. Log values of the results were used for statistical analysis. The results below the level of linearity range (40 copies/mL, 1.602 log10copies/mL) were regarded as 1.602 log10copies/mL for optimal comparison.

Results: Among 181 specimens, 103 samples showed results below the level of linearity by any or both of the method (Table 1). The mean difference between two assays was 0.044 log10copis/mL (95% confidence interval, 0.067 to 0.021). The Pearson's correlation coefficient of two assays were $R = 0.993$ $P < 0.0001$, 95% confidence interval, 0.990 to 0.995). On linear regression, intercept was -0.0156 (95% confidence interval, -0.0661 to 0.0350), and the slope was 1.0242 (95% confidence interval, 1.0060 to 1.0425).

Conclusion: The results of HIV-1 viral loads using two commercial real-time PCR assays by CTM and ABB were comparable and well correlated. It can be considered as an alternative method for patient monitoring.

4.4. TTID
Update on other transfusion related viruses

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DECLINING TENDENCY OF HUMAN T-CELL LEUKEMIA VIRUS TYPE I CARRIER RATES AMONG BLOOD DONORS IN MASHHAD, IRAN

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Human T-cell leukemia virus type I (HTLV-I) is a retrovirus unevenly distributed around the world. Recently North East Iran, particularly the region of Mashhad, has been recognized as a new endemic region with 2–3% of the population and 0.7% of the blood donors infected with HTLV-I. We report the results of screening 232 648 blood donors in Mashhad for antibodies to HTLV-I, from January 2004 to December 2006. The donors were 90% male (n = 211,265) and 10% female (n = 21,383), with a mean age of 30 years (range: 18–65). The number of blood donations was 73489, 77502 and 81657 in the years of 2004, 2005 and 2006, respectively. Anti-HTLV-I antibody was performed by using ELISA. The positive samples were confirmed by Western blot (WB) test. Among the 232 648 blood donors analyzed, 1054 (0.45%) were repeatedly reactive by ELISA and confirmed by WB testing. The numbers of HTLV-I seropositive blood samples were 365, 343 and 346 in 2004, 2005 and 2006, respectively with a prevalence of 0.5%, 0.44% and 0.42% in these years. One hundred sixty three out of 1054 seropositive donors were females (15%) and 891 were males (85%). The prevalence of HTLV-I infection in males and females was 0.42% and 0.76% respectively. This decline in HTLV-I prevalence in Mashhad, from 0.77% in 1999 to 0.42% in 2006, could be attributed to better strategies for donor screening, sufficient education to the population about the routes of transmission and modes of protection, and other unknown reasons which need more studies to be determined.

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EPIDEMIOLOGY OF HUMAN T-LYMPHOTROPIC VIRUS I/II AMONG BLOOD DONORS IN TAIWAN

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Background: Systematic screenings for antibodies of Human T-lymphotropic viruses (I+II) among all donated blood have begun from January of 1996 in Taiwan. The epidemiology of HTLV infected people among blood donors has not been described before.
Aims: The aim of this study was to determine the seroprevalence, demographic information and co-infection rate of HTLV with human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus among blood donors in middle and southern Taiwan.
Methods: There were 1 899 527 blood donors included in this study from January 2007 to December 2008. The antibodies to HTLV (I+II), HIV(1.2.O), hepatitis C and hepatitis B surface antigen (HBsAg) were detected by enzyme immunoassay. Confirmation of HTLV- I+II enzyme immunoassay (EIA) positive cases were detected by Western blot assay.
Results: The prevalence rates of HTLV, HIV, HBV and HCV was 0.0064 %, 0.12 %, 0.47 % and 0.14 %, respectively. HTLV prevalence showed a significant and linear increase with age ($P < 0.001$). In addition, the prevalence rates in female were significant higher than those in male ($P < 0.001$). The co-infection rates of HTLV with HIV, HBV, and HCV were 0 %, 0.124 % and 0.375 %, respectively.
Conclusion: The findings of our study suggest that the prevalence of HTLV among blood donors was lower than other infectious agents, and the co-infection rate of HTLV with HCV was higher than the co-infection rate of HTLV with HBV in HBV high prevalence country. More retrospective studies of demographic information should be taken in the future.

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PHYLOGENETIC ANALYSIS OF HUMAN PARVOVIRUS B19 AMONG BLOOD DONORS IN HOKKAIDO, JAPAN

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Background: Human parvovirus B19 (B19) is a small non-enveloped DNA virus that is transmitted to susceptible individuals through blood transfusion and plasma-derived products. In Japan, donated blood has been screened for this virus using a receptor-mediated hemagglutination assay (RHA) from 1997 to 2007 and a chemiluminescence enzyme immunoassay (CLEIA) from 2008. Recently, B19 has been classified into three genotypes, 1, 2 and 3, based on its sequence homology. This study presents the results of genotyping of B19 in Hokkaido, Japan from April 1996 to March 2009.
Aim: To clarify the distribution of B19 genotypes in B19-infected individuals from Hokkaido, Japan.
Methods: Blood donors were screened for B19 using RHA or CLEIA (detection limit: approx. 10^10 IU/mL, 10^6 IU/mL, respectively) from April 1996 to March 2009 in Hokkaido, Japan. Reactive samples were tested by TaqMan real-time PCR assay. Universal PCR with some modification are performed according to the previous report (Koppelman et al., Vox Sanguinis 2007 93, 208 – 15). A total of 96 randomly selected B19 DNA-positive samples were analysed further by PCR direct sequencing and phylogenetic analysis based on the various sequences of NS1/VP1u region of nt 2254 – 2978 (numbering according to GenBank accession no. AF162273).
Results: Sequencing analysis based on NS1/VP1u region showed that only genotype 1 of B19 was detected in blood donors in Hokkaido. This genotype 1 was at least segregated into four minor subgroups. The nucleotide identities between isolates from each subgroup ranged between 98.2% and 99.3%. The intragenotypic homology in each subgroup was > 98.4%. One subgroup was consecutively detected from 1996 to 2009 and the other subgroups disappeared in the early period or the other emerged recently. The regional epidemic in Hokkaido of these subgroups was not observed.
Conclusions: Only B19 genotype 1 with four subgroups was a strain circulating in Hokkaido during the past 13 years. The two new genotypes of B19, genotype 2 and 3 were not detected. A time of emergence differed with each four subgroup.

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EXTENDED STUDY TO EVALUATE THE NECESSITY OF HTLV SCREENING IN KOREAN BLOOD DONORS

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Background: Seroprevalence of human T-lymphotropic virus (HTLV), known to be transmissible by transfusion, was considered to be very low in Korea. However, with the identification of a HTLV-1 confirmed positive case among 15 173 donors (0.007%) during a study performed in 2006, the Korean Ministry for Health, Welfare and Family Affairs (MIHWAF) decided

to conduct an extended study until approximately 10% of donors have been tested to evaluate the necessity of HTLV screening in Korea.
Materials and methods: Serum samples were collected from December 2007 to January 2008 from a total of 353 001 donors. Serological screening was done with the ABBOTT PRISM HTLV-I/HTLV-II assay (Abbott Diagnostics). Samples reactive repeatedly on serological screening were tested further by Western blot (WB) analysis (HTLV Blot 2.4, MP Diagnostics) and a line immunoassay (INNO-LIA HTLV I/II, Innogenetics N.V.). These samples were also tested for HTLV proviral DNA by a nested polymerase chain reaction (PCR) method.
Results: Among 309 samples positive on serological screening, 284 samples (0.08%) were repeatedly reactive. 33 samples (0.009%) were confirmed positive by WB, line immunoassay and PCR. 13 samples gave indeterminate results. There was no regional or gender difference in seroprevalence ($P > 0.05$), but seroprevalence increased with age ($P < 0.001$).
Conclusions: As the observed seroprevalence did not differ with that of 2006, the extended study confirmed the results of the study performed in 2006. This seroprevalence is higher than that of some countries where HTLV screening is mandatory. Based on the result of this study, all donors donating whole blood are screened for HTLV in Korea since April 2009.

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INVESTIGATION OF THE INCIDENCE OF HUMAN PARVOVIRUS B19 IN KOREAN BLOOD DONORS

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Background: The safety of plasma derivatives has been reinforced since 1980s by NAT (nucleic acid amplification test) screening of source plasma and implementation of various pathogen inactivation/removal steps in the plasma fractionation process. Some countries have been screening the human parvovirus B19 (B19V) in blood donors to secure the safety of transfused blood and fractionated products.
Aims: We studied the incidence of B19V DNA in Korean blood donors to evaluate the necessity of B19V screening in Korean blood donors.
Materials and methods: Plasma samples were collected during March 2008 to July 2008 from 10 032 plasmaphersis donors. NAT of B19V DNA was done using LightCycler 2.0 (Roche, Mannheim, Germany). B19V Quantification kit (Roche, Mannheim, Germany) was used as reagent. Anti-B19V IgM and IgG was tested in 938 randomly selected donors using recomWell Parvovirus B19 IgM, IgG assay (MikrogenR, Neuried, Germany). Donors with positive results in NAT and anti-B19V test were confirmed by line immunoassay using recomLine Parvovirus B19 IgG/M kit (MikrogenR, Neuried, Germany).
Results: The incidence rate of B19V DNA was 0.1% (10/10,032). Level of virus titer in B19V DNA positive donors was low (less than 10^5 IU/mL). Only one donor showed high titer of B19V DNA (1.33-10^8 IU/mL). 64% of the tested donors had anti-B19V IgG antibodies. Nine of ten B19V DNA positive donors had anti-B19V IgG/M antibodies.
Conclusion: This study showed that the incidence of B19V in Korean blood donors was not high and many donors have anti-B19V antibody. Therefore, the implementation of B19V NAT as a donor screening test seems not to be required.

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PREVALENCE OF CHLAMYDIA PNEUMONIAE IN PERIPHERAL BLOOD MONONUELEAR CELLS BY RT-PCR IN MULTITRANSFUSED PATIENTS

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Background: Chlamydia pneumoniae is a common pathogen that has been associated with upper and lower respiratory tract infections. Recent evidence suggest that C.pneumoniae associated with some chronic disease such as cardiovascular disease, sarcoidosis, reactive arthritis, lung cancer and some neurologic disease (multiplesclerosis or alzheimer). It has been suggested that C.pneumoniae could be disseminated by circulating monocytes. Resent studies have shown that apparently healthy blood donors harbor C.pneumoniae within their peripheral blood mononuelear cells (PBMNs). Therefore there is possibility of its transmission by blood transfusion Specially in multitransfused patients.
Aims: In the present study we evaluate prevalence of C.pneumoniae DNA in groups of patietns who frequently receive blood (thalassemia major patients).
Method: Study subjects were adult thalassemia major patients (n = 113). Informed consent was obtained from all patients. The presence of C.pneumoniae DNA in PBMNs from 10ml blood of patients was assessed by real-time reverse transcriptation polymerase chain reaction (RT-PCR). PCR master mix containing primer specific for C.Pneumoniae 16SrRNA(-sense:5?GGA-CCT-TAG-CTG-GAC-TTG-ACA-TGT,antisense: 5? CCA-TGC-AGC-ACC-TGT-GTA-TCT-G).
Results: By using RT-PCR, we detected the presence of C.pneumoniae DNA in 5 of 113 (5.65%) thalassemic patients. Our study group, were 61 males and 52 females. Sixty seven (75.7%) of patients had history of splenectomy. There was no significant correlation between the presence of C.pneumoniae DNA and age, sex, duration or number of blood transfusion and history of splenectomy.
Conclusion: According to some studies presence of C.pneumoniae DNA seems to be frequent in apparently healthy blood donors. But furthermore studies are needed on blood recipients to define whether or not transmission has occurred.

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COMPARISON OF TWO COMMERCIAL SCREENING ASSAYS FOR DIAGNOSIS OF HUMAN T-CELL LYMPHOTROPIC VIRUS TYPES 1 AND 2

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Background: Human T lymphocytotropic virus HTLV is a virus from retroviridae family, and more than 20 million people are infected with this virus worldwide. It can cause T-cell leukemia/lymphoma in adults,tropical spastic paraparesis and HTLV associated myelopathy(HAM/TSP). It can be transmitted by blood products and via blood transfusion.
Aims: The aim of this study was comparison specificity of the two ELISA commercial assays; HTLV 1/2 ELISA 3.0(MP Diagnostics) & Vironostika HTLV-1/2(BIOMERIEUX) for HTLV 1/2 detection.
Methods: A cross-sectional study was conducted from march 2007 to July 2007 among blood donors. At first the serum of blood donors was checked by HTLV 1/2 ELISA 3.0(MP Diagnostics) and positive cases were comfirmed by HTLV BLOT 2.4 WESTERN BLOT ASSAY(MP Diagnostics kit). Then positive samples were tested by Vironostika HTLV-1/2(BIOMERIEUX).
Results: A total of 463 repeatedly reactive donors samples with HTLV 1/2 ELISA 3.0(MP Diagnostics) were evaluated by HTLV BLOT 2.4 for confirmation. 70 samples among these positive samples were confirmed, 323 samples had indeterminate results and 70 cases were negative for HTLV BLOT 2.4. Then 463 repeatedly reactive donors samples with HTLV 1/2 ELISA 3.0(MP Diagnostics) were analyzed by Vironostika HTLV-1/2 (BIOMERIEUX). 42 cases among 463 samples had positive results with Vironostika. These 42 cases were evaluated with HTLV BLOT 2.4 for confirmation. 39 samples and 3 cases among 42 samples had positive & indeterminate for HTLV BLOT 2.4 respectively.
Conclusion: For important of blood safety, the assay with high sensitivity and specificity is recommended to be used. These results should be considered for choosing the most accurate serological screening assays in order to obtain efficiency of the algorithm for HTLV 1/2 diagnosis and prevention of its transmission via blood & blood products transfusion.

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QUANTIFICATION OF HUMAN T-CELL LYMPHOTROPIC VIRUS TYPE 1 (HTLV-1) PROVIRUS LOAD IN SEROPOSITIVE BLOOD DONORS AND ESTIMATION OF INFECTIOUS VIRAL LOAD FOR TRANSFUSION-TRANSMITTED INFECTION

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Background: Serological screening and prestorage leukocyte reduction for donated blood have undoubtedly decreased the risk of transfusion-transmitted infection (TTI) for HTLV-1 in Japan. However, the provirus load in blood component that would cause TTI is still unclear.

Aims: HTLV-1 provirus load was measured in blood samples collected before leukocyte reduction that were obtained from seropositive blood donors. From the distribution of provirus load among blood donors, provirus load for infectivity was estimated using the historical data on the frequency of transfusion-transmitted infection.

Methods: DNA samples were obtained from peripheral blood mononuclear cells or blood clots of stored samples obtained from 74 HTLV-1-seropositive individuals. All blood samples were obtained before leukocyte reduction. HTLV-1 provirus load was determined using TaqMan PCR for the HTLV-1 pX region and human CD81 gene to estimate the amount of cellular DNA. Previous data showed that seroconversion occurred in approximately 80% of patients transfused with one unit of fresh red-cell concentrate from HTLV-1-seropositive blood donors (Okochi K et al. AIDS Res 1986;2:S157–61). It is, therefore, expected that 80% of our blood samples will be in the category of units with infectious risk, which allows us to estimate the viral load for infectivity by transfusion.

Results: The HTLV-1 provirus loads in HTLV-1-seropositive blood donors ranged from less than 0.01 to 4.9 copies (average 0.83) per 100 leukocytes. Eighty per cent of blood samples evaluated contained at least 0.06 copies of HTLV-1 provirus per 100 leukocytes. Assuming that the number of leukocytes per unit of red-cell concentrate was $1\text{-}10^9$ before leukocyte reduction, a minimum of $6\text{–}10^5$ HTLV-1-infected cells would have been found in the unit that caused TTI.

Conclusions: In 2007, universal prestorage leukocyte reduction was introduced for all blood components in Japan. The number of residual leukocytes after leukocyte reduction is confirmed to be less than $1\text{–}10^6$ in 99% of unit currently issued from Japanese Red Cross Blood Center. If serological screening is omitted, the maximum number of HTLV-1-infected cells found in blood components would be $4.9\text{–}10^4$ per unit. This figure is substantially lower than the infectious virus load estimated ($6\text{–}10^5$ infected cells). The combination of serological screening and universal leukocyte reduction virtually eliminated the TTI risk for HTLV-1 in Japan.

4.5. TTID Bacterial contamination

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THE STUDY OF EFFICIENCY OF GMP FOR PHLEBOTOMY IN DECREASING THE RATE OF BACTERIAL CONTAMINATION OF PLATELET UNITS PRODUCED IN TEHRAN BLOOD TRANSFUSION CENTER BY USING BACT/ALERT

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Background and objectives: Bacterial contamination evaluation of blood products is regarded as important point of blood safety. Since platelets should be stored at room temperature that makes them an excellent growth medium for bacteria, it is mentioned as a major problem in transfusion medicine. Regarding that the transfusion risk of a bacterial contaminated platelet concentrate is 100 to 1000 time higher than viral pathogen such as HIV,HBV,HCVand HTLV. For reducing this risk attempt on implementation of quality audit and detection of contaminating agents affecting platelets during preparation procedure have important role.
Material and methods: At the first step of this interferential - applied study samples were taken from 1332 platelet bags were inoculated in Bact ALERT vials for detecting aerobic and obligate anaerobic bacterias. At the second step after implementation of GMP for phlebotomy 272 blood units were obtained from donors under quality audit. Samples were taken from platelet units which were produced from these blood bags were inoculated in Bact ALERT vials like previous step. Finally Results were compared
Findings: The rate of bacterial contamination in platelet concentrates was 1.9%. It contained 1.1% for aerobic bacterias and 0.8% for anaerobic bacterias. Inapproprate disinfection of donors skin was detected as effective role for Bacterial contaminations Of platelet units. At the second step of this study after implementation of GMP for phlebotomy and improving skin disinfection procedure, the rate of bacterial contamination in platelet concentrates changed to 0%.
Conclusion: Regarding the role of bacteria to risk the blood safety (particularly platelet concentrate) it is necessary to diminish the risky agents as low as possible by implementation of GMP for preparing this product and developing platelet safety by using appropriate detecting procedures.

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DEVELOPMENT OF AN INTERNALLY CONTROLLER ASSAY FOR BROAD RANGE DETECTION OF BACTERIA IN PLATELET CONCENTRATES

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Introduction: At the moment, bacterial contamination of blood products is the most common microbiological risk of transfusion. The prevalence of contamination of cellular blood products is approximately 1 in 3000 donations. The risk is greatest for platelet concentrates (PC) as they are stored at room temperature under constant agitation to preserve function and vitality. These conditions make PCs an excellent growth medium for bacteria. In the Netherlands, screening of PCs for the presence of bacteria is done by automated culturing with the BacT/Alert culturing system (BioMerieux). Although the system is sensitive, in theory it can detect 1 colony forming unit (CFU) per 5 to 10 ml PC, its use is restricted by long assay times. Slow growing bacteria or low bacterial loads are not always detected by the system. Previously a real-time PCR assay based on the 16s rRNA gene was developed as a fast alternative for culturing of PCs. However, this assay was not as sensitive as the BacT/Alert culturing system. To improve the sensitivity of the real time PCR assay, a reverse transcriptase step was added to detect RNA.
Methods: Total nucleic acids were isolated using the MagNA Pure LC automated extraction system (Roche). Hereafter, reverse transcriptase was used to make cDNA, together with random hexamer primers. The real-time PCR was performed with a previously developed 16S rRNA gene primer and probe set that detects all bacteria relevant for bacterial contamination in PCs. A RNA bacteriophage internal control (IC) was used to control RNA isolation and amplification. Two model bacteria, Staphylococcus epidermidis and Escherichia coli were used to determine the sensitivity of the assay in PCs. The total amount of RNA and DNA in growing bacteria and bacterial cultures treated with antibiotics was determined to investigate whether there are differences in sensitivity of the test dependent on the bacterial viability.
Results: With the real time PCR based on detection of DNA, 150 CFU/ml of E. coli and 700 CFU/ml of S. epidermidis could be detected in PCs. By detecting RNA, sensitivity improved to 3 CFU/ml for *E. coli* and 70 CFU/ml for S. epidermidis. The ribosomal RNA of bacteria grown in the presence of antibiotics broke down very slowly. Therefore, the test is suitable for detection of bacteria even if the bacteria are not actively growing.
Conclusions: - Sensitivity of 16S rRNA PCR assay improved considerably by detecting RNA instead of DNA.
- Because RNA of bacterial cultures treated with antibiotics breaks down very slowly, the test remains sensitive, even if the bacteria are not actively growing.
- Because of the short turn around time of the real time RT PCR assay it can be used as alternative for the BacT/Alert and/or as a rapid test shortly before transfusion.

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APPLICATION OF A RAPID BACTERIAL DETECTION SYSTEM USING A NEWLY DEVELOPED BIO-IMAGING TECHNIQUE FOR RED CELL CONCENTRATE

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Background: Bacterial detection systems or inactivation techniques are necessary to reduce the risk of transfusion-transmitted bacterial infections. However, an inactivation technique for red cell concentrate (RCC) is under development, and bacterial culturing takes several days. In addition, the expiration date of RCC is only 21 days after collecting blood considering the risk of bacteria growing at the low temperature such as *Yersinia enterocolitica* etc. although we have already introduced prestorage leukocyte reduction and the diversion of the initially drawn blood. It will be very beneficial to extend it to 28–35 days by implementing the bacterial testing for the 21 day older RCC in preparation against the shortage of RCC in the near future. Therefore, we developed a rapid bacterial detection system for a platelets concentrate (PC) and applied to RCC.
Methods: Gram-negative (Serratia marcescens, Serratia liquefaciens, Yersinia enterocoltica, and Pseudomonas fluorescens) and gram-positive (Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus dysgalactiae, and Propionoum acnes) bacteria were spiked into RCCs so that the final bacterial concentrations were 10 cfu/mL, 100 cfu/mL, and 1 000 cfu/mL. After hemolyzing, the pretreated RCCs were filtrated, stained with CFDA (carboxylfluorescein diacetate) and auto-analyzed on the bio-imaging system, the microFinder.
Results: The spiked bacteria were all detected after complete removal of hemolyzed red blood cells (RBCs). It took 15–30 minutes for the hemolyzing process, 15 minutes to stain and catch the bacteria, and 15 minutes for the auto-analysis.
Conclusion: Removing RBCs completely by hemolyzing is an important process to increase the sensitivity and specificity of this bacterial detection

system. Although further examinations are needed for the other hemolyzing methods and bacteria, this system using the microFinder will be very useful for rapid bacterial screening of 21 day older RCCs before supplying to hospitals.

P-145

INVESTIGATION ON BACTERIAL CONTAMINATION IN BLOOD COMPONENTS

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Background: Blood can help bacteria growth, especially the platelet, it must be stored in (22 ± 2)' and shaked. If the platelet has been contaminated by bacteria, it can easily provide an ideal environment for bacteria growth. We began the study of blood contamination by bacteria at 2005.
Aims: To investigate the ratio of bacterial contamination in blood and platelets after donation, and identify the kind of bacteria.
Methods: Samples of blood components from 2005 to 2008 were cultured by the automatic incubator Bacter/Alert 3D 120. The positive blood were sampled and cultured again. Bacteria in double positive samples were identified the strain. Results A total of 10475 samples were cultured, and 11 of them were positive (positive rate: 0.11%). A total of 932 whole blood samples were cultured, and 2 of them were positive (positive rate: 0.2%). A total of 4056 concentrated platelets samples were cultured, no positive was found. A total of 5487 apheresis platelets samples were cultured, and nine of them were positive (positive rate: 0.16%); 4 in 9 positive samples were identified as anaerobic bacterium, and the others were aerobic bacteria.

Table 1: Blood components contaminated by the bacteria

name	total	anaerobic bacterium		anaerobic bacterium	
		positive	positive rate (%)	positive	positive rate (%)
whole blood	932	2	0.2	---	---
concentrated platelets	4056	0	0	---	---
apheresis platelets	5487	5	0.09	4	0.07
total	10475	7	0.07	4	0.04

Conclusions: The ratio of bacterial contamination in whole blood and platelet was far higher than virus contamination in our blood bank. Most of the contaminated bacteria were considered of coming from donor's skin. Disinfection of blood-draw point at donor's skin and discard the first 10~20 ml blood were the most important procedure for preventing the blood products from being contaminated. Bacteria culture including anaerobic bacterium and anaerobic bacterium should be carried out, in order to ensure the safety of transfusion.

P-146

TRANSFUSION ASSOCIATED BACTERIAL INFECTION BY PLATELET CONCENTRATES CONTAMINATED WITH *STREPTOCOCCUS DYSGALACTIAE* SSP. EQUISIMILIS

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Case report: A MDS patient received apheresis-derived platelet concentrates (PCs) that were 4 days old. A few minutes after the start of transfusion, small aggregates were observed at the bottom of the PCs bag and the flow became very slow. A total of approximately 10 mL of PCs entered into the patient blood when the flow finally stopped. The patient developed angialgia, hypotension, fever (> 40°C) and chest pain 4 hours later. Fortunately, those symptoms were improved on the next day by medication including antibiotics.
Samples of the recipient blood and PCs were subjected to blood culture and *Streptococcus dysgalactiae* ssp. Equisimilis was detected in the both samples. DNA analysis showed they were identical, revealing that posttransfusion bacterial infection was caused by *Streptococcus dysgalactiae* ssp. Equisimilis that was contaminated in PCs. PCs in the bag contained more than 10^6 CFU per mL of bacteria and pH was 5.5. However, the appearance of the PCs looked normal without color abnormality, opacity or cessation of swirling.
The aggregates observed at hunging to the girdle were the only unusual appearance in this case. The aggregates were observed not only in the bottom of PCs bag but in the segment tube. The PCs bag had been kept hanging to the girdle for a few minutes.It should be noted that the abnormal appearance of the PCs bag that was contaminated with *Streptococcus dysgalactiae* ssp. Equisimilis could not be detected by observation of platelets swirling that was usually performed in blood centers and hospitals. It could be detected when PCs bag was hung to the girdle for transfusion at bedside.
Conclusion: The observation of swirling at pre-transfusion is an important countermeasure to reduce the risk of transfusion-associated bacterial infection. However, platelets swirling was observed despite a large amount of bacteria in the PCs bag in this case. In addition to the observation of platelets swirling, pH test before transfusion and confirmation of existence of small aggregates in PCs bag before and after transfusion is important to reduce the risk of transfusion-associated bacterial infection.

4.6. TTID
Parasites and transfusion

P-147

FOUR YEARS EXPERIENCE ABOUT MALARIA IN SRILANKAN BLOOD DONORS

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Background: Blood transfusion provides an ideal artificial vector for malaria parasite to transmit from asymptomatic blood donor to a recepient. For several decades Srilankan blood donors are screened for *Plasmodium vivax* and *P falciparum* to prevent transmission of malaria. Also we are using risk factor questions to differ donors who are at risk for infection with malaria parasite. Risk factors are classified as history of malaria for last three years and history of febrile illness in last 1 week duration.
Aim: To asses the prevelence of malaria among blood donors.
Method: A Retrospective analysis was done for all blood donors from colombo north teaching hospital Sri Lanka from 2004 to 2007. At the time of blood donation a blood film was prepared to identify malaria parasite which is the causative agent for malaria infection. From the thin blood film we can detect the malaria species and the thick blood film help to detect parasetemia. These blood films are raed by welltrained and experienced technical officers in malaria campaign in Sri Lanka. This method is less cost and having high specificity and sensitivity. There were total number of 28,829 blood donors at colombo north teaching hospital from 2004 to 2007. But there were no any positive cases of malaria among them. This study shows 28829 blood donors had undergone for malaria screening test for four years but non of them were positive for malaria. These results were compared with malaria positive cases in total blood donors in Sri Lanka.

Total blood donors in Sri Lanka	Total number of malaria positives
2004: 1,92919	0
2005: 2,07380	1
2006: 2,46752	1
2007: 2,60212	0

Conclusion: There were no positive cases of malaria for last four years at colombo north teaching hospital blood donors. But the total blood donors in Sri Lanka having two reported cases for malaria. At present in Sri Lanka when the malaria parasites are positive the blood and blood products of that donor is discarded. Also there are no reported cases with malaria infection after blood transfusion for last 4 years.
All these facts show that there is a very minimal risk for transmit malaria infection from blood and blood products in Sri Lanka.

4.7. TTID
Newly emerging pathogens

P-148

WEST NILE VIRUS: THE EXPERIENCE OF THE ITALIAN BLOOD SYSTEM

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Background: On 20th September 2008 IgM and IgG antibodies against West Nile virus (WNV) were detected in the serum of a patient living in the district of Bologna (Emilia Romagna [ER] region). On 29th September 2008, this was confirmed to be the first human case of WNV neuroinvasive infection (NI) ever occurred in Italy. On 3rd October 2008 a second human case of suspected WNV NI was identified in a patient living in the district of Ferrara (ER). On 30th October a third human NI was diagnosed in the province of Rovigo, Veneto Region. This report aims at describing the epidemiological surveillance strategy and the precautionary measures adopted for blood safety (BS).
Methods: In October 2008, the National reference centre for exotic animal diseases reported various horses affected by neurological disorders referable to West Nile Disease (WND) in several stables in ER, Veneto and Lombardy regions. Moreover, WNV had been detected in wild birds in the ER region. Grading of the risk was adopted according to the French Ministry of Health's guidelines on the procedures against the circulation of WNV, and the following precautionary measures for BS were put in place:
grade 1: bird mortality due to WND: no precautionary measures;
grade 2: horse morbidity: blood donors' serum storage for at least sixty days;
grade 3: human morbidity: WNV NAT and 28-day deferral for blood donors resident or having been for at least one night in the affected provinces during the 28 days preceding the donation.
Results: The above measures were introduced in ER on 1st October 2008 and on 30th October 2008 in Veneto. On 3rd October the NBC alerted the European Commission's Health Threats Unit and, on 8th October, 11 European national blood competent authorities (ENCA) had accordingly modified their donor selection criteria, whereas 5 ENCA had adopted a ''watch and wait''strategy.
WNV NAT was promptly introduced to test all blood donations from donors living in the areas of Bologna, Ferrara and Rovigo, at the same time testing all available samples from blood donations collected during the two preceding weeks. No donor tested positive out of > 12 000 screened.
The NBC indicated to all Blood Centres nationwide to defer for 28 days donors who had been for at least one night in the interested areas.
As to cord blood donations, WNV NAT test was indicated for all mother-donors resident or having been for at least one night in the above provinces during the 28 days prior to delivery.In the first week of December 2008, all precautionary measures were suspended. Active animal and human surveillance is permanently carried out in the above areas.
Conclusion: The reported experience once more shows that new emerging pathogens may be a threat to public health, not only because of their impact on the population, but also because they have a relevant potential to threaten BS and to be a challenge to the maintenance of the blood inventory and to the comprehensive management of a regional and national blood system.

P-149

A STUDY OF WEST NILE VIRUS (WNV)INFECTION IN IRANIAN BLOOD DONORS

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Background and objectives: West Nile virus (WNV) is a mosquito - transmitted virus that can cause disease in human and horses. A majority of people infected with WNV will have no symptoms or may only experience mild symptoms, such as a headache. About 20 % of infected human develop flu- like illness characterize by fever, while in the elderly and immunocompromised, the WNV can cause more serious neurologic disease and can be fatal. West Nile Virus infection is endemic in the Middle East. West Nile virus can be transmitted by transfusion through infected blood components.
The objective of this study was to find the WNV-RNA incidence and anti-WNV prevalence among Iranian blood donors to determine whether this emerging infection is a possible risk for the blood supply in Iran.
Materials and methods: Serum samples from 500 blood donations who had attended to Tehran Blood Transfusion Center for blood donation, were collected between May and October 2005. Serum samples were examined for IgM and IgG antibodies to WNV using ELISA method. The samples were tested for the presence of WNV RNA by the Real- Time RT-PCR assay. All the data were analyzed statistically using Chi-Square test.
Results: All 500 donors were negative for West Nile virus-specific IgM antibody at donation. No WNV RNA-positive samples were detected. The percentage of seropositive for IgG antibodies to WNV was 5% at donation.
Conclusions: No evidence of the presence of West Nile virus-specific IgM antibody and WNV RNA in blood donor samples was found. For increasing the safety of blood donation, the surveillance of this emerging infection is an essential to protect the blood supply in the future.

P-150

STANDARDISED ASSESSMENT OF EMERGING INFECTIONS AND THE SAFETY OF BLOOD

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Background: Increased travelling, climate change, media coverage and improved communication via internet contribute to a steady flow of reports on emerging infectious diseases (EIDs). EIDs may pose a threat to the safety of blood and blood products. To facilitate fast evaluation of reports on EIDs, a dedicated committee was installed by Sanquin Blood Supply Foundation in the Netherlands.
Aims: The 'Working group on emerging blood-borne infections' (WEBI) was established to perform a fast qualitative risk assessment of reported EIDs and the safety of the Dutch blood supply.
Methods: On a daily basis the secretary and two donor physicians of the WEBI monitor messages and reports from a defined set of national and international institutions and medical alert services (WHO, CDC, ECDC, ProMed, Eurosurveillance, etc). The secretary stores incoming information in a database and presents highlights and a summary to the members of the WEBI by e-mail. Biweekly the WEBI convenes by teleconference and decides whether a report on an EID, and accompanying advice, needs to be prepared for the Medical Advisory Board (MAB), which is advising the Executive Board of the Sanquin Blood Supply Foundation. As determined by the nature of an EID, one of three reporting formats is used: a so-called signal, a quick-scan, or a full review.
Results: In 2008 the WEBI secretary evaluated over 1000 reports, messages and articles on EIDs; resulting in 256 signals being presented to the members of the WEBI; who discussed these signals in 710 e-mail messages, 19 teleconferences and 1 live conference. As a result, in 2008 the WEBI presented 6 quick-scans to the MAB containing concise information on an EID and proposals for specific safety measures. Quick-scans were presented

on Q fever, Chikungunya, Crimean Congo Hemorrhagic Fever, Chagas' disease, Leishmaniasis and Tick-Borne Encephalitis.
Conclusions: By defining an explicit flow and selection of information, it is possible to rapidly and qualitatively assess in a standardised way the relevance of emerging infections and possible interventions regarding the safety of the blood supply.

P-151
ABSENCE OF DENGUE VIREMIA IN BLOOD DONORS FROM SÃO PAULO - BRAZIL

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Background: Dengue has re-emerged in Brazil in the last two decades, reaching epidemic levels in several cities. Serotypes 1,2 and 3 are already detected in successive outbreaks and hundreds of hemorrhagic cases are observed yearly with associated fatalities. In contrast, transfusional cases have never been described in Brazil, and in the literature there are only a few documented reports.
Aims: To investigate blood donors for the presence of dengue RNA in plasma minipools.
Methods: In our service, blood donations are screened in a routine basis for HIV and HCV RNA by an ''in-house''RT-PCR method. Plasma minipools of 6-12 donations, collected in between November 2007 and March 2008, were spiked with an artificial RNA and retrotranscription performed with random hexamers. Resulting cDNA contained a representation of all RNAs present in the sample, allowing us to employ the stored cDNA to investigate for dengue genomes. A real-time PCR targeting the 3' UTR from all four serotypes (dengue-generic, Lai, Y.L. et al., J. Clin. Microbiol. Jan. 2007) with a detection limit of 1,000 copies/mL was applied to 5.321 pools prepared from 23.568 donations.
Results: We haven't identified any dengue-RNA reactive pool.
Summary/conclusions: This result shows that in the largest Brazilian city, during the summer season, dengue-RNA was not identified in a significant cohort of blood donors. In the same period, only a few vetorial cases occurred in the area, corroborating our finding and suggesting that in such an epidemiological situation, NAT screening of blood donors for dengue is not necessary.

5.1. Immune Haematology Fetal-maternal immunology and transfusion

P-152

RED CELL ALLOIMMUNIZATION AMONG ANTINATAL MOTHERS IN SRI LANKA

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Background: Sensitization to clinically significant antibodies results in complications during pregnancy leading to Haemolytic disease of fetus and newborn. Antenatal antibody screening of pregnant mothers is usually done on Rh D negative females at the booking visit.
Aim: This study was carried out to identify the clinically significant antibodies and their distribution among antenatal mothers.
Method: The retrospective study was carried out at the Reference Immunohaematology Laboratory National Blood Centre Sri Lanka. Data was collected from Antibody screening Identification and titration records of six month period from 1st December 2008 to 31st May 2009.
Results: Twenty eight pregnant mothers were found to have developed clinically significant alloantibodies against red cell antigens. Twenty eight of them were Rh D negative and one Rh D positive. Twenty four were sensitized against Rh D, Three against D+ C and one against c. Thirty five percent (35.71%) of them were between the ages 30–34. Thirty five percent (35.71%) of them were in their third pregnancy. Thirty two percent (32.19%) were in their fourth pregnancy. Twenty eight (28.57%) of them were between 30–35 weeks of Period of Gestation. Antibody titration was performed on 27 patients. The initial Anti D titer was 32 and 64 in six patients each, and 128 in four patients. The highest initial Anti D titer to detect was 1024 in one patient.
Rising antibody titer was observed in 11 patients. In nine patients only the initial antibody titration was done, and no follow up samples were received.
Rh phenotype was performed in all 28 pregnant mothers and 26 were rr, one r''r and one R1R1. Fifteen patients have sent blood samples from their partner for Rh Phenotyping and 13 have not sent.
Conclusion: According to the results the most common antibody to develop in Rh negative mothers was anti D. Non detection of antibodies other than Rh system was due to not doing routine antenatal antibody screening in all pregnant female. Majority of antibodies were detected at the late pregnancy. It was also one reason why the follow up samples were not received. It is recommended to do the antibody screening of all antenatal mothers irrespective of their Rh D status at the booking visit.

P-153

A REVIEW OF HEMOLYTIC DISEASE OF THE FETUS AND NEWBORN DUE TO MN INCOMPATIBILITY IN JAPAN

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Background: Hemolytic disease of the fetus and newborn (HDFN) resulting from MN incompatibility is very rare, and in previously reports, ranged from asymptomatic to lethally hydropic with severe hemolytic anemia. The clinical significance of anti-M or -N is not completely recognized.
Aims: This study retrospectively investigated the laboratory and clinical findings of Japanese HDFN cases attributed to anti-M or anti-N.
Methods: The Japanese language literature was electronically searched (Ichushi Web, version 4) from 1975 through 2008. Data abstracted from relevant cases included maternal anti-M or -N titres at delivery, and fetal direct antiglobulin test (DAT), hemoglobin, total bilirubin and reticulocyte count at birth. Therapeutic interventions were also abstracted.
Results: Twenty eight cases of HDFN due to anti-M (n = 27) or -N (n = 1) were reported in Japan since the first Japanese case report in 1975. Of 26 pregnant women, 14 (54%) had previous histories of multiple fetal demise, spontaneous abortion, or HDFN due to anti-M. The median maternal antibody titer was 1:64 at delivery and was under 1:16 in 6 (23%) pregnant women. Of 28 babies, 2 (7%) were stillborn and 3 (11%) died of apparent heart failure or severe hydrops fetalis due to anemia within two days of birth. Survivors included 8 (30%) with severe hemolytic anemia (< 6g/dl) and/or hydrops fetalis. Of 26 live-birth neonates, 20 (77%) were DAT negative, and 25 (96%) required medical interventions such as exchange blood transfusion (n=18, 69%) and/or phototherapy (n = 13, 50%) for hyperbilirubinemia, immunoglobulin (n = 5, 19%) or steroid therapy (n = 2, 8%). Nine (35%) babies with persistent anemia received multiple antigen-negative RBCs transfusions (n = 8, 31%) and/or erythropoietin therapy (n = 3, 12%) in the first two months after birth. The reticulocyte count of babies with anemia was still below the reference interval. One neonate suffered from kernicterus but the other 22 babies had no sequelae after medical intervention.
Conclusion: HDFN resulting from MN incompatibility in Japanese population is not rare, with 13 (46%) reported cases of HDFN due to anti-M or or -N manifesting as severe hemolytic anemia and/or hydrops fetalis over the past thirty-four years.

P-154

CASE REPORT- HAEMOLYTIC DISEASE OF THE NEWBORN CAUSED BY ANTI C RHESUS ANTIBODY

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Introduction: Haemolytic disease of the newborn is a condition in which the life span of the infant's red cell is shortened by the antibodies derived from the mother by placental transfer. Anti c is an important antibody that can cause haemolytic disease with anaemia and high bilirubin levels, which can cause kernicterus. Mother can be stimulated to form antibodies by previous pregnancies or blood transfusions.
Case report: A 34 year old Mrs. R admitted to the Obstetric unit at term with dribbling. This was her 5th pregnancy with two children. She gave birth to her 3rd baby next day by vaginal delivery. She had post partum haemorrage (PPH)) and transfused fresh frozen plasma (FFP.) Day one baby developed jaundice and blood samples demonstrated high bilirubin level and low Haemoglobin. Infection screening remained negative. Bilirubin level progressively increased till day five while on phototherapy and gradually improved in next two days. The final diagnosis was haemolytic disease due to anti c isoimmunization. The baby was discharged home on day 08 Baby was healthy at followed up visit.
Method: Past obstetric history of Mrs. R and transfusion records of all transfused blood components were reviewed to analyse any association for Rhesus isoimmunization. Genotyping of all the family members were done.
Results: Mrs. R is group O+ with genotype CDe/CDe with anti c and E antibodies. Her partner is O negative with cde/cde. All her babies are O+ with CDe/cde. Her youngest child's Direct antiglobulin test was positive with C3d specificity. After delivery of her 1st child she develop PPH and transfused 2 units of Phenotypically unmatched blood. Her second pregnancy was ended up in an incomplete abortion and surgical evacuation. Her 3rd pregnancy was a normal vaginal delivery. She developed PPH and transfused with 2 units of phenotypically unmatched blood and FFP. Baby developed jaundice and found to have anti c and E in mother. Her 4th pregnancy was also ended up in an incomplete abortion and evacuation of retained products. At that time she received phenotypically matched blood.
Discussion: With in the Rhesus blood group system the most immunogenic antigen after D are c and E. Anti c is found most commonly in female with the R1R1 genotype CDe/CDe which occur in 20% pregnant women. Such women also have the propensity to make anti E. The management of haemolytic disease due to alloimmunization by c antigen is the same as for RhD but yet there is no way to prevent this condition.

Alloimmunization of mother can occur due to transfusion of phenotypically uncross matched blood, surgical interventions carried out in pregnancy and at the time of delivery. Alloimmunization to red cell antigens could be prevented in a manner similar to that with anti D. But in practice it would be very costly. Therefore, the best choice is that women of reproductive age receive primary prevention against the development of antibodies by application of a selective blood transfusion policy, bearing in mind the frequency of occurrence of antigens c and E.

P-155

THE IMPROVEMENT OF SAFE TRANSFUSION IN NEWBORN AND INFANT LESS THAN FOUR - MONTH OLD IN SIRIRAJ HOSPITAL

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Background: In our Department, to provide blood for newborn with ABO hemolytic disease of the newborn (ABO-HDN), we perform blood grouping (ABO and D), direct antiglobulin test (DAT) and screening for IgG anti - A or anti - B without performing elution test. Maternal serum is used for antibody screening and crossmatching. For infant less than one year old, infant's serum is used for crossmatching without performing antibody screening and elution test.
Aim: To detect IgG maternal antibodies in newborn and infant less than four month old in order to improve the safety of blood transfusion.
Methods: We studied 234 blood samples from newborn with hyperbilirubinemia for ABO (cell grouping), Rh (D) typing, direct antiglobulin test (DAT) and IgG anti -A and anti - B. Lui freeze thaw elution test was performed in every case. For maternal blood samples, besides ABO and Rh (D) typing, antibody screening for alloantibodies was also tested. Acid elution test (DiaCidel, DiaMed, Switzerland) was done when maternal alloantibodies were detected. The same protocol was used in the study of 70 blood samples from infant less than four months old but without maternal samples, using infant's serum for antibody screening.
Results: In 154 cases of newborn with ABO-HDN (mother O, newborn A or B), positive DAT and positive Lui freeze thaw elution test was detected in 55 cases (35.7%). Interestingly, 80 cases of negative DAT newborn had positive Lui freeze thaw elution test (80.8 %). IgG anti - A and anti - B were found in 102 cases of ABO- HDN (66.2 %) which 50 cases had negative DAT (49 %). We also found 3 cases with alloantibodies in maternal serum, they were identified as anti-E and anti- Mia, anti-E and anti- Jk a, respectively. DAT and acid elution tests were all positive. In 70 cases of infant group, negative DAT and negative antibody screening were found in all cases. Only two cases of negative DAT had positive Lui freeze thaw elution test (2.9%).
Conclusion: To improve safety transfusion, elution tests should be introduced as routine procedure in order to confirm ABO-HDN. In addition, to provide blood for newborn and infant, antibody screening in maternal or infant's serum should be included as well.

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AN EVALUATION OF ANTENATAL SENSITIZATION EVENTS AND THE EFFICACY OF ANTENATAL ANTI -D IMMUNOGLOBULIN PROPHYLAXIS IN THE MANAGEMENT OF RHESUS ISO IMMUNIZATION IN A SRI LANKAN TEACHING HOSPITAL

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Background: Haemolytic disease of the newborn resulting from the transplacental passage of maternal allo-antibodies directed against fetal red cell antigens varies from mild derangements in laboratory tests to in-utero anaemia causing cardiac failure, hydrops fetalis and intrauterine death.
Although there are a number of recognized events that are associated with Feto-Maternal Haemorrhage (FMH) during pregnancy, such as spontaneous or therapeutic abortion, antepartum haemorrhage, amniocentesis, abdominal trauma and ectopic pregnancy a number of studies have shown that 'silent sensitization' occurs in the absence of a recognizable precipitating event. Therefore the National Institute for Clinical Excellence (NICE) has advocated the use of Routine Antenatal Anti-D Prophylaxis (RAADP) during the course of pregnancy in an attempt to reduce Rhesus iso immunization to an absolute minimum.
However, as anti-D immunoglobulin therapy is expensive the current Sri Lankan guidelines do not advocate provision of RAADP in Sri Lanka due to financial constraints and there is a wide variety of practices depending on the clinician choice.
Aims: To study the antenatal sensitization events and the differences in the prophylaxis of Rhesus iso immunization in different units of De Zoysa Maternity Hospital (DMH), Sri Lanka where RAADP is offered in some units and to assess the effectiveness of RAADP in preventing Rhesus iso immunization in pregnancy.
Methodology: A both prospective and retrospective analytical study was carried out using a pre tested objectively designed questionnaire. Data were collected from the bed head tickets of all Rhesus negative mothers delivered at the DMH from 1st of June 2007 to 31st of May 2008. Sample size was 490.
Results: Range of age distribution was from 18 to 46 years with a mean age of 28.9. One hundred forty two (28.6 %) were primigravidae.
Forty mothers had previous sensitizing events. Thirty two (6.5%) had spontaneous miscarriages which was the commonest sensitizing event while ectopic pregnancy (1.2%), therapeutic miscarriage (.8%), and antepartum haemorrhage (.4%) were also present as other sensitizing events. 14 were given Rhogam for sensitizing events.
Out of the study sample only 18 (3.6 %) were positive for unexpected antibodies by 28 weeks and antibody titers were done in only eight (1.6 %). Out of the 472 (96.4%) mothers eligible for RAADP at 28 weeks, only 90 (18.1 %) mothers had received RAADP. Unexpected antibodies appeared in two mothers (RAADP given = 0, RAADP not given = 2) after 34 weeks and Direct Antibody Test was positive in only 10 (RAADP given = 2, RAADP not given = 8) fetal blood samples. Prophylactic anti D immunoglobulin therapy has reduced the incidence of positive unexpected antibodies antenataly and the number of positive direct antibody tests. But the reduction was not statistically significant ($P > 0.05$).
Conclusion: Prophylactic anti-D immunoglobulin treatment does not significantly reduce the incidence of positive unexpected antibodies and direct antibody test in our study. We recommend large scale studies to assess the cost effectiveness of antenatal prophylactic anti D immunoglobulin in the settings of developing countries.

P-157

SURVIVOR OF HDN DUE TO C ANTIBODY

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Severely jaundiced, febrile, Female Baby, Transferred from a Rural Hospital to the Teaching hospital Karapitiya, Galle, on Day One. Further Investigations revealed that the baby has HDN (Haemolytic Disease of Newborn) due to anti c antibody and septicemia. After transfusion with R1R1 blood baby became well and went home.
It is not so uncommon to have HDN due to anti c antibody. But the reason why this baby was affected, and why couldn't we prevent this particular incident was very interesting.
Also this particular case became a very good eye opening sort of an example to our higher Authorities to consider the implementation of starting the antibody screening for all pregnant mothers in Sri Lanka.

5.2. Immune Haematology Platelet immunology

P-158

THE EXPRESSION OF THE PLATELET GP SPECIFIC ANTIBODIES AND HLA ANTIBODIES IN PTR PATIENTS

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Objective: To study the expression of the platelet antibodies in PTR patients and the genotypes of HPA and HLA-I antigen, then discuss the pertinence of PTR with HPA and HLA-I antigen.
Methods: A total of 17 patients of PTR ware selected. An easy PCR-SSP assay was used to detect single-nucleotide polymorphism or deletion in HPA and HLA systems. The platelet GP specific antibodies and HLA antibodies in serum were tested with a solid phase ELISA.
Results: There were six patients only expressed HLA antibodies and 4 patients expressed the platelet GP specific antibodies in serum, especially GP II b/IIIa expressed the most. The gene frequencies obtained from 17 PTR patients were 0.676 and 0.324 for HPA-3a and -3b; the gene of HLA-A-02, A-24, A-11 and HLA-B-60, B-13, B-46 much expressed in PTR patients. Conclusion The expression of the HLA and platelet GP specific antibodies resulted in PTR, so found out the pertinence of PTR with HPA and HLA-I antigen, it simple quote is meaningful for clinic to guide platelet transfusion.

Tab.1 The expression of HLA antibodies and the platelet GP specific antibodies in 17 patients

				Sreum			
ID	Gender	Age	HLA-Antidodies	GP II b/IIIa	GPIa/IIa	GPIb/IX	GP IV
1	M	76	+	+	+		
2	F	50	+	+			
3	M	13					+
4	M	79	+				
5	M	36	+				
6	F	69			+		
7	F	62			+		
8	M	34					
9	F	45					
10	F	58	+				
11	F	51	+	+			
12	F	45	+				
13	F	27	+				
14	M	60					
15	F	23	+				
16	M	62					
17	M	52		+			

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FREQUENCY OF HPA-15 (GOV) PLATELET ALLOANTIGEN IN CHINESE CANTONESE

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Background: The human platelet antigens (HPA) systems consist of a group of immunological markers on the platelet membrane receptor glycoprotein (GP) and any polymorphism in the GP gene can provoke alloimmunization like following pregnancy or upon a transfusion. Anti-HPA-15a/15b (Gov b/Gov a) antibodies are considered to play an important role in alloimmune disorders in some studies. In this study, PCR with sequence-specific primers was employed to determine the frequency of allelic polymorphisms of the HPA-15 in Chinese Cantonese.
Method: Study subjects comprised 200 volunteer healthy donors in Guangzhou Blood Center (140 males, 60males, mean age: 28.74 ± 7.10 years) with giving informed consent. Three milliliters of the Blood samples were collected in EDTA anti-coagulant vacuum tubes and samples DNA was extracted by DNA extraction kit (Wizard Genomic DNA Purification kit, Promega BiotechCo. Ltd). A 225bp-fragment containing the HPA-15 was expected to be obtained by PCR amplification of genomic DNA using the oligonucleotide primers for HPA-15a 5'-TTC AAA TTC TTG GTA AAT CCT GG-3'(sense) and 5'-ATG ACC TTA TGA TGA CCT ATT C -3'(anti-sense) and HPA-15b:5'-TTC AAA TTC TTG GTA AAT CCT GT-3'(sense) and 5'-ATG ACC TTA TGA TGA CCT ATT C -3'(anti-sense). A pair of internal positive control primers 5'-TGC CTT CCC AAC CAT TCC CTT A-3' and 5'-CCACTCACGGATTTCTGTTGTGTTTC-3' were included in PCR to amplify a 427bp-PCR product from human growth hormone gene. The PCR thermocycler program consists of an initial step of 94' for 5 minutes, followed by 32 cycles. Each PCR cycle comprised denaturation (95', 30sec), annealing (61', 50 sec) and extension (72', 30sec). After then, a final extension step of 72' for 7mins preceded. The amplification products were visualized by ultraviolet illumination following electrophoresis on 1.5% agarose gels. Two microlitres loading buffer (30% v/v glycerol stained with bromophenol blue and xylene cyanol) was added to each 10μL PCR reaction mixture and 2.5 μL of each sample was loaded onto a 1.5% agarose gel prestained with 0.5 μg/mL ethidium bromide. The gel was electrophoresed for 15 minutes at 150 V in 0.4TBE (0.036M Tris-Borate, 0.0008M EDTA). Results were recorded by ultraviolet photography.
Results: In the study, the 15b/15b genotype is the most prevalent (45.00%) followed by 15a/15b (29.5%) and 15a/15b (25.5%). The allelic frequencies for HPA-15a and 15b are 42.25% and 57.75%, respectively.
Conclusion: As compared with some ethnic groups, Chinese Cantonese was found to have a relatively high prevalence of HPA-15b which might predispose to a higher risk of allomoimmunization although there is no documented a single case of neonatal alloimmune thrombocytopenia or posttransfusion purpura by serologic means in China.

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INDICATOR OF INCOMPATIBLE ANTIGEN FLUORESCENCE INTENSITY BY THE LUMINEX METHOD (LABSCREEN SINGLE ANTIGEN) FOR EFFECTIVE PLATELET TRANSFUSIONS IN HLA-MATCHED TRANSFUSION

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Background: Based on CREG(cross-reactive groups of HLA antigens), we selected donors for HLA-matched platelet (PC-HLA) by HLA matched tests including LCT and AHG-LCT. However, we experienced cases in which HLA-matched tests were negative but the LABScreen single antigen (SA) (One Lambda,USA) test showed positive with the HLA visual Software(One Lambda,USA). The SA test is a higher sensitive method of anti-HLA antibody detection. We examamind the fluorescence intensity of the incompatible antigen with the SA test in cases described previously. The level of the fluorescence intensity for successful PC-HLA transfusions was analyzed. In addition, it was suggested that the fluorescence intensities more than 500 reveal positive reaction with the HLA visual Software.
Methods and results: Case 1. MDS, HLA-A24, A26, B51, B-. The HLA antibody specificity was multispecific. We selected donors having B52 as acceptable antigen and supplied three times of PC-HLA, but the transfusions were refractory. The antibody specificity was identified as being positive to B52 and the fluorescence intensity was 7365 by the SA. Transfusions of B52-negative PC-HLA twice showed effective. Three months later, we supplied transfusions of B52-negative PC-HLA having A31 twice, but the transfusions became refractory. The antibody specificity was identified as being positive to A31. The fluorescence intensity was 12589 by the SA. The antibody specificity of the blood specimen drawn three months before showed A31 positive. The fluorescence intensity was 3588 but the transfusions were effective.
Case 2: AML, HLA-A2, A24, B60, B75. The HLA antibody specificity was multispecific. We selected donors having B35, B48, B61 and B62 as acceptable antigens and supplied seven times of PC-HLA. The effect of platelet transfusions was effective. However, the antibody specificity was identified as being positive to B35, B48, B61 and B62. Their fluorescence intensities were 5966, 5625, 5706 and 3760, respectively by the SA.

Conclusion: These findings suggested that fluorescence intensities less than 6000 of incompatible antigens showed the tendency that such a PC-HLA transfusion was successful.

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ADHESIVE INTERACTION BETWEEN PERIPHERAL MONONUCLEAR CELLS AND ACTIVATED PLATELETS IN THE PRESENSCE OF ANTI-HLA CLASS I ANTIBODY

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Background: Febrile transfusion reactions in patients with anti-HLA class I antibodies are attributed the residual leukocyte in platelet concentrates. Even after the introduction of leukoreduction of platelet products, the febrile reactions are still observed. Because the adhesive interaction between platelets activated and monocytes is shown to cause production of inflammatory cytokines in vitro, we examined the possibility that activated platelets by anti-HLA class I antibody could cause adhesive interaction with peripheral mononuclear cells (PBMNCs) and generation of IL-1beta, a proinflammatory cytokine.
Materials and methods: HLA-A2 positive or negative platelets were incubated with HLA-A2 negative PBMNCs at a ratio of 100:1 in the presence of anti-HLA-A2 serum at 37°C. The binding of platelets to monocytes was analyzed by flow cytometry. After gating CD14-positive monocyte fraction, and CD42b-positive cells in the fraction were designated as monocyte-platelet aggregates. The amount of platelets bound to monocytes was evaluated by MFI value of CD42b-positive cells. The level of IL-1?beta in the culture supernatant was determined by ELISA. To analyze the role of P-selectin (CD62P) on platelets and its ligand, P-selectin glycoprotein ligand-1, on monocytes (CD162), in the adhesive interaction anti-CD62P monoclonal antibody (mAb) (clone AK4) and anti-CD162 mAb (clone PL1) were used.
Results: The anti-HLA-A2 serum caused platelet-monocyte aggregates in a dose dependent manner. In the presence of 10% anti-HLA-A2 a percentage of monocytes bound to platelets were significantly ($p<0.01$) higher in HLA-A2 positive platelets (87% ± 10.5) than that in HLA-A2 negative platelets (40.4% ± 9.8). In addition, the anti-serum increased platelet-monocyte aggregates as well as amount of platelets bound to monocytes in a time dependent manner. The addition of either anti-CD62P mAb or anti-CD162 mAb significantly inhibited formation of platelet-monocyte aggregates as well as amount of platelets bound to monocytes. When the HLA-A2 positive or negative platelets were incubated with HLA-A2 negative PBMNCs in the presence of 10% anti-HLA-A2 for 16 hr, the level of IL-1?beta in supernatant of coculture was significantly ($P < 0.05$) higher in HLA-A2 positive platelets (37.5 ± 12.1 pg/mL) than that in HLA-A2 negative platelets (7.3 ± 6.9 pg/mL).
Conclusion and discussion: The binding of anti-HLA A2 antibody matching to the A2 positive plateletin the presence of A2 negative platelets PBMNCs, caused platelet-monocyte aggregates in a dose and time-dependent manner. P-selectin on platelets and its ligand, P-selectin glycoprotein ligand-1, on monocytes were at least in part attributed to this formation of platelet-monocyte aggregates. Since the incubation of PBMNCs with platelets in the presence of anti-HLA class I antibody induced generation of IL-1?beta, this process may be involved in the febrile transfusion reactions in the patients with anti-HLA class I antibody, when received platelet products in a cognate antigen-antibody relationship.

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HUMAN PLATELET SPECIFIC ANTIGEN (HPA-1) POLYMORPHISM IN MALAY ISCHEMIC STROKE PATIENTS

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Introduction: Ischemic heart disease and cerebrovascular disease are the leading causes of morbidity and mortality among both adult men and women in the developed western world. Recent evidence indicates that the incidence of this disease is steadily increasing among Asian populations. However, the majority of cases of ischemic stroke are mutifactorial in aetiology. Recently, the PlA2 (HPA-1b) allele of GPIIb-IIIa was reported to be an inherited risk factor for acute coronary artery events, but the association with ischemic stroke is less clear. HPA-1 is part of the GPIIb/IIIa complex (integrin $\alpha IIb\beta 3$), which is the numerically predominant platelet integrin, and mediates platelet aggregation by binding adhesive proteins, most notably fibrinogen and von Willebrand factor. Polymorphisms in platelet glycoprotein influence platelet function. However, assessments of their exact contribution to atherothrombotic events, including stroke, often yielded inconsistent results.
Aim: The aim is to study HPA-1 polymorphism in Malay ischemic stroke patients and normal population and determine the association of HPA-1a/b genotypes with stroke.
Methods: A prospective case control study was done by collecting 2 mls of EDTA-anticoagulated blood from 91 ischemic stroke patients and 104 samples from normal blood donors among Malay population. DNA was isolated using phase lock gel method and HPA-1 genotype was determined by allele-specific oligonucleotide PCR (ASO-PCR) method.
Results: In ischemic stroke the allele frequencies were HPA-1a/b was 6.6%; and in blood donors, the frequencies were 6.7%. There were no statistically significant differences for the analyzed HPA polymorphism frequencies either between ischemic stroke patients or blood donors.
Conclusion: Our results indicate that the HPA-1b polymorphism is not associated with an increased risk for stroke in Malay population. Given the demographic and ethnic heterogeneity in the distribution of the HPA polymorphic variants, and likely their pathogenic capacity, additional studies are required, involving larger numbers of subjects together with other populations, to assess the role of HPA polymorphic variants as risk factors for stroke.

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FLOW PRA ANALYSIS OF ANTI-HLA ANTIBODIES IN A MULTIPLE MYELOMA PATIENT WITH PLATELET TRANSFUSION REFRACTORINESS

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Background: Platelet transfusion refractoriness (PTR) is a complication of chronic platelet support in which immune factors such as anti-HLA class I antibody and anti-human platelet antigen (HPA) antibody cause poor post-transfusion platelet increments. Here we report the transition results of anti-HLA antibodies in a multiple myeloma patient who developed PTR and was treated with HLA-matched platelet transfusions.
Case report: A 61-year-old woman was diagnosed as myeloma (IgG-lambda type) in 2003. She received induction chemotherapy consisting of vincristine, adriamycin, and dexamethasone and obtained partial response (PR). Subsequently, she underwent high-dose chemotherapy followed by tandem autologous peripheral blood stem cell transplantation, and achieved very good PR in 2004. However, relapse occurred in 2006. At that time, she was treated with melphalan and predonisolone (MP) or thalidomide, but her disease progressed rapidly with pancytopenia and renal dysfunction. She was referred to our hospital in October 2006. Laboratory examination revealed pancytopenia (Hb level was 5.7 g/dl, neutrophil count 747/μl, and platelet count 0.7×104/μl) with markedly increased plasma cells (5561/μl) in the peripheral blood. Serum monoclonal IgG (7451 mg/dl) were increased. Her blood type was O, Rh D positive, and HLA type was A*1101, A*2601, B*4001, B*5901, DRB1*0803, and DRB1*1401. Platelet transfusion was effective at first and corrected count increment (CCI) value was 28 620 at 24 hours after transfusion, but she became refractory to platelet transfusions from random donors (CCI, -7155 to 1272 at 24 hours). Therefore, we screened serum anti-platelet antibody by mixed passive hemagglutination assay (MPHA) with chloroquine-untreated platelets and tested anti-HLA class I and class II antibodies by flow

cytometry assay (FlowPRA, One Lambda). The result of anti-platelet antibody was strongly positive, and anti-HLA class I antibody (83.8% positive) and anti-HLA class II antibody (98.4% positive) were detected. The specificity of anti-HLA class I antibody was B62. She was diagnosed as platelet transfusion refractoriness due to anti-HLA class I antibody. After treatment with HLA-matched platelet transfusions, platelet counts were maintained at 2-7×104/μL (CCI, 1,986–73,140 at one hour). She was then treated with bortezomib and dexamethasone (BD) for refractory myeloma. Notably, anti-HLA class I and class II antibodies were decreased to 78.2% and 93.4% (at day 28 after BD therapy), 62.4% and 78.2% (at day 50), 10.4% and 69.5% (at day 81), 6.0% and 76.3% (at day 110), and 1.9% and 69.6% (at day 143) by FlowPRA. Anti-platelet antibody was found to be negative by MPHA at day 115. At the end of three cycles of BD therapy, plasma cells in the peripheral blood disappeared and serum IgG level decreased to 1735 mg/dl, indicating the therapeutic effect of partial response. In accordance with these results, platelet transfusions from random donors showed clinical effects at the time of thrombocytopenia during the fourth BD therapy (CCI, 34,980–38,160 for one hour).
Conclusions: This case suggest that BD therapy induced a significant reduction of M-protein and HLA class I antibody, and led to the improvement of myeloma and PTR. Detection of anti-HLA antibodies, especially by Flow PRA methods, is useful for monitoring the course of PTR.

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ANALYSIS OF PHENOTYPES AND GENETIC ABNORMALITIES OF CD36 OF PLATELET-PHERESIS DONORS IN TAIWAN

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Background: CD36 (GP IV) is a type B scanvenger receptor located on the surface of many types of cells, including platelets, endothelial cells and monocytes. CD36 deficiency is divided into two subgroups according to the phenotype. In type I deficiency patients, no CD36 was detected on their monocytes and platelets. While in type II deficiency, only monocyte CD36 is expressed in the absence of platelet CD36. More than 20 mutations in the coding region of the CD36 gene have been reported. The type I subjects may produce isoantibodies against CD36 following pregnancy or transfusion, causing refractoriness to platelet transfusion, post-transfusion purpura or neonatal immune thrombocytopenia. In previous reports, subjects with platelet CD36 deficiency were identified in 1-3% of the Taiwanese population. However, no detailed data about the phenotypes or the molecular basis of CD36 in Taiwanese have been reported.
Aims: We recruited the platelet-pheresis donors for investigation the phenotype and molecular basis of CD36 deficiency in Taiwanese population.
Methods: Six hundred platelet-pheresis donors (male 510, female 90) were recruited for this study. The phenotype of CD36 expression was determined by flow cytometry using FITC-conjugated CD36, PE-conjugated CD42b and PE-conjugated CD14. Molecular study of the genomic DNA was analyzed in exons and respective boundary regions through Exons III to XIV by PCR direct sequencing.
Results: Of the 600 donors investigated, the type I and type II deficiencies were found in 3 (0.5%) and 6 (1%) respectively. Sixty-nine donors (11.5%) were found to have weakened expression of CD36 on their platelets (MFI <50% of normal) but nearly normal expression on monocytes. Molecular analysis of CD36 gene identified two previously reported mutations: deletion of 539AC and 12-bp deletion at nt1438-1449; and a novel mutation: the substitution of T for A at nt 1373 (1373A?T). A pilot survey of the three mutations in the weakened expression subgroup showed that deletion of 539AC is the most frequent mutation found in the weakened expression subgroup donors (around 30% of them carried this mutation).
Conclusions: In this study, we presented the frequency of different phenotypes of CD36 deficiency and the finding of the molecular mutation study. The CD36 deficiency pheresis donor data may be useful in the future for providing platelets for patients with CD36 isoimmunization.

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THE FIRST CASE OF HPA-15B ANTIBODY DETECTION IN JAPAN

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Background: The involvement of the HPA-15 system, which locates on CD109, in NAIT has been reported in various populations. In Japan, however, although of the relatively high risk of HPA-15 incompatible platelet transfusion or pregnancy to occur, presently, no cases of anti-HPA-15 antibody detection have been reported. Whereas MAIPA (monoclonal antibody immobilization of platelet antigens) is the preferred method for platelet immunology in US and Europe, in Japan, the most applied method is MPHA (mixed-passive hemagglutination), which has high sensitivity for detection of HPA alloantibodies. However most of the reported cases of HPA-15 incompatibility are based on MAIPA or immunoprecipitation, thus there is a possibility that they are not efficiently detected by the MPHA. And currently, the causative antibody is not detectable in approximately half of the suspected NAIT cases in Japan.
Aim: In this study, we aimed to test this hypothesis and examined the sera of mothers from NAIT cases, previously with undetected HPA antibodies by MPHA, using the MAIPA method.
Methods: Sera from 90 mothers of suspected NAIT were tested by the rapid-MAIPA for the presence of anti-HPA-15 alloantibodies
Results: Anti-HPA-15b antibodies were detected in 2 cases. These samples were sent to the MAIPA's reference lab of the ISBT in Germany (Dr. Santoso), and one had the specificity confirmed, but could not be confirmed in the other. The confirmed case was a mother in the first pregnancy diagnosed as hydatid mole-coexisting fetus, and the baby was born with suspect of NAIT. The hydatidiform mole was enucleated. The female infant was treated by randomized platelet transfusions and gamma-globulin administration, and the symptoms improved. The familial analysis revealed compatibility of HPA-15 genotype between the mother and the baby (both HPA-15a/a), but incompatibility with the paternal one (HPA-15a/b). Also a strong anti-HLA class I antibody was detected in mother's serum, the probable causative antibody of NAIT. Then, the hydatid mole's tissue was genotyped and found to be HPA-15b positive. In the other case with suspected anti-HPA-15b antibody, although the materno-fetal incompatibility of HPA-15 genotype was observed, the specificity of the antibody could not be confirmed in the reference lab.
Conclusion: Although not the causative antibody of the NAIT, the present case is the first case of anti-HPA-15 antibody detected in Japan. Interestingly, the hydatid mole was speculated as the responsible for the antibody production, and the coexisting anti-HLA class I antibody was the causative of NAIT. Careful antenatal management at an early stage and postpartum care will be necessary in the next pregnancy. Both MAIPA and MPHA are very sensitive and specific methodologies for platelet antigen-antibody detection. However, there is need to improve the sensitivity and specificity of both methodologies, or eventually combine them, for the detection of alloantibodies of the HPA-15 system.

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SURVEY OF ANTI PLATELET SPECIFIC ANTIBODIES PREVALENCE IN PATEINTS WITH HEMATOLOGIC DISORDERS BY FLOWCYTOMETRY AND TOTAL ANTI PLATELET ANTIBODIES BY IF

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Platelet Specific antibodies may be produced in different situation (pregnancy, blood transfusion and transplantation); that may result in

reduced survival of transfused platelets Antibodes against platelet. specific antigens were reported in Multitransfused patients, it has been shown that refractoriness to random donor platelet caused by immumzation, occurs in 17% to 25% of patients. It is suggested detection of platelet specific antibodies are usefule to monitoring and managing of treatment protocols. The aim of this study was detection of platelet speicific antibodies by flocytometry and immunoflouresence in patients with hematologic disorders (Acute leukemia, Aplastic Anemia & ITP). In this descriptive study platelet specific antibodies were detected by flowcytometry, using 62 serums drawn from patients with different whose response to platelet transfusion was a poor response, and 20 patientns with ITP. The serum samples were incubated with acid - treated (for destroying HLA -I antigens) and with PBS - treated platelets in separate tubes, then suitable secendry FITC conjugated antibodies were added and then the samples were analyzed by partec PASIII flowcytometr to evaluat of antibody subtypes.
In Indirect Immunofluorscence Test serum sample were incubated with paraformaldehyd fixed platelets, Then suitable FITC - labeled total antiglobulin was added and then the samples were examined by fluorescence microscope.
Our results showed 36 out of 82 (43.9%) patients had platelet specific antibodies in their serum (by flow cytemetry) and 74.4% of patient had total antiplatelet antibodies (by IF) that indicated immunization to platelet specific antigens.
The frecuency of each antibody isotype by flowcytometry was found the following percent: lgM (40.2%), lgG (30.5%) lgA (12.2%). There wase a significant relationships between the presence of platelet specific antibodies with the number of infused platelets units and disease subgroup.
Immunizaton to platelet specific antigens may be on of the important resulting to platelet refractoriness in these patients. More studies on Platetel specific antibodies with more number of sample, platelet cross - match and use of single match donor platelet concentrate for these patients are suggested.

5.3. Immune Haematology Red cell immunology

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STUDY ON GENETIC STATUS OF A CHIMERIC INDIVIDUAL WITH A3B3 PHENOTYPE

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Background: Chimerism, the presence of two genetically distinct cell lines in an individual, either is inherited or acquired through transplantation or transfusion. A case of a 4-year-old boy,who was sustained from dislocation of the hipbone and was diagnosed with serologic blood group AB in preparation for transfusion. His mother simultaneously delivered a female baby by natural spontaneous vaginal delivery, but the twin died of asphyxia anoxia on her second day. And the child's mother and father showing blood group O, AB respectively, were investigated.
Aims: To reveal genetic status of a rare chimeric individual with disputed parental phenotype.
Methods: Blood grouping was preformed with standard tube test and gel centrifugation test cards on the proposita's blood and on blood samples from his father and mother. The full ABO coding regions(exon1-7) and the enhancer-active region binding of CCAAT-binding factor NF-Y to a 43-bp repeat unit, located at the 5'-nontranslated region of the ABO gene, were analyzed by direct DNA sequencing in these samples. And then PCR products from proposita, spanning from intron5 to 3'UTP were cloned into the pGEM-T Easy vector by using a cloning kit. Amplified and cloned products were sequenced by using the Dye Termintor method. HLA-ClassI (A, B) and II (DRB1) alleles were typed with commercially available kits by sequence-specific primers(SSP) according to the manufacturer's instructions. Multiplex amplification of 15 short-tandem-repeat(STR) markers was performed for three individuals of the family, addition to the amelogenin locus.
Results: The proposita's RBCs were typed as A3B3 with a mixed-field agglutination. The main blood group was B but a smaller amount of A cells was clearly visible. Direct genomic sequencing show B101 and O01 alleles in proposita, A102 and B101 in his father, and homozygous O01 in his mother. Among 21 cultivated clones of PCR products about 2.1kbp length from proposita, genotype of one clone is A102 allele, genotype of 6 clones is B101 allele, genotype of 15 clones is O01 alleles. Two paternal and one maternal ABO haplotypes were found. And two maternal and one paternal haplotypes were determined in HLA-A gene locus of the proposita. HLA-A genotype of the proposita is A2ï¼Œ33ï¼Œ31 while his father is 24ï¼Œ33 and his mother is 2ï¼Œ31.
The results of autosomal STR loci tested show two haplotypes in both parents with an additional haplotype in the proposita at D8S1179, D13S317 and vWA. The cumulative parentage index for the child and her parents is 50877164482 and the plausibility for the putative parents as the biologic parents is 99.9999 percent. The family members displayed no abnormal finding in tests performed.
Conclusion: By investigation of DNA polymorphisms, double parental contribution of nuclei appears to be responsible for a rare case of chimerism.

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AN DELAYED HAEMOLYTIC TRANSFUSION REACTION DUE TO MISSING ANTI-JKB IN LISS/COOMBS MICROCOLUMN GEL TEST

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Background: The gel microcolumn assay is being used to detect unexpected serum antibodies and to determine ABOï¼Œ Rh phenotype, to crossmatch in many blood centers and transfusion services. However, antibodies of the Kidd blood group show too weaker reactions to detect in the gel test sometimes. We report a case of delayed hemolytic transfusion reactions ï¼DHTRï¼‰ due to missing Anti-Jkb in Liss/coombs microcolumn gel test. There has not been reported before.
Case report: A 34-year-old pregnant woman with $\hat{I}^2$-thalassemia received red cells transfusion because of chronic anemia. On Feb 12, 2009 4 units red cell were transfused (hb65.0g/L). The transfusions of 4 units were well tolerated (Hb94.2g/L). On Feb 24, 2009 another 4 units RBCS had to be transfused (Hb62.2g/L). Antibody screening and cross-matches again were negative in Liss/coombs gel test. Two days later, she had a high fever of 39.5â"*f*, and urine with bloody red color was observed (Hb52g/L). The woman had two pregnancies, and regularly received red cell transfusions.
Methods: Blood samples were collected within six hours. Blood grouping, antibody screening and identification, cross-matches, DAT, were carried out with blood samples using the tube test. We performed the detection of RBC alloantibody screen by the standard tube indirect antiglobulin test (IAT) technique, and to compare that to the performance of the microtube column systems, and then further investigation of blood samples from blood recipients and the samples of the 4 units(On Feb 24, 2009). The kidd blood group was determined using IgM human monoclonal anti-Jka, anti-Jkb. The Jk(a-b-) phenotype were detected using the urea lyses test.
Results: Anti-E and anti-Jkb could be detected by the standard tube technique (IAT), while anti-E only could be detected in Liss/coombs gel test. Further testing was performed in the laboratory. Anti-Jkb was observed agglutination (strength 1+) with RBCs from donors who were homozygous for Jkb in microtube column system, while anti-Jkb would have been missed, if we had used RBCS from the donors who were heterozygous for Jkb. Additionally, the pregnant woman[simplequote]s phenotype is A, CCDee, Jka-b-. The direct antiglobulin test was negative. Both blood units were A CCDee, Jk(a+b+), therefore only the anti-Jkb could have caused the DHTR. In the woman family her mother and her old sister were Jk(a-b-), her father was Jk(a+b+).
Conclusions: Kidd antibodies, which are often difficult to detect, are a hazard in blood transfusion. The frequency of the Jka and Jkb alleles, in Chinese blood donors was 0.4829 and 0.5161, respectively. The frequency of Jkb is around 3% in Chinese. Anti-Jkb has been responsible for severe and fatal immediate hemolytic transfusion reactions, and is regularly associated with DHTR. Kidd antibodies are often difficult to work with. Probably a major reason why Kidd antibodies are such a common cause of DHTR is their tendency to fall rapidly to low or undetectable levels in the plasma, Some anti-Jkb also demonstrates a hint of dosage.

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MOLECULAR GENETIC ANALYSIS ON BEL SUBGROUP IN A CHINESE INDIVIDUAL

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Background: Frequency of weak B type in different ethnic origin is variant. B subgroup is common, which frequency is higher than that of A weak group in Chinese Han population. Their serologic characters show negative results or only weak agglutination reactions between red cells and anti-B sera. Accompanied with widely commercial high concentrate reagents, more and more weak subgroups can be identified in routine group typing. But some weaker-expression A or B antigen still can't be confirmed, which leads to ABO mistyping.
Aims: To elucidate the molecular genetic background of a rare B elution phenotype and identify a novel ABO allele.
Methods: A blood sample typed as O from a healthy female donor, which cells occurred 2+ agglutination reactions against several sera of O type patients by MGT methods during compatibility testing, was collected in our blood group reference laboratory. Her blood group was diagnosed by series serological tests including direct and reverse blood grouping by monoclonal antisera or by polyclonal blends of human serum, adsorption-elution test and salivary blood group substances. Genomic DNAs were genotyped by direct DNA sequencing for all exons of ABO gene. A

fragment of 2170bp spanning exon6, exon7 and the intervening intron6 of ABO gene was amplified by a primer pair and analyzed by cloning and haplotype-sequencing. And the sample was further detected for nucleotide sequencing of the 5'regulatory region containing the CBF/NF-Y enhancer at ABO gene locus.

Results: A discrepancies between forward and reverse typing in routine ABO grouping showed its RBCs were not agglutinated by anti-B monoclonal serum, but weakly by polyclonal anti-B serum. Adsorption-elution tests preformed by testing B RBCs against anti-B produced elutes-reaction moderately with B cells. The Lewis phenotype is (a-b+). The sample contained H substance. Based on the serologic characteristics and ABO subgroup definition, the woman 's phenotype was classified Bel phenotype. Products of direct PCR and clone PCR were simultaneously sequenced to separate any PCR-induced errors from the actual sequence polymorphism. One haplotype sequences was O02 alleleand the other revealed that ABO sequences differed from the B101 allele only by a nucleotide substitutions in exon7, 905A > C, which results in an amino acid change from Asp to Gly at 302 locus. The allele was defined as Bel06 new allele according to Blood Group Antigen Gene Mutation Database and registered as number FJ009674 on Genbank. The sample had four 43-bp minisatellite repeats within the CBF/NF-Y enhancer region.

The result of the first repeat G/C at nt41 in CBF/NF-Y region shows B101/Oï¼01 or 02ï¼‰ heterozygosity.

Conclusions: A base pair substitution at nt905 position was associated with weak ABO antigen express. Mutations at the amino acid 302 position can probably alter activity of the ABO enzymes. This finding suggests that the amino acid at the position of 302 may be critical for the specificity of a glycosyltransferases and is possibly located in the active site of the enzyme.

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THE ASSOCIATION BETWEEN DIABETIC RETINOPATHY AND GENE POLYMORPHISMS IN AUBERGER ANTIGENS, AN ADHESION MOLECULE

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Background: Diabetic retinopathy(DR) is one of the most common chronic complications of diabetes with severe microvascular pathological changes, and it is a complex multi-factor, multi-gene, multi-stage disease. During the DR pathological process, cell adhesion molecules(CAM) on cells surface show abnormity. Up to date, some study have shown genetic factors play import role during the occurrence or development of DR. The genome DNA sequence on any unrelated individuals is 99.9% identical, and genetic differences of the remaining 0.1% could be accounted for the disease susceptibility and drug response. Lutheran blood group belongs to the immunoglobulin superfamily(IgSF) of receptor and adhesion molecules. Single pair of antigens, Aua/Aub(LU18/LU19), show polymorphisms while other antigens are of very high or very low incidence.

Aims: The relationship between diabetic retinopathy and the gene polymorphisms encoding Auberger antigens in Lutheran blood group were determined to screen possible susceptible genetic factor or prevention factors for the early diagnosis and therapy of DR.

Methods: Peripheral blood samples from 143 patients with type II diabetes mellitus(51 with versus 92 of high HbA1C level without diabetic retinopathy) were collected while 368 unrelated healthy blood donors as controls. We performed single nucleotide polymorphisms (SNPs) analyses with PCR genotyping-based sequence methods for Exon12 sequences at the Lutheran gene locus. Student's t test was used to compare age at diagnosis of diabetes, disease duration after diagnosis of diabetes, and HbA1C at diagnosis of diabetes between subgroups of patients. Analyses of gene frequency counts were preformed using the chi-square test. Multiple logistic regression analysis was preformed to evaluate the relationship between those with versus those without retinopathy. Variable in sampling associated with the estimated odds radio(OR) was assessed by 2-sided 95% confidence intervals(CI).

Results: Among 51 DR individuals, 44 showed homozygous nt1615A, 2 showed homozygous nt1615G, and 5 showed nt1615A/G heterozygosity. The gene frequencies of the Aua and Aub were 0.9118 and 0.0882 respectively. The gene frequencies of the Aua and Aub of high HbA1C level individuals without DR were 0.8370 and 0.1630 respectively. The gene frequencies of Aua and Aub in Chinese healthy subjects were 0.8695 and 0.1304 respectively. The genotype distribution in each group was in Hardy-Weinberg equilibrium. The frequency of nt1615G in DR group was significantly lower that that in other groups. OR is 0.655 between diabetic retinopathy patients and common population, and OR is 0.553 between diabetic retinopathy patients and only high HbA1C population.

Conclusions: Nucleotide 1615 polymorphisms at of Lutheran gene encoding Auberger antigen might be associated with the prevalence of retinopathy in patients with type II diabetes mellitus. It is possible that Ala539 within the fifth IgSF domain of Lu-glycoproteins can protect the occurrence of retinopathy.

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PREVALENCE AND FACTORS ASSOCIATED WITH POSITIVE DIRECT ANTIGLOBULIN TEST IN THAI PATIENTS

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Background: Direct antiglobulin test (DAT) is performed to determine whether an anemic patient with evidence of hemolysis is experiencing an autoimmune and/or alloimmune hemolytic anemia.

Aims: The purpose of this study was to investigate the prevalence and factors associated with positive DAT in Thai patients.

Methods: All together, 158 Thai patients were included in this study. EDTA blood samples were obtained from patients who were either anemic, for reasons other than blood loss, recently transfused, or had serum antibodies detected during routine pre-transfusion tests from different hospital blood banks. These complicated samples were sent to the National Blood Centre of the Thai Red Cross Society to investigate and find compatible blood components. Each blood sample was tested for DAT by conventional tube technique. To increase the validity and reliability of the evaluation, two laboratory technicians performed DAT in parallel and were blinded for the DAT results. The grading of the agglutination reactions were 4+, 3+, 2+, 1+, w+ and negative, respectively.

Results: The age of the patients ranged from 9 to 88 years with the mean age of 56 years, a total of 61 males and 97 females (ratio = 1:1.6). One hundred and four samples were positive for DAT (65.8%); male (25.9%) and female (39.9%). However, factors including age, sex and ABO blood group were not associated with positive DAT results. Additionally, strong DAT positive results (> 2+) were found in patients with autoimmune diseases and anemic patients with previous transfusion within three months.

Conclusions: In this study, the prevalence of positive DAT in Thai patients is relatively high due to the patient selection. Even though the DAT is not routinely performed in pretransfusion testing, patients with history of previous transfusion and who cannot find compatible blood, the DAT would benefit.

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FLOW CYTOMETRY ANTIBODY SCREENING USING POOLED RED CELLS

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Background: For red cell alloantibody screening, the column agglutination technique (CAT) is used extensively, and flow cytometry (FC) screening has recently been demonstrated to be accurate, rapid, and cost-effective.

Aims: We attempted to determine whether the high sensitivity of FC allows pooling of screening red cells, which is generally not an acceptable technique in CAT.
Methods: A commercial two-cell screening panel was utilized for the preparation of individual cells ('CSi'), as well as pooled cells diluted 1 in 2 ('CSp'), and 1 in 3 ('CS1/3'). Another panel was pooled from 120 randomly selected group O donors ('RSp').
Results: Comparing the endpoint titrations of serial dilutions, CS1/3 was found to be 1 dilution, on the average, less sensitive than CSi. In 33 CAT-positive patient samples, sensitivities between CSi and CSp did not differ significantly without polyethylene glycol (PEG) (30/33, 26/33, respectively, P = 0.125), although did differ significantly with PEG (32/33, 25/33, respectively, P = 0.016). The percentages of reactive cells among the total cells from RSp were roughly proportional to the relevant antigen frequencies of the local donors.
Conclusions: A trend toward reduced sensitivity was seen using pooled red cells even by FC. Pooled cells from randomly-selected group O donors can be employed as another approach to investigate the characteristics of known antibodies.

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EVALUATION OF POSITIVE DIRECT ANTIGLOBULIN TEST IN PATIENTS WITH AUTO-IMMUNE HAEMOLYTIC ANAEMIA AND PROVISION OF TRANSFUSION SUPPORT

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Background: Auto-Immune Hemolytic Anemia (AIHA) are characterized by decreased red cell survival and presence of auto antibodies directed against red cell antigens. The Direct Antiglobulin Test (DAT) with other serological investigations carried out in blood bank will help to determine the type of haemolysis. And this study has been conducted to evaluate of positive DAT and to select blood product for transfusion support of patients with AIHA.
Study Design and Methods: Total number of 99 consecutive patients diagnosed as AIHA with a referral to reference immune haematology Laboratory In National Blood Center for serological investigation with DAT and for red cell products were retrospectively analyzed.
Results: A total of 86 adult Patients and 13 pediatric patients were included in this study. Warm Auto-immune Hemolytic Anemia (WAIHA) was the commenst type auto immune hemolytic anemia with both C3d and IgG specificities. Cold Agglutinin Syndrome (CAS) is the second common type and only C3d specificity could be found. Paroxysmal cold Hemoglobin Urea (PCH) which was found only in pediatric population in this study consisted only C3d specificity in positive DAT. Mixed type of AIHA and drug induced AIHA could be the minor categories of this study.
Discussion: The DAT will be helpful to determine the type of AIHA and to select blood products for transfusion support and to avoid unnecessary transfusions.

P-174
CHARACTERISTICS OF RED BLOOD CELL (RBC) ALLOANTIBODIES DETECTED IN PATIENTS OF THE REGIONAL CENTRE OF TRANSFUSION MEDICINE AND BLOOD BANK (RCTMBB)

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Background: The Reference Serology Laboratory (RSL) of the RCTMBB is a reference centre for 32 local serological laboratories (LSL). Whenever local laboratories face difficulties in used tests, they refer a case to the RSL in order to identify or confirm the specificities of RBC alloantibodies.
Aim: Analyze the interdependence between the specificity of the detected alloantibodies and both the age and the sex of patients. The specificity of alloantibodies detected in 323 patients in 2004 was compared to the specificity of alloantibodies in 469 patients in 2008. In 2004, all LSL carried out serological tests by standard serological tubes methods. In 2008, nine LSL started using microcolumn technique.
Material and methods: The identification of alloantibodies specificity was carried out by means of appropriate indirect antiglobulin test (IAT) and a 11-set test cell reagents group 0 (RCTMBB and DiaMed) of the microcolumn gel tests (DiaMed), as well as by means of the enzymatic test LEN using the standard tube method.
Results: The introduction of the microcolumn technique in 2008 in LSL resulted in the increase in confirmation of RBC alloantibodies (60,9%) in comparison to the results obtained in 2004 (41,19%), with a comparable age range of both female (average in 2004: 48; in 2008: 53,69) and male (average in 2004: 52,73; in 2008: 55,35) patients. The presence of alloantibodies was more often detected in women (in 2004: 72%; in 2008: 69%) than men (in 2004: 28%; in 2008: 31%). The most common alloantibodies detected were these directed against the antigens of systems: Rh (in 2004: 52,39%; in 2008: 60,84%), Lewis (in 2004: 26,7%; in 2008: 14,64%), Kell (in 2004: 6,05%; in 2008: 8,56%) and P (in 2004: 7,81%; in 2008: 7,79%). The presence of these alloantibodies was detected in women: in 2004: anti-D (21,18%), anti-E (16,67%), anti-Lea (11,81%) and in 2008: anti-E (22,61%, anti-D (21,54%) and anti-K (9,84%). In men, results were as follows: in 2004: anti-Lea (20%), anti-E (18,18%), anti-Leb (10%) and anti-D (8,18%) while in 2008: anti-E (35,67%), anti-P1 (11,46%), anti-Lea (10,83%) and anti-D (6,37%). Polyspecific allloantibodies were detected in 114 (14,4%) cases: 107 patients (93,8%) had two types, 6 (5,3%) had three and 1 person had four. The most frequent combination was anti-D+C in 55 cases (51,4%).
Conclusions: The analysis of tests' results shows that:
1. the frequency of anti-D RBC alloantibody detection in female and male groups was the same in 2004 and in the 2008:
2. the increase in anti-E alloantibody detection in male patients in 2008 in comparison with 2004 was observed;
3. the introduction of the microcolumn technique, using automatic or semi-automatic lines, into routine serological diagnostics in local serological laboratories resulted in the increased efficiency of RBC alloantibody detection in patients whose blood samples were referred for verification.

P-175
SEROLOGICAL PROBLEMS IN AB0 TYPING IN MINOR INCOMPATIBILITY BONE MARROW TRANSPLANT (BMT) BETWEEN DONOR-RECIPIENT

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A detection in the recipient of a BMT transplant of two blood cell populations differing in antigens proves mixed chimerism.
Aim: To prove the existence of mixed chimerism in the blood group of the AB0 system, resulting from the BMT in minor incompatibility in type A to 0 (4 cases) and in type AB to 0 (1 case) as well as showing reduction of alloagglutinins.
Material and methods: The research was carried out in five allogeneic BMT transplant recipients in a minor AB0 incompatibility of a A to 0 (4 cases), AB to 0 (1 case). In all these cases, a difference between a donor and a recipient was observed in reference to the phenotype of antigens in Rh and MNS systems. The research was carried out by means of a microcolumn gel tests (DiaMed) and by means of a tube technique, using monoclonal reagents.
Results: In all the described cases, in the pre- and post-transplant period the direct (DAT) and indirect (IAT) antiglobulin tests in recipients were negative. The presence of alloantibodies was not detected. Tests to detect chimerism resulting from a prior transplantation were carried out three months after the last transfusion.
In AB0 minor-mismatched transplantation of the A to 0: 1. six months and one year after the BMT, a change in the phenotype of red blood cells towards the donor's type in Rh and MNS systems. During the determination of a blood group in the AB0 system with anti-A reagent, mixed

agglutination was obtained, and with reagent red blood cells we obtained negative reactions with group A red cells;
2. two years after BMT, mixed agglutination with anti-A reagent was detected and lack of alloagglutinins anti-A was observed. Stable mixed chimerism was restricted to an antigen of the AB0 system.
In AB0 minor-mismatched transplantation of the AB to 0: 1. six months and one year after the BMT, a change in the phenotype of red blood cells towards the donor's type in Rh and MNS systems. During the determination of a blood group in the AB0 system with anti-A and anti-B reagents and with reagent red blood cells agglutination was not detected;
2. two years after BMT, mixed agglutination with anti-A reagent was detected and with reagent red blood cells lack of alloagglutinins anti-A and anti-B was observed.
Conclusions: 1. Mixed chimerism detected in recipients of BMT justifies a systematic control of a blood group in the AB0, Rh and other systems, and of alloantibodies.
2. The detection of mixed chimerism in an AB0 system in recipients of BMT should result in the preparation of a summary of a blood group systems for transfusion.

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FREQUENCY OF BLOOD GROUP ANTIGENS REPORTED TO IMMUNOHAEMATOLOGY REFERENCE LABORATORY SRI LANKA

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Background: Extended Red cell Phenotyping is usually done for transfusion dependent patients before starting their first transfusion. The facility to do extended Red cell phenotype is available only in Reference Immunohaematology Laboratory, National Blood Centre, Sri Lanka.
Aim: The study was carried out to identify the frequency and distribution, pattern of red cell antigens.
Method: The retrospective study was done at Reference Immunohaematology Laboratory, National Blood Centre, Sri Lanka. The data was collected from Immunohaematological records from 1st December 2008 to 31st May 2009. Phenotyping for Rh D, C, E, c, e, K, k, Fya, Fyb, Jka. Jkb, M, N, S and s antigens was performed using commercially available monoclonal Phenotyping reagents.
Results: Forty nine extended red cell Red cell Phenotypes were performed during the six months period. Seventeen patients (34.69%) were between the ages of one month to six months. Majority of patients were Thalassaemics before straining the transfusion therapy, accounting eighty one percent (81.63%). Twenty six (53.06%) were O Rh D positive and Ten (20.04%) were A Rh D positive. Frequency of red cell phenotypes (See Table 1).

Table 1: At the end of the Results

Blood Group system	Red Cell Phenotype	Number	Percentage %
Rh	R_1R_1	26	53·06
	R_1r	13	26·53
Kell	kk	49	100
Kidd	$Jk^{(a+b-)}$	20	40·81
	$Jk^{(a+b+)}$	14	28·57
Duffy	$Fy^{(a+b-)}$	21	42·85
	$Fy^{(a+b+)}$	20	40·81
MNSs	MNSs	14	28·57
	MMss	12	24·48

Conclusion: The frequently encountered extended Red cell phenotype was R1R1 kk Jk(a+b-) Fy(a+b-) MNSs.
Thalassaemia Major is the commonly encountered Transfusion dependent condition in Sri Lanka. Estimated number of new cases per year is between 60 and 80. Forty requests came from diagnosed Thalassaemia patients for six month period. Therefore almost all newly diagnosed patients have get their extended phenotype done before straining the transfusion therapy.

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EVIDENCE SEARCH FOR TRANSFUSION MATCHED OR NOT FOR RECIPIENTS HAVING COLD-REACTIVE ANTIBODIES SUCH AS ANTI-M, ANTI-P1, ANTI-LEA AND ANTI-LEB

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Background: Although anti-M, anti-N, anti-P1, anti-Lea and anti-Leb, usually active at cold temperature, are not considered clinically significant, few systematic studies on such cold-reactive antibodies have been reported in Asian populations. Matched (i.e., antigen-negative) red cells have been transfused to patients who have a cold-reactive antibody in Japan.
Aim: To certify that cold-reactive antibodies such as anti-M, anti-N, anti-P1, anti-Lea and anti-Leb can or not cause hemolytic transfusion reactions, collaborative study has been done.
Methods: From 2007.4 to 2009.4, screening of unexpected antibody in recipients is routinely tested. A total of 14 anti-M, 1 anti-N, 20 anti-P1, 37 anti-Lea and 14 anti-Leb without active at 37C were transfused with random red cells. Antigens of transfused red cells were retrospectively determined. Recipients transfused with antigen-positive red cells against cold-reactive antibody were followed whether hemolytic reactions were occurred or not after transfusion by markers as ALT, LDH, total bilirubin and urine occult blood.
Results: Of the 86 recipients with cold-reactive antibody, 57 were transfused with antigen-positive red cells. A recipient with anti-N was not able to follow-up. No recipients developed hemolysis among 13 anti-M, 12 anti-P1, 19 anti-Lea and 12 anti-Leb who received antigen-positive red cells.
Conclusion: No hemolytic transfusion reaction was observed after transfusion of non-matched red cells with cold-reactive antibodies including anti-M, anti-P1, anti-Lea and anti-Leb. These results do not indicate that patients having such cold-reactive antibodies may be supported with antigen-negative red cells, however, we should include more numbers of such transfusions to draw a conclusion.

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ABO SUBGROUP DETECTION: CAN WE RELY ON AUTOMATED BLOOD GROUPING ANALYZER ALONE?

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Background: ABO subgroups in Chinese are rare but are occasionally encountered. ABO grouping is routinely examined by both automatic analyzer (Olympus PK7300 Automated Microplate System) and manual method for first time donors (FT) in Hong Kong. For repeat donors (RD), ABO grouping is tested solely by auto-analyzer; the results are checked against records in the blood bank computer system.
Aims: We conducted a pilot study to explore if the current ABO grouping algorithm for first time donors by both automatic and manual methods could be replaced by automated procedures solely without losing important data in order to save manpower.
Methods: From August 2008 to April 2009, donor samples with indeterminate results by automated analyzer and/or discrepancy ABO results by manual methods were sent to Reference Laboratory for ABO group resolution by conventional serological methods.
Results: In the period, 154 824 donation samples (FT 31,996; RD 122 828) from 117 078 donors were ABO grouped. Of these 154 824 groupings by

auto-analyzer for both FT and RD samples, 982 (0.63%) were indeterminate. Of these 982 samples, 48 (4.9%) were due to ABO subgroups. Of the 31,996 FT donation samples with manual grouping, 193 (0.60%) showed forward and backward typing discrepancy; of which 13 (6.7%) were attributed to ABO subgroups. Two cases of ABO subgroups revealed by manual method were missed by auto-analyzer whereas there was none vise versa.

Case 1: - A3B (FT donor): 3+mf with anti-A by manual method
- Strong atypical anti-A (3-4+) in donor's plasma
- Auto-analyzer interpreted as group B

Case 2: - ABsub (FT donor): 4+mf with anti-B by manual method
- Only slightly weakened reaction (4+mf) against anti-B
- Auto-analyzer interpreted as group AB

Of the 117,078 donors, 40 ABO subgroups with a frequency of 0.034% were identified. The distribution of these cases is as follows:
- A subgroup: n = 4 (including 1 case of Ael, 2 cases of AelB and 1 case of AsubB)
- B subgroup: n = 31 (including 4 cases of Bv, 6 cases of B3, 14 cases of Bsub, 2 cases of AB3 and 5 cases of ABsub)
- H-deficient phenotype: n = 5 (A: 0, B: 3, AB: 1, O: 1)

Conclusions: ABO subgroup occurs at a frequency of 0.034% in Hong Kong Chinese donors. High throughput automated blood group analyzers are always reliable and give accurate ABO blood group results except in the case of ABO subgroups that typing can be less precise. The 2 above-described cases were exceptional: AsubB with strong atypical anti-A occurred in Case 1 and only very mildly weakened forward reaction in Case 2. We maintain the current testing algorithm and plan to evaluate the performance of other automated systems in this aspect.

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PREVALENCE OF RED CELL ALLOANTIBODIES AND THEIR SPECIFICITY IN A POPULATION OF MALAYSIAN PATIENTS

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Introduction: The prevalence of allo-antibodies to red cell antigens differ among populations depending on their racial composition, frequency of transfusions, parity and techniques used for antibody screening. Blood group polymorphisms differ between regions in Asia and also show significant differences from the western world.

Methods: We aimed to identify the prevalence of red cell allo-antibodies within a patient population which is composed predominantly of Malay, Chinese and Indian ancestries. Antibody screening results of 95 676 (44% female, 66% male) individual patients tested in the hospital over a 5-year period from 2004 to 2008 was retrospectively reviewed. Antibody screening was performed by an IgG mono-specific indirect agglutination method on a 3-cell screening panel using column agglutination technology. Samples tested positive were subjected to antibody identification procedures.

Results: Positive antibody screen was seen in 3.2% (3 042/95 676) of the patients tested. Forty-one percent (1 250/3 042) of the samples from patients were interpreted as false negatives giving weak reactions on antibody screening and negative on further testing with antibody identification panel cells. The overall allo-antibody prevalence was thus calculated as 1.9% (1 792/95 676). The prevalence among males and females was 1.2% (507/42 322) and 2.4% (1 285/53 354) respectively. Overall, there was no significant difference for prevalence of allo-antibodies between pregnant and non-pregnant females. 1 904 antibodies were identified of which the specificity could be confirmed with certainty in 92%. The most common allo-antibody identified was against the Lewis blood group, accounting for 39.2% of all antibodies identified. This was followed by anti-E (18.1%) and anti-D (10.8%). However, a significantly higher prevalence of anti-D was observed among pregnant mothers, indicating that many of the anti-D identified was due to passive immunization with anti-D used for routine RhD antenatal prophylaxis. The other common antibodies identified were anti-Mia (4.7%), anti-M (4.1%) and anti-c (4.0%). Other clinically significant antibodies identified include anti-Jka, anti-C, anti-S, anti-Fyb, anti-K, anti-Fya, anti-e, anti-Jkb, anti-N, anti-s and anti-Kpa.

Conclusion: The antibody prevalence in the East-Asian patient population differs from the western population, in that we see more antibodies directed to the Lewis and MNS blood group antigen system. Antibodies to the Rh system, in particular anti-D, still remain a significant problem in our population due to the heterogeneity of races, which may be in contrast to a purely oriental population. Anti-K meanwhile remains of little concern to East Asians. Antibody screening policies and patient management will need to be tailored according to local antibody prevalence.

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THE FIRST CASE REPORT OF MCLEOD PHENOTYPE IN TAIWAN

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Background: McLeod syndrome is characterized by the absence of Kx antigens, a weakened expression of Kell blood group antigens and neuromuscular dysfunction. McLeod phenotype has an X-linked mode of inheritance and not been reported in Taiwanese.

Aims: We reported one male patient of McLeod phenotype with a strong anti-Kx red cell antibody. In search of family donors for compatible red cells, one of his brothers was found to inherit McLeod phenotype also.

Methods: Irregular antibody screening and cross-matching were performed with manual polybrene method and Ortho BioVue system.

Results: A 69 years old male patient who suffered from osteoarthritis of both hips for a long period and planned to receive a hip arthroplasty. He had received blood transfusions about 10 years ago in other hospital. A blood order for scheduled surgery was required and pre-transfusion test was performed. Irregular antibody screening showed strong red cell alloantibodies which reacted with all screening cells and panel cells. The red cell antibodies reacted strongly (3+) in LISS anti-human globulin test with all tested red cells with a normal Kell phenotype. Phenotyping of patient's red cells showed a weakened expression of Kell blood group antigen (K- kw+), Kp(a- b-). The absence of Kx antigen and specificity of anti-Kx in the patient's serum were established by a reference immunohematologic laboratory. In search for possible donors in the patient's family, it happened that one of the patient's brothers had the same phenotype and cross-matching of the patient's serum with this brother's red cell was compatible. Phenotyping of the proband's brother also showed a McLeod phenotype. In tracking the medical history, both of them had persistent elevated creatinine kinase (in the range of 700–800 IU/L), which had even prompted one of them to visit clinics several times without a conclusive diagnosis. A neurological checkup in both cases revealed movement disorder and neuromuscular dysfunctions which were compatible with McLeod syndrome. Blood smears of them showed mild acantohcytosis (around 1%). Neither brother had manifestations of chronic granulomatous disease (CGD).

Conclusions: We reported two brothers of McLeod phenotype. Our finding also supports the previous report that non-CGD McLeod patient can make anti-Kx after transfusion.

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RETROSPECTIVE COMPARISON OF ALBUMIN AND POLYETHYLENE GLYCOL IN THE INDIRECT ANTIGLOBULIN TEST FOR THE PREVENTION OF DELAYED HEMOLYTIC TRANSFUSION REACTION

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Background: Delayed hemolytic transfusion reaction (DHTR) usually occurs more than 24 hours after RBCs transfusion. DHTR arise from red cell

antigen incompatibilities outside of the ABO system. Incompatible RBCs sometime induce allo-immunization and are destroyed by a secondary alloantibody in the reticuloendothelial system. Pretransfusion screening is important to prevent DHTR. IgG alloantibodies are best detected with the indirect antiglobulin test, for which various enhancement solutions exist, e.g., albumin (Alb), low ionic strength solution (LISS), and polyethylene glycol (PEG). All IAT enhancement solutions increase the sensitivity of alloantibody detection in some way, but it is not clear which solution most effectively prevents of DHTR.

Aims: The aim of this study was to compare the sensitivity of pretransfusion screening with Alb-IAT and PEG-IAT for the prevention of DHTR.

Methods: We investigated 11 459 RBCs transfusion recipients and 109 346 of the RBCs units they received from January 1989 to December 2008. Over these nineteen years, our hospital used two different enhancement solutions in IAT pretransfusion screening. Alb-IAT was used for 8 years, from 1989 to 1996, and PEG-IAT was used for 12 years, from 1997 to 2008. The incidence and presentation of DHTR during the two intervals were compared.

Results: In total, 33 patients were investigated. The incidence of DHTR during the Alb-IAT and PEG-IAT years was 0.37 % (17 of 4,651 patients) and 0.17% (14 of 8,038 patients), respectively ($P < 0.05$). The major alloantibody specificities associated with DHTR were directed against Rh and Kidd system antigens, which were better detected by PEG-IAT, thus accounting for the lower incidence of DHTR from 1997 to 2008. DHTR were associated with a positive direct antiglobulin test, a drop in hemoglobin and an increase in bilirubin by the production of alloantibodies in spite of a compatible RBCs transfusion.

Conclusion: Within the limits of a retrospective study, our data suggests that pre-transfusion screening with PEG-IAT is more effective than Alb-IAT for preventing DHTR.

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COLLABORATIVE STUDY ON ERYTHROCYTE IRREGULAR ANTIBODIES IN JAPAN: RESULTS FROM JAPANESE STUDY GROUP OF ALLO-IMMUNITY TO ANTIGEN DIVERSITY IN ASIAN POPULATIONS

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Background: An investigation on erythrocyte irregularity antibodies (Abs) has been undertaken in Asia including Japan. Although several studies have been reported previously, the relationship to blood transfusion and pregnancy has not been well elucidated. It is important to understand recent movements including frequencies and patterns of erythrocytes irregular Abs in this multi-institutional study. Collaborative study in Asia will bring us better understanding on racial difference and allo-immunity. Furthermore, it will improve international collaborations in the field of transfusion medicine.

Methods: In addition to information on institutes including number of bed, operation and transfusion, we investigated number of blood grouping test, cross match test, and erythrocyte irregular Ab analysis. We also investigated methods adopted in erythrocyte irregular Abs screening and determination. Furthermore, we studied the frequencies of erythrocyte irregular Abs, and compared them with post transfusion or during pregnancy.

Results: Twenty-nine institutes were registered, and 214 909 cases were analyzed in this study. Number of bed varies from 135 to 1388 beds, number of operation from 162 to 3 951 cases per year, and number of Abs screening test from 219 to 14 900 cases per year. Ab screening methods adopted in these institutes were as follows; gel column in 3 institutes, beads column in 14, traditional tube in 8, and beads column plus traditional tube in 4. Ab determination methods adopted were as follows; gel column in 4 institutes, beads column in 6, traditional tube in 15, and beads column plus traditional tube in 4. Erythrocyte irregular Abs were determined in 3 392 cases (1.6%). The frequency of Abs in 3,392 cases were as follows; anti-E 27%, Lea 26%, P1 11%, M 6%, E+c 4%, Fyb 4%, Dia 3%, Leb 3%, and D 2%. Anti-P1 Ab was more frequently observed in the institutes adopted saline method in room temperature than those not adopted. No difference in the frequency of Abs was observed between the institutes adopted enzyme methods and not adopted. In pregnancy, anti-D (5%) and E+c (6%) Abs were more frequently determined. In post-transfusion cases, anti-E (38%), E+c (8%), Jka (4%) and E+ Jka (1%) Abs were more often determined.

Conclusion: The frequency of Abs was different among institutions and from previous reports. This discrepancy might be explained by the methods adopted and the current developments occurring around blood transfusion practices. International collaborative study on them will be informative.

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ESTABLISHMENT AND CHARACTERIZATION OF MURINE MONOCLONAL ANTI-FYA BY IMMUNIZATION WITH SYNTHETIC PEPTIDES

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Aims: To screen Fya negative red blood cells (RBCs), monoclonal anti- Fya is useful. We tried to establish mouse monoclonal anti-Fya by immunizing with synthetic peptides.

Materials and methods: C3H mice were immunized with a synthetic 10 mer peptide containing the Gly (Fya antigen) residue conjugated to multiple antigen peptides (MAP). After immunization, splenic cells were fused with mouse myeloma cells (P3-U1) and cloned by limiting dilution. Anti-Fya producing hybrid cells were identified by mouse indirect antiglobulin test (IAT). The epitopes of Fya antigen recognized by these monoclonal antibodies (MoAbs) were examined by a SPOTs analysis. The established and purified anti-Fya (OSK47 and OSK47-1) were cross-linked with rabbit anti-mouse IgG and used for screening Fya negative RBCs on the machine (PK7300) by saline method.

Results: OSK47 and OSK47-1 reacted with Fy(a+b-) RBCs but not with Fy(a-b+) RBCs by IAT. The titers of OSK47 with Fy(a+b-) and Fy(a+b+) RBCs were 1:64 and 1:8, respectively. Those of OSK47-1 were higher than those of OSK47. The reactivities with treated RBCs with trypsin, sialidase, and DTT were resistant, whereas the other enzyme-treated RBCs were sensitive. Immunoglobulin subclass of OSK47 and OSK47-1 were IgG2b and IgG1,?respectively. As for the epitopes recognized by these MoAbs, OSK47 recognized LEA(Leu-Glu-Ala) and OSK47-1 did NLE(Asn-Leu-Glu). A total of 4,799 donors' RBCs were screened and 48 were found to be Fya negative. These 48 RBCs were further examined by using a commercial anti-Fya and confirmed to be Fya negative.

Conclusions: We established two murine monoclonal anti-Fya, and showed that they were useful to screen for Fya negative RBCs on the machine.

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CLINICAL SIGNIFICANCE OF ENZYME TECHNIQUES IN IRREGULAR ANTIBODY SCREENING

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Background: It is known that enzyme techniques are a highly sensitive assay for detection of weak or developing Rh antibodies. Therefore, many facilities in Japan prefer to use enzyme techniques for irregular antibody screening. However, the enzyme techniques often detect cold antibodies and benign autoantibodies which are regarded as clinically non-significant.

Aims: The purpose of this study is to evaluate clinical significance of enzyme techniques in irregular antibody screening.

Methods: Of approximately 200 000 blood donors, 123 specific antibodies were detected by enzyme techniques (1-stage bromelin or 2-stage papain) and were also tested by indirect antiglobulin test (Saline-IAT), polyethylene glycol test (PEG-IAT) and MTS gel system (MTS-IAT). Clinical significance of the antibodies is evaluated according to detection rate of each IAT (IgG antibody active at 37C), antibody titration, flow cytometric analysis of Ig isotypes, levels of cell-bound IgG and monocyte monolayer assay (MMA).
Results: Of 123 samples, 71 (Rh: n = 52, Diego: n = 9, Jra: n = 6, S: n = 3, Lewis: n = 1) (58%) were positive by Saline-IAT, PEG-IAT and MTS-IAT (group A), 34 (Rh: n = 30, Lewis: n = 3, S: n = 1) (28%) were positive by PEG-IAT or MTS-IAT (group B), and 18 (Rh: n = 9, Lewis: n = 8, P1: n = 1) (14%) were negative by all IAT (group C). Detection rate of each IAT was 58% (Saline-IAT), 84% (PEG-IAT), and 80% (MTS-IAT). In group A, both antibody reactivities and levels of cell-bound IgG were very high and most Ig isotypes were IgG1 and IgG3, both were lower in group B and C. MMA-positive rate of group A and B were 87%, 24%, while group C were 0%.
Conclusion: Among antibodies reactive in the enzyme techniques, clinically significant antibodies were able to be detected by PEG or MTS-IAT. Although the techniques employed for antibody screening must be broad enough to detect clinically significant antibodies, the antibodies that are detected only by the enzyme techniques are not considered clinically significant.

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EVALUTION OF THE SENSITIVITY OF AN AUTOMATED COLUMN AGGLUTINATION TECHNOLOGY USING READY-TO-USE 3% REAGENT RED BLOOD CELLS IN DETECTION OF ANTI-MIA AND ANTI-E IN RECIPIENTS

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Background: Anti-Mia and anti-E are the two most prevalent red cell alloantibodies of clinical significance in Taiwan. An in-vitro diagnostic product of 3% reagent red blood cells (RBCs), MeDiPro Antibody Screening Cells, is currently the only one able to detect anti-Mia in Taiwan, but its application in an automated Column Agglutination Technology (CAT) using glass bead test has not yet been standardized and remains controversial.
Aims: The combined use of an automated CAT and the MeDiPro reagent RBCs were assessed to determine the detection sensitivity of clinically significant alloantibodies.
Methods: Plasma samples containing anti-Mia or anti-E were randomly selected from recipients. Samples with detectable autoantibodies or alloantibodies inactive at 37C were excluded. Three techniques using the MeDiPro reagent RBCs (3 ± 0.5%, Formosa Biomedical Technology Corporation) for antibody detection (AD) were employed in each sample. The automated CAT was run on the Ortho AutoVue Innova System using the BioVue Polyspecific Anti-Human Globulin cassettes. Two conventional tube techniques for antibody screening employed including the manual Polybrene test (MP) and the indirect antiglobulin test in low ionic strength solution (LISS-IAT). The MP was regarded as a reference method.
Results: Thirty-three samples were enrolled and the alloantibodies identified were anti-E (9), anti-Mia (15), anti-E/-c (7) and anti-E/-Mia (2). The concordance rates of serological activities in AD were 72.7% (24/33) among three techniques and 90.9% (30/33) between LISS-IAT and CAT. The overall sensitivity of AD was 81.8% (27/33) for LISS-IAT and 72.7% (24/33) for CAT. For those discrepant samples (6 in LISS-IAT and 9 in CAT), all were found to contain one or two antibodies of low concentrations (less than 2 plus serological activity in MP). Therefore, the sensitivity of AD greatly reduced to 66.7% (12/18) for LISS-IAT and 50.0% (9/18) for CAT in a low titer subgroup (6 anti-E, 9 anti-Mia, 2 anti-E/-c and 1 anti-E/-Mia). Among them, 7 (77.8%) out of 9 samples containing anti-E and 3 (30%) out of 10 samples containing anti-Mia had false-negative findings by CAT.
Summary: The performance of CAT for AD was similar to LISS-IAT. Both automated CAT and LISS-IAT seemed inferior to MP in detection of low titer of clinically significant alloantibodies using 3% reagent RBCs. The clinical significance of this finding merits further investigation.

P-186
THE PREVALENCE OF HAEMOLYSINS IN BLOOD GROUP O DONORS IN LOKOJA, NIGERIA

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Background: The presences of haemolysins in banked blood are not detected during normal reverse tube cross- matching of recipient blood. But will cause haemolysis at body temperature after blood transfusion. Such reactions are not desirous in an emerging safe blood transfusion practice. Reports of haemolysins in the blood of Africans have been well documented in the literature. It is against this backdrop that samples of blood group O donors were tested for the presence of haemolysins in order to avert possible haemolytic reactions when such are given to patient with blood groups A and B.
Aims: The aim of this study is to determine the prevalence of Haemolysins in the serum of blood group O donors in the Lokoja centre of the Nigerian National blood transfusion service. This is with the view to making our findings an advocacy and educational tool among the clinicians and end users of blood and blood products. Such findings will enhance the safety of our blood and blood products in African blood transfusion service.
Materials and methods: We conducted a cross-sectional pilot study on the serum of blood group O donors. The presence of haemolysins were tested using the tube method, at an incubation of 37 degrees for 90–120 minutes and observed for haemolysis
Results: Haemolysins were identified in 22% of the blood group O donor population of the sample. This incidence is higher than previous reports in the literature.
Summary and conclusion: The uses of Blood group O as a universal blood in all patients continue to be the norm in most clinical settings, particularly in the rural Hospitals and clinics in Nigeria. This study demonstrates the potential danger in such practise. The inclusion of haemolysins testing is recommended as a routine in blood processing as the procedure is simple cheap and user friendly. However, a more extensive study will be required in Nigeria and Africa to find the real incidence of Haemolysins, and ensure more safety of blood and blood products in the emerging blood transfusion practice in Africa.

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THE SEROLOGIC AND GENETIC ANALYSIS OF RARE A2 SUBTYPE IN TWO TAIWANESE FAMILIES

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Background: The A2 is a very rare phenotype in the ABO blood group system in the Oriental population. The frequency of A2/A2B phenotype is much higher in Caucasian populations than in Oriental populations. It was believed that no A2/A2B phenotype was present in Taiwan till 1987. Until now is still very few A2 cases were reported.
Aims: In our study, we used serologic and molecular genetic methods to analyze the two A2 families in Taiwan for disclosing the phenotype and genotype of this rare blood subtype.
Methods: The peripheral venous blood samples from17 members of two families were collected into standard hematological EDTA tubes. Using murine monoclonal anti-A and anti-B antibodies(ImmucorGamma) and A1 and B cells(Ortho)on an automatic analyser (AutoVue; J&J). If unusual expression was detected, then perform manual tube methods using anti-A, anti-B, anti-AB (ImmucorGamma) and A1 cell, B cell, anti-H(Ortho), in addition, the anti-A1(lectin) saline method (Sanquin) with a stabilized extract prepared from the seeds of Dolichos Biflorus was used to identify the A2 phenotype. After blood typing, DNA sequencing and PCR were performed to further investigate the relationships between the genetic and phenotypic characteristics of all samples.

Results: There are eight people in family1, one with A2 phenotype and the other with A2B phenotype were discovered by the standard ABO haemagglutination sereologic test with discrepancies between forward and reverse typing, consequently we found that two individuals did not react with anti- A1 and this confirmed their A2/A2B phenotype. After sequencing, the A2 allele is characterized by a single base deletion at nt 1061, The deletion generates a frameshift and results in an additional domain at the carboxyl terminus of the mature protein. There are nine people in family2, three are with A2 phenotype that three individuals did not react with anti- A1, another with A2B phenotype. After sequencing, A2 allele is with 1009A > G that leads to amino acid substitution of arginine For Glycine. But there are five 1009A>G cases in family 2.

Conclusions: The Anti- A1 Occurring ratio (6.9%)in Taiwanese much lower than Caucasian could result in the lower A2 frequency in Taiwanese. A2 allele maybe has different type in Taiwanese, but it still need proceeding more study and research. Most of the A2-forming polymorphisms occur near the 3-end of exon 7. For instance, the 1059delC, 1054C > T, 1054C > G, and 1009A > G have been suggested to alter the A transferase activity and we reported the same result. Complete ABO gene analysis including the promoter region is required to explain the discordance.

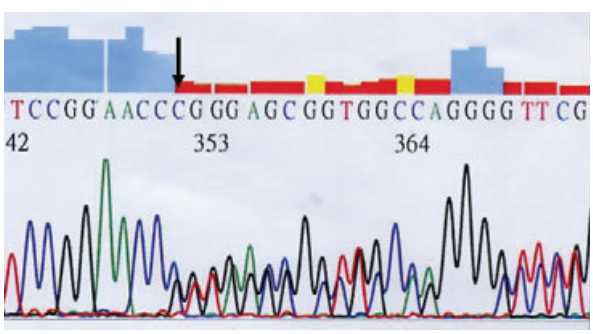

Figure 1:

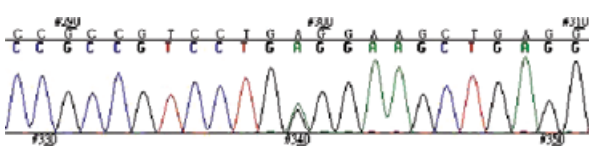

Figure 2:

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SEROLOGICAL BEHAVIOR, DEMOGRAPHICAL PATTERN & ASSOCIATED RISK FACTORS OF AUTOIMMUNE ANTIBODIES TO RED BLOOD CELL ANTIGENS

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Subject: Serological behavior, demographical pattern & associated risk factors of autoimmune antibodies to red blood cell antigens.

Background: Autoimmune red blood cell antibodies are those antibodies that are directed against an individual's own red blood cells. The formation of auto antibodies results due to the breakdown of immune tolerance to self red cell antigen. Autoantibody gives rise to problematic grouping, serology & cross match.

Aim: To analyze:

1. serological behaviors
2. demographical pattern &
3. Associated risk factors of red cell auto antibodies.

Method: Data were collected from all pretransfusion compatibility testing requests received at National blood Center from all hospitals in Sri Lanka during Jan 2009 & May 2009. Those that had positive auto antibodies at the time of testing were analyzed irrespective of the hemolytic status.

Results: Total of 95 patients was found to have auto antibodies. Out those 65.3% were females & 34.7% males. Male to female ratio was 1:1.88. Age ranged between 1.5 to 85 years of whom 30.5% were in >60y age group & 27.4% were in 20–40 age group. Out of 95 19 were found to have cold auto antibodies & 31 had mixed type while 45 had warm auto antibodies.52.6% males and 47.3% females had cold autoantibodies.41.9% males and 58% females had mixed type autoantibodies. 24.5% males and 75.5% females had warm auto antibodies. In 1–20 age group 47.6% had warm auto antibodies, 38% had mixed type and 14.2% had cold auto antibodies. In 20–40 age group cold auto antibodies were detected in 65.3% & mixed type in 19.2% & warm in 15.3%. In 40–60 and > 60 age groups distribution of cold, warm and mixed type was similar. Of the 19 who developed cold auto antibodies C3d alone was detected in 8 samples IgG alone in 1 both in 1 where neither IgG nor C3d specificities were detected in 9 cases. Of the 31 mixed type of auto antibodies IgG alone were found in 1 case & C3d alone in 13 case s & both IgG & C3d in 17 cases. Of the 45 warm auto antibodies 7 were IgG alone, 12 were C3d alone & both specificities were detected in 26 cases.

Of the 95 cases 48.4% had detectable hemolysis.9.5% of 95 auto antibody positive cases had associated malignant diseases.

Conclusion: 1. Females are more prone to develop auto antibodies.

2. In cold and mixed type both males and females had a similar distribution where as in warm type, proportion of females was significantly higher than males.
3. Warm auto antibodies were the most common.
4. In the age group 20–40 cold type was the predominant (65.3%).
5. In all other age groups warm and mixed types were the predominant and both types had similar distributions.
6. Most of the cold auto antibody type had C3d specificity alone or neither IgG nor C3d specificities. In mixed & warm type most of them had both IgG & C3d specificities. Of the warm auto antibody 23% had only C3d specificity

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THE FREQUENCY OF THE BLOOD GROUP ANTIGEN DIEGO A IN ROSARIO, ARGENTINA

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The Diego a (Dia) antigen is very rare in Caucasians but relatively common in populations of Mongoloid ascendance with frequencies ranging from 1-10% in Asians to 5-54% in Amerindians. The anti-Dia can be responsible for severe cases of Hemolytic Disease of the Newborn (HDN) and transfusion reactions. Since red cells panels used for irregular antibodies screening in Argentina do not contain Di(a+) cells, most anti-Dia antibodies remains undetected.

Motivated by the recent diagnosis of a second case of HDN caused by anti-Dia en el Servicio de Hematología y Medicina Transfusional del Hospital Italiano Garibaldi and several reports in the region, we decided to determined the Dia frequency in the population of Rosario, the third major population by number in the country, with more than 1.1 millions inhabitants.

We phenotyped 335 unrelated healthy blood donors from the Hospital Italiano Garibaldi (n = 190) and from the Centro de Sangre de la Municipalidad de Rosario (n = 145), using conventional Liss-Coombs method in tube and two human antiserum anti-Dia.

As expected from previous studies, the homogeneity test (Chi2) between both groups of donors show no difference (p=0.97) for the Dia occurrence. The Diego a antigen frequency in Rosario was 6.3%. The frequencies for the Di(a+b-), Di(a+b+) and Di(a-b+) phenotypes estimated by Hardy-Weinberg law were 0.4%, 5.9% and 93.7% respectively.

These results were similar to the reported frequencies in La Plata (Argentina) and several cities from Japan, Korea and Brazil, where most of the alloimmunization and HDN cases were reported.
In Rosario, where 3.5% of pregnancies and 5.5% of transfusions are potentially incompatible for the Dia antigen, the use of red cells panel expressing the antigen seems justified. Additionally, we implemented the use of Di(a+) cells for antibodies screening in our Service.

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RH PHENOTYPE FREQUENCIES OF THAI BLOOD DONORS

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Background: Rh antigens are only presented on the red cells. They are the most clinically significant in transfusion medicine next to ABO system. The distribution of Rh antigens differs among different populations. It is important to know phenotype frequencies in the population. These will be helpful to plan and select the suitable blood for alloimmunized patients or to select the most compatible blood for multitransfused patients to prevent alloimmunization.
Aim: To determine the phenotype frequencies in the Thai blood donors in the Blood Transfusion Center of Srinagarind hospital, Khon Kaen University.
Materials and methods: Rh (C, c, E, e) phenotype was performed in 6,668 Thai blood donors. All samples were not repeated subjects and had D positive antigen. The C, c, E and e antigens were typed by specific gel test (anti-C, anti-c, anti-E and anti-e) (KKU gel, Thailand).
Results: The frequencies of C, c, E and e antigens were 95.29%, 34.43%, 31.15% and 96.18%, respectively. The Rh phenotype frequencies were CDe (60.50%), CcDEe (21.09%), CcDe (7.86%), CDEe (4.83%), cDE (2.80%), cDEe (1.41%), CcDE (0.78%), cDe (0.49%) and CDE (0.24%).
Conclusions: This study showed the Rh phenotype frequencies in the largest sample size of Thai blood donors. The most common Rh phenotype (CDe) in Thai population was different from the White and Black populations. Typing in regular blood donors is useful to reserve the rare blood type (cDE) in the stock or can recruit them when it is needed to transfuse to the rare blood type patients.

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A CASE REPORT-HIGH TITER ABO ISSOAGLUTININ CAUSING ABO GROUPING AND CROSSMACH PROBLEMS

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Background: Transfusion of ABO identical blood component is common practice in all hospital blood banks. However in emergencies or shortage of ABO identical blood supply non ABO identical blood components are used. Usually ABO isoagglutinin titer is not performed as a routine practice.
Case report: A 38 year old male who was diagnosed as SLE with cerebral lupus was on prednisolone and other treatments presented with acute onset haematemesis to the local hospital. On admission his Hb was 6.7 g/dl and patient was symptomatic. They have transfuse one pint of B Rh positive and one pint of O Rh positive pack cells for this patient prior to transfer to tertiary care hospital for further management. The patients Hb was falling and heamatemesis was not settled, further blood transfusions were planned. During the process of ABO grouping an ABO grouping discrepancy was detected. For resolution of this problem blood sample was sent to the immunoheamatology reference laboratory.
Forward blood grouping reveal mixed filed agglutination with anti B and anti AB and reverse grouping compatible with blood group B. DAT (direct agglutination test) was positive with a +1 grading. An antibody elusion was done which reveled IgG anti B antibodies as the reason for the positive DAT. All the cross matches with group B Rh positive pack cells were incompatible but group O Rh positive pack cells were compatible.
After these investigations the O Rh positive pack transfused to this patient was traced and assessment of the IgG anti B heamolysin titer was done by titration method and It was 256.
Discussion: This case demonstrates that high titer isoagglutinin in pack cells were able to cause grouping discrepancies and cross match incompatibilities. Some group O donors may have high titers of anti A, and anti B antibodies in their plasma which could cause haemolysis of A and B cells, particularly if large volumes of plasma are transfused. So isoheamagglutinin titers should be determine before switching in to ABO non identical transfusions.

5.4. Immune Haematology Granulocytes immunology

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COMPARISON OF EM AND SQUARE ROOT ALGORITHM APPLIED TO ESTIMATE HLA-ABDR HAPLOTYPE FREQUENCY

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Background: Expectations-Maximization(EM)algorithm and square root algorithm are two major methods to estimate haplotype frequency (HF) in population study. However, the accuracy of the two methods to estimate HLA-A-B-DR HFs were not compared, especially in small sample size.
Aims: To investigate the accuracy of the two HF estimate algorithms when they are applied to estimate HLA-A-B-DR HFs.
Methods: One hundred families that consisted at least one of the parents and one child who had been typed for HLA-A, -B, -DR loci were selected. One individual of each family was included in this study. A total of 8934 HLA-A, -B, -DR typed individuals were selected from Sichuan branch of CMDP. The haplotypes of the 100 individuals with pedigree information were deduced following the rules of Medelian segregation. EM and square root algorithm were applied to estimate the HFs of the 100 individuals which are compared to the pedigree results. Only HFs beyond the reliable frequencies calculated by the method described by R. F. Schipper(1998) were compared. The 20 common HFs in the 8934 indviduals determined by two algorithms were compared by chi-square test. All the EM algorithm were exerted by arlequin and square root exerted by in-house designed software.
Results: In 100 individuals, 11, 14 and 10 haplotype of which frequencies beyond the reliable frequences(0.0149) estimated by pedigree information, EM and square root algorithm were found, and the sum of the reliable frequences are 0.3100, 0.3042, 0.3001, respectively. For chi-square test require most of the number tested is more than 5, only the top 5 HFs were tested and $P > 0.05$ both. Every theoretical haplotype has a value determined by the square root algorithm, but most of them(1991 in 3718) were negative and sum up to -1.5126. The positive values sum up to 2.5157.
In a sample size of 9034, the negative HF values(4831 in 9360) estimated by sqaure root algorithm sum up to -0.1269, 4529 postive frequencies sum up to 1.1255, 838 reliable frequencies (> 0.000166) sum up to 1.0192. 691 reliable frequencies in 9034 individuals estimated by EM algorithm sum up to 0.9985. The frequencies of top 20 haplotypes(EM: sum up to 0.3156; SR: 0.3100) in the 9034 individuals determined by two algorithms were similar($P > 0.05$).
Conclusions: EM is superior to square root algorithm for HF estimates. However, the results of both algorithm for the highest HFs are comparable.

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SUCCESSFUL TREATMENT OF LIFE-THREATENING MULTIPLE LIVER ABSCESSES IN A PATIENT WITH CHRONIC GRANULOMATOUS DISEASE BY BONE MARROW TRANSPLANTATION WITH GRANULOCYTE TRANSFUSION

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Background: Chronic granulomatous disease (CGD) is the most common inherited disorders of phagocytic function caused by abnormal nicotinamide adenine dinucleotide phosphate oxidase. CGD is characterized by the formation of glanulomas due to recurrent bacterial/fungal infections from early childhood. Hematopoietic Stem Cell Transplantation (HSCT) has been shown to be curative for patients with CGD. However, HSCT has considerable risk of transplantation-related morbidity and mortality in patients with life-threatening infection. We hereby report the successful treatment in the combination of HSCT and granulocyte transfusion therapy (GTX) for a CGD patient with life-threatening multiple liver abscesses.
Case: A 26-year-old man was diagnosed as CGD (gp91phox deficiency) at the age of one year due to the recurrent severe infections. He suffered from intractable multiple liver abscesses for six months and was referred to our hospital to receive a partial liver excision and bone marrow transplantation (BMT) from an HLA-matched related donor. Although he underwent partial hepatectomy including abscesses, further multiple liver abscesses were recognized one month later. And then, he received BMT with reduced intensity conditioning regimen (RIC). The active infections persisted during the nadir of leukocyte count after the transplant. Granulocytes donated once by his brother who was also a donor of BMT were transfused to him because his liver abscesses became worse. Seven days after GTX, neutrophil engraftment was achieved, and then the abscesses gradually disappeared. He was transfused 8 units of red cell concentrations and 290 units of platelet concentrations during the clinical course. Ten months after BMT, he remains in good condition. He had no infections, GVHD, or therapy-related complications.
Methods: He had received GTXs in the previous hospital four months before BMT for treating intractable liver abscesses. We examined his serum as mentioned bellow before the BMT and the next GTX. Fluorescence beads were utilized in identification of Anti-HLA class1 /2 antibody and HLA DNA type. Anti-human neutrophil antigen(HNA)antibody was determined by granulocyte immunofluorescence test (GIFT) using healthy person's granulocyte and immunofluorescence test (IF) using cell lines transfected with HNA gene. HNA typing was determined by GIFT using Monoclonal Antibodies (MoAbs) to HNA. Identification of gp91 was determined by IF using the anti- Flavocytochrome b558 antibody. These tests were analyzed by flow cytometry.
Results: The recipient had antibodies which had multi-specificity to HLA before the BMT. Both the recipient and the donor had the same HLA (A0206,2402B0702,5401 Cw0102,0702 DR0101,1502). The recipient had no anti-HNA antibodies right before the GTX. The recipient's HNA were HNA-1a,1b,2a. Although the gp91 expression of the recipient was 0.3%, it increased 94.7% at 21 days after the BMT.
Conclusion: There are only a few reports on GTX after HSCT in CGD. BMT using RIC combined with GTX is an effective treatment for CGD patient with life-threatening infections. We, clinical technicians, should understand the necessity of examinations about leukocytes/granulocytes when GTX is considered. Furthermore, we will support safe and effective GTX in the future.

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IDENTIFICATION OF AN ANTI- HUMAN NEUTROPHIL ANTIGEN (HNA)-2A ANTIBODY BY THE ANALYSIS METHOD USING CELL LINES TRANSFECTED WITH HNA GENE

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Background: The frequency of human neutrophil antigen (HNA)-2a positive population is 99.5% in Japanese. It is very difficult to identify anti-HNA-2a antibody using Granulocyte Immunofluorescence Test (GIFT-FCM) due to the difficulty of obtaining a HNA-2a negative panel cells. Here we report a case of neutropenia due to anti HNA-2a antibody that detected by FCM using cells transfected with HNA genes.
Methods: A 78 years old man was suffering from moderate neutropenia and thrombocytopenia. A few milliliters of the patient's serum was

obtained and examined it for anti-HNA antibodies. (1) GIFT-FCM;The patient's serum was incubated with three types of panel neutrophils already determined as HNA-1a/1a and 2a positive, HNA-1b/1b and 2a positive or HNA-1null and 2a positive, respectively. And then, it was analyzed by flowcytometer after incubation with FITC-labeled anti-human IgG antibody. (2)GIFT inhibition assay using monoclonal antibodies: Three types of panel neutrophils were incubated with TAG4 (HNA-2a-MoAb, neutrophil specific antigen), TAG5 (positive to all neutrophils, monocytes, platelets), CD32MoAb, CD55MoAb (positive both neutrophils and platelets), and CD59MoAb (positive to neutrophils but negative to platelets), respectively. After then, the patient's serum was added to them and the samples were analyzed by flowcytometer after incubation with FITC-labeled anti-human IgG antibody. (3)Immunofluorescence test(IF)-KY cell line assay: The patient's serum was added to KY-2a and KY-mock(K562 cells transfected with HNA-2a cDNA, with vacant cDNA by retrovirus vectors, respectively) and was analyzed by flowcytometer after incubation with FITC-labeled anti-human IgG antibody.
KY cell lines were provided by Osaka Red Cross Blood Center.
Results: The patient's serum reacted positively to all panel neutrophils. The inhibition tests were positive in panel cells preincubated with monoclonal antibodies which react to neutrophils alone or both neutrophils and platelets. The ratio of fluorescence intensity (FI) with KY-2a to FI with KY-mock was 18.69.
Discussion: It was indicated that the antibody in the patient's serum recognizes at least HNA-2a. Furthermore, there might be antibodies which recognized the common antigens on neutrophils and platelets, and that lead to both neutropenia and thrombocytopenia. When there are anti-HNA antibodies which bind to all panel cells in serum, it is very difficult for us to identify the specificity of each antibody. In a patient with such antibodies, it is able to identify the specificity using cells transfected with HNA gene.
Conclusion: The method using GIFT-FCM combined with GIFT-Inhibition and IF-KY cell line assay was very useful.

P-195

DISTRIBUTION OF HLA CLASS 1 ANTIGENS AMONG SRI LANKANS

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Background: National Blood Transfusion Service of Sri Lanka provides HLA typing facility since 1987 for patients and organ donors of government and private sectors. Histocompatibility laboratory of the National Blood Centre is the only HLA typing laboratory in the country. Since there is no documented evidence of HLA antigen frequencies in Sri Lanka, it would be beneficial to analyze the frequencies of HLA antigens among this group. HLA antigens are tested by a serological method, named lymphocytotoxicity test.
Objectives: To determine the distribution of HLA class 1 antigen frequencies, HLA-A, -B and -Cw, in the study population.
Method: A retrospective analysis was done using a sample of tissue donors and recipients attended for HLA typing at Histocompatibility Laboratory. Thousand number (1000) of consecutive study units were taken as the sample during a period from January 2006 to July 2007. Data was extracted by using a data extraction sheet. HLA typing work sheets and Patient and Donor Registers were used as sources. Data were analyzed using SPSS v13.
Results: In the study sample, 766, 112, 112 and 10 were Sinhalese, Tamils, Muslims and other ethnic groups respectively. There were thirteen HLA-A antigens in the study sample. HLA-A33,-A24,-A2 and -A1 had frequencies between 15-10%. HLA-A9 frequency was between 10-5%. HLA-A3, -A26, -A28, -A11, -A36, -A10 had frequencies between 5-1%. HLA-A34, -A29 had frequencies less than 1%. No HLA-A antigen was detected in 4.4%. Numbers of detected HLA-A antigens were twelve each among Sinhalese and Muslims, and eleven among Tamils. HLA-A29 was not detected among Sinhalese. HLA -A29 and -A34 were not detected in Tamils. HLA -A36 was not detected in Muslims and HLA-A29 was detected only among Muslims. The commonest antigens were A33, A1, A2, A24 and A9 among all, though the frequencies were different. Further, twenty five HLA-B antigens were found. HLA-B15 had a frequency between 15-10%. HLA-B35,-B57,-B7, -B51,-B44,-B60,-B12,-B5 had frequencies between 10-5%. HLA-B13, -B40,-B62,-B17,-B55,-B18 had frequencies between 5-1%. HLA-B14, -B37,-B8,-B21,-B63,-B27,-B16,-B41,-B45,-B52 antigens had frequencies less than 1%. Numbers of detected HLA-B antigens were 25, 18, and 21 among Sinhalese, Tamils, and Muslims respectively. The Sinhalese had all twenty five antigens, HLA-B37,-B8,-B21,-B27,-B41,-B45,-B52 were not detected among Tamils and HLA-B8,-B63,-B45,-B52 were not detected among Muslims. The commonest was B-15 among all groups, and other shared common antigens were B-35 and B-7. There were seven Cw antigens and Cw-7, Cw-3, Cw-4 were the commonest among all the racial groups.
Conclusions: The study showed a statically significant difference in the distribution of HLA-A 33, -A24, -B35,-B57,-B44,-B60 and -B65 antigens among races in Sri Lanka. As there were no HLA-A antigens detected in some of the individuals, it is necessary to develop our own anti-sera for HLA typing or need molecular method of typing to detect them.

5.5. Immune Haematology DNA based analysis

P-196

GENOTYPING METHOD FOR COMMON POLYMORPHISMS OF HAPTOGLOBIN AND DISTRIBUTION OF HPDEL IN ASIA

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Background: The haptoglobin gene (HP) has two common alleles, HP1 and HP2, that account for three phenotypes, HP1, HP2-1, and HP2. Because HP2 is generated by an intragenic duplication of HP1 and of existence of HPR with high homology to HP, the genotyping is not so easy and time-consuming.
Aims: To develop simple and rapid method for genotyping of HP common alleles.
Methods: We used TaqMan PCR system and 129 samples of known genotypes for HP genotyping based on comparative Ct method that allows us to evaluate the relative number of copies of the HP2 specific sequence to those of the HP promoter region.
Results: We clearly differentiated HP genotype groups by this method. We could simultaneously detect HPdel, one of causes for anaphylactic reactions to transfusions due to the production of antihaptoglobin antibodies. From the previous studies, the HPdel distribute in East Asian (0.8 to 3.0%) and Southeast Asian (1.0 to 1.5%) populations.
Summary and conclusions: We successfully developed HP genotyping method. This assay produces results in less than one hour and is suitable for high-throughput analysis. In addition, this assay is applicable for routine diagnosis prior to transfusions to prevent anaphylactoid shock caused by anti-HP antibodies in Asian populations.

P-197

DRB1*14 AND RELATED HAPLOTYPES DIVERSITY IN SICHUAN HAN POPULATION

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Background: Some DRB1*14 alleles are dif?cult to distinguish between HLA-DRB1*14 and other alleles families. DRB1*1401 and 1403 were involved in nonpermissive mismatche combinations identified recently.
Objective: To investigate the diversity of HLA-DRB1*14 alleles family and HLA'''A-B -DRB1*14 haplotypes in Sichuan Han population. And to determine the probability of identifying allele matched donors for patients with DRB1*14 alleles and possible mismatch combinations in Sichuan transplantation population.
Methods: A total of 217 individuals were randomly selected from 1818 DRB1*14 positive samples in a database of 11247 Sichuan Han individuals. To provide at least a 99% probability of detecting a rare allele occurring in less than 3% of unrelated DRB1*14-positive individuals, more than 152 samples were required. DRB1*14 alleles were identified in 217 individuals by DNA sequence analysis to obtain complete exon 2 sequences. Maximum-likelihood HLA-A-B-DR haplotypes frequencies in 11247 individuals and haplotypes constituting each genotype were estimated by the expectation maximization (EM) algorithm. HLA-A-B-DRB1*14 haplotypes were inferred according to HLA-A-B-DR haplotypes and high resolution results of HLA-DRB1*14. The probability of identifying allele matched donors for patients with DRB1*14 alleles and possible mismatch combinations in Sichuan transplantation population were determined base upon HLAï¼A-B-DRB1*14 haplotypes.
Results: 225 DRB1*14 alleles in 217 samples were determined. Only 10 of the known DRB1*14 alleles were detected, including DRB1*140101/1454(50.7%), DRB1*1402(0.4%), DRB1*1403(3.6%), DRB1*1404(12.0%), DRB1*140501(27.6%), DRB1*1406(1.8%), DRB1*140701(2.2%), DRB1*1412(0.4%), DRB1*1418(0.4%) and DRB1*1425(0.9%). The three most frequency alleles comprise about 90% of DRB1*14 positive samples. Approximately 90% of the known DRB1*14 alleles were not observed and are probably found at frequencies of less than 3% in DRB1*14 positive populations in Sichuan han population.
3607 haplotypes including 127 DR14 positive haplotypes were founded in 11247 han individuals. In 213 DRB1 heterozygous individuals, 59 different A-B-DR14 haplotypes were detected and can be splitted into 106 different A-B-DRB1 *14 haplotypes. The most frequent haplotypes were A2-B46-DRB1*140101/1454ï¼29/213ï¼‰ï¼ŒA11-B46-DRB1*140101/1454ï¼47/213ï¼‰followed by A24-B54-DRB1*140501ï¼46/213ï¼‰and A2-B60-DRB1*140101/1454ï¼6/213ï¼‰. 66 haplotypes have only one copy in the samples studied.
Patients with A2-B46-DRB1*140101/1454 haplotypes were most likely found a DRB1*14 allele matched donor in Sichuan. While patient-donor pairs shared A2-B46-DR14 or A11-B13-DR14 haplotypes, many mismatch combinations involving DRB1*140101/1454, 1404ï¼Œ140501, 1406ï¼Œ140701and 1412 may arise. DRB1*140301 and 1425 may mismatched with DRB1*140101/1454 or 140501 in different haplotypes.
Conclusions: The diversity of HLA-DRB1*14 alleles in Sichuan Han population was limited and was similar to Guangzhou han population. And extensive diversity of HLAï¼A-B-DRB1 *14 haplotypes were also demonstrated. Many mismatch combinations may be founded according to different haplotypes.

P-198

ABO-VARIANT ALLELES RESPONSIBLE FOR PHENOTYPES WITH VERY WEAK EXPRESSION OF A IN JAPANESE: MOST OF AEL ALLELES MAY BE GENERATED BY CROSSING-OVER BETWEEN O02 AND EITHER A101 OR A201 ALLELES

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Background: The ABO blood group system is one of the major blood group systems, and it is important in transfusion medicine. Since the elucidation of the molecular genetic basis of the ABO blood system, various suballeles have been reported. We have found many variant phenotypes with very weak expression of A [Ax, Ael, O(A), AxB, AelB and B(A)] in Japanese donors, and we also have analyzed the sequences of exons 6 and 7 of the alleles responsible for the variant phenotypes.
Aim: The aim of the present study is to demonstrate the variant alleles responsible for very weak-A phenotypes in Japanese and to examine the generation of three Ael alleles.
Methods: Blood samples of ABO variants with very weak-A phenotypes were selected from Japanese blood donors. Genomic DNA was prepared by the conventional method. We analyzed the sequences of exons 6 and 7 of the ABO alleles by direct sequencing.
Results: We found 153 variant samples with very weak A-phenotypes. The DNA sequence obtained from those samples demonstrated 13 kinds of variant alleles (n = 153) in Japanese. More than 90% (n = 139) of the alleles were variants with T646A, and this allele-group comprised the following four kinds of alleles: A(ProAATA), Ael-3 (n = 92); named A(LeuAACG), Ael-1 (n = 38); A(ProAACG), Ael-4 (n = 6), and A(ProACGC), Ax-1 (n = 3). The sequence from nt261 to nt526 of the Ael-3 allele was identical to that of A101, and the sequence from nt646 to nt1096 was identical to that of 002. The sequence of Ael-1 and Ael-4 between nt261 and nt1096, except nt646-681, were identical to those of A102 and A101, respectively, and the sequences between nt646 and nt681 of the two alleles were identical to that of 002.
Conclusion: This study shows that 13 kinds of ABO-variant alleles responsible for phenotypes with very weak expression of A are present in Japanese. We examined the generation of three Ael alleles characteristic for Japanese; i.e., Ael-3, Ael-1, and Ael-4. The Ael-3 allele may be generated by single crossing-over between A101 and 002 alleles, and Ael-1

and Ael-4 may be generated by double crossing over between A102 and O02, and A101 and O02, respectively.

P-200

STABLE EXPRESSION OF RECOMBINANT RHD ANTIGEN ISOLATED FROM CORD BLOOD IN K562 CELL LINE

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Background: Rh (Rhesus) is a highly complex blood group system in man deeply rooted in transfusion medicine, through implications in alloimmune transfusion reactions, hemolytic disease of the newborn, auto-immune hemolytic anemia and through the non-immune hemolytic condition associated with Rh-deficiency syndrome. The highly immunogenic Rh antigens are expressed as a part of a protein complex in the RBC membrane. This complex is a tetramer, consisting of two molecules of RhAG and two molecules of Rh proteins. To express RhD in RBC membrane, expression of RhAG is essential. This co-expression only occurs in the erythroid lineage. K562 cell line has erythroid lineage.
Aims: K562 cell line was chosen to express RhD protein in this study.
Methods: Cord blood was used as a source of RHD gene in which nucleated RBCs is rich. Mononuclear cells were isolated using Ficol method. RNA was extracted by trizol followed by cDNA synthesis. RHD gene was isolated with specific primers containing EcoR I and Not I sites. The RHD cDNA was ligated to pcDNA3.1 vector and cloned into E. coli TOP10. The recombinant pcDNA-RHD construct was transfected to K562 cell line. Stable cells expressing RhD were selected in the presence of geneticin. RT-PCR and western blot analysis were performed to detect recombinant RhD.
Results: Stable cells expressing recombinant RhD was established. RT-PCR results showed exogenous expression of recombinant RhD and further confirmed by western blot analysis.
Conclusion: Overall, our results revealed that K562 is suitable for expression of RhD. The recombinant RhD may be helpful to further investigate the molecular basis of RhD protein.

P-201

MOLECULAR CHARACTERIZATION OF ABO BLOOD GROUPS AMONG INDIANS: THE FIRST REPORT

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Background: ABO blood group system was discovered first among all other blood group systems and is clinically the most important. The ABO blood group system was first discovered by Karl Landsteiner in 1900. Four major phenotypes are derived from two antigens A and B of this system. Several polymorphisms affect the specificity of the gene product i.e. glycosyltransferase. Various lethal mutations result in the formation of blood group "O". The ABO genotyping study by DNA-based methods enables the identification of both maternally and paternally derived alleles without family study.
Aims: To characterize the common A, B and O alleles by molecular analysis in the Indian population.
Methods: One hundred samples from the mixed population and 87 samples from Parsi population were included in this study. Most of the polymorphic sites in ABO blood group system studied so far lie in exons 6 & 7 of ABO gene. The ABO genotyping of 100 samples from mixed population was first confirmed by PCR-RFLP and then a catalog was prepared for SSCP by taking the known genotypes. In PCR-RFLP method, the polymorphic sites from exons 4, 6 & 7 were amplified and then the post PCR fragment were digested with different restriction enzymes to identify the genotypes.
PCR-SSCP was then used as molecular method for ABO genotyping in other populations by referring the catalog. Three fragments from exon 6 & 7 having nine polymorphic sites (i.e. 261, 297, 467, 526, 646, 657, 681, 1059 & 1096) were amplified in PCR-SSCP. Seven common ABO alleles were identified from these nine polymorphic sites. This technique can also detect many rare alleles having the polymorphisms within the regions bounded by three primer pairs.
Result: In mixed population the A1O1, BO1, O1O1 & O1OIV were found to be the most common genotypes. In Parsi population, A1OIV, BO1 and O1OIV were found to be the most common genotypes. The A1 or A101 allele was found to be more prevalent over the AIV or A102 and A2 or A201 allele in A blood group i.e. 91.66% of the total A group in mixed population. However, in O blood group the O1 or O101 & OIV or O201 were seen to be most commonly found alleles rather than the non-deletional O2 allele in both the population. The O1 allele is 73.07% in mixed population and 78.57% in parsi population of total O group. One O variant and one A variant allele was also detected in our study.
Conclusion: In addition to serological study ABO genotyping is also a valuable complement for correct determination of ABO blood group status and to define an individual's ABO genotype without laborious family study. It is also a useful tool for resolution of typing discrepancies and correct molecular characterization of weaker variants of A & B blood groups. This is the first study of its kind reporting the detailed distribution of ABO alleles in the Indian population.

P-202

MOLECULAR CHARACTERIZATION OF ABO ALLELES IN ONE TRIBAL POPULATION FROM WESTERN INDIA

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Background: The ABO blood group system was first discovered by Karl Landsteiner in 1900. Four major phenotypes are derived from two antigens A and B of this system. The A and B blood group antigens are inherited as co-dominant characters according to mendelian rules, and their absence results in blood group O.
Indian population is comprised of non-tribal and tribal groups. There are more than 3000 distinct, strictly endogamous groups. Lot of work on distribution of ABO blood groups among non-tribal and tribal groups has been done in India. However here is the first attempt to study ABO alleles at molecular level among one tribal group from western India.
Aims: To detect the common ABO alleles by molecular analysis in one tribal population, Dhodias from Western India.
Methods: Hundred and one samples from the Dhodia tribal population were included in this study. Molecular methods i.e. PCR-RFLP and PCR-SSCP were used for genotyping.
In PCR-RFLP the polymorphic sites from exon 4, 6 & 7 were amplified and then digested by different restriction endonucleases. The nucleotide sites for restriction enzyme digestion were 188/189, 261, 467, 526, 703 and 1096. The post PCR fragments were then run on a 10% PAGE and viewed under UV trans illuminator after staining with ethidium bromide. In PCR-SSCP three fragments (279bp, 192bp & 165bp) covering exons 6 & 7 were amplified. The ABO genotyping based on PCR-SSCP analysis can discriminate 7 common ABO alleles (A1, AIV, A2, B, O1, OIV & O2) defined by using nine polymorphic sites in a single lane format. This technique can also detect many rare alleles having the polymorphisms within the regions bounded by three primer pairs.
Result: In Dhodia tribe the AO1, BO1, O1O1 & O1OIV were found to be the most common alleles in A, B & O blood groups. The A1 or A101 allele was found to be prevalent over the AIV or A102 and A2 or A201 allele in A blood group i.e 95.65% of the total A group. However, in O blood group, the deletional alleles i.e. O1 or O101 & OIV or O201 were more common alleles than the non-deletional O2 allele. The O1 allele is 79.41% in this tribal population.
Conclusion: This is the first study of its kind reporting the detailed distribution of ABO alleles at molecular level in the Indian tribal population.

6.1. Clinical Transfusion Maternal health and blood transfusion including pediatric transfusion

P-203

THROMBOPHILIAS AND PREGNANCY COMPLICATIONS

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Aims: Determination of thrombophilia and evaluation of maternal and fetal outcomes in women with history of pregnancy complications.
Material and methods: A total of 35 patients with fetal loss syndrome, 11 patients with preeclampsia, 14 with venous thromboembolism (VTE), 9 patients with placental abruption and 30 healthy controls were tested.
Results: Thrombophilia was found in 82% patients with fetal loss syndrome, 96% patients with recurent preeclampsia and in 100% patients with placental abruption and VTE.
Anticardiolipin antibodies were fond in 29,6% women with fetal loss syndrome, 14% in women with preeclampsia, 26,2% in women with placental abruption and VTE.
FV Leiden were found in13%women with fetal loss syndrome, 15% in women with preeclampsia, 35% in women with placental abruption and VTE. In control group 4%, 1,7%. Women with history of pregnancy complications received treatment in the preconception period and during pregnancy including low molecular heparin, aspirin in low doses, antioxidants, vitamin B, folic acid. Patients had no recurrence of placental abruption or VTE, nobody had a severe preeclampsia, 91% patients with fetal loss syndrome delivered after 37 g.w.
Conclusion: Thrombophilia might be the main pathogenic mechanism of recurent pregnancy complications. Preconception treatment with low molecular weight heparin, antioxidants, low doses of aspirin and vitamins allows preventing recurrent pregnancy complication and fetal loss in most cases.

6.2. Clinical Transfusion (Therapeutic) aphaeresis

P-204

SPORADIC (IDOPATHIC) TYPE THROMBOTIC THROMBOCYTOPENIC PURPURA DOES NOT SHOW TYPICAL PENTAD FEATURES AND RESPONDS POORLY TO PLASMA EXCHANGE

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Background: Observations of the presenting features and clinical course of TTP suggested a pentad of clinical features for diagnosis: thrombocytopenia, microangiopathic hemolytic anemia, neurologic and renal abnormalities, and fever. Now only thrombocytopenia and microangiopathic hemolytic anemia, without another apparent etiology, are sufficient criteria to establish a clinical diagnosis and begin treatment. A severe deficiency of ADAMTS13 (a disintegrin-like and metalloprotease with thrombospondin type 1 repeats) as detected by current assays, less than 5% of normal activity, may be specific for TTP. ADAMTS13 deficiency caused by an autoantibody provides a possible explanation for the effectiveness of plasma exchange (removal of the autoantibody by apheresis; supply of ADAMTS13 by plasma replacement), a role for ADAMTS13 activity measurements to guide treatment decisions has been suggested. However, the sensitivity of severe ADAMTS13 deficiency in patients with idiopathic or sporadic TTP can be clinically suspected as they respond poorly to plasma exchange with persistant high LDH level and very low platelet count and acute relapses during plasma exchange for identifying all patients who have an appropriate clinical diagnosis of TTP Aims: To evaluate the success and effectiveness of TPE in TTP. and do the follow up study on patients with poorly responds to TPE and their further management.
Methods: A total number of ten consecutive patients with provisional diagnosis of TTP admitted between 01.01.2007 to 30.06.2008 and referred to blood bank National Hospital Sri Lanka for TPE were retrospectively analyzed. Single volume daily plasma exchange done using cryo poor plasma as a replacement fluid in addition to immune suppression therapy with methyl prednisone and TPEE for each patient was continued for a minimum two days after complete remission of the desirable end point in platelet count and lactate De-Hydragenase levels were taken as parameters and then tapered off. The treatment out come, prognosis, number of plasma exchange and ADAMS 13 level in patients with poorly respond were done.
Results: Number of patients under gone TPE was 10. Two patients who responded poorly with acute relapses during plasma exchange had 23 and 40 TPE cycles respectively and other eight patients were ended up with less than 15 cycles with median of 10 cycles. ADAMTS 13 activity of theses two patients were less than 5 % and those two patents were started with Retuximab 375 mg/m^2 slow iv infusion quarter weekly for 4 weeks until the ADAMTS 13 activity reaches more than 80 %.
Conclusion: The effectiveness of plasma exchange has been attributed to the removal of ADAMTS 13 autoantibodies and replacement of ADAMTS 13 activity and however, plasma exchange also seems to be effective for patients who do not have a severe deficiency of ADAMTS 13 activity. A severe ADAMTS13 deficiency can be clinically suspected as they respond poorly to plasma exchange even though there is no facilities to detect ADAMTS 13 activity and a role for ADAMTS13 activity measurements has been suggested to guide the treatment decisions and long term follow up for complete remission is essential.

P-205

THE CONDITIONING PLASMA EXCHANGE FOR ABO-INCOMPATIBLE RENAL TRANSPLANTATION

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Background: According to the 2006 annual report from the Japanese Society for Clinical Renal Transplantation, a total of 1136 transplants including 923 cases with living donors were performed. Among these, 215 transplants (23.5%) were from ABO-incompatible donors, which continue to grow in number. Although it must be critical to efficiently reduce anti-A or B antibody titers before surgery for ABO-incompatible organ transplantation, a standard protocol for the conditioning plasma exchange (PE) has not been established.
Aims: To evaluate the efficacy and safety of the preoperative PE as well as to standardize the conditioning PE protocol, we retrospectively analyzed the data from ABO-incompatible transplants that had been performed in our institution between January 2005 and May 2009.
Methods: Anti-A or B antibody titers were measured by the agglutination assay using Micro Tiping System (Olympus). The conditioning PE was scheduled for one to two weeks before surgery based on the biginning levels of anti-A or B titers. By using a COBE Spectra (Gambro BCT), 1 to 1.5x plasma volume was removed per treatment replacing with 100% volume of either 4% albumin solution or ABO-matched fresh frozen plasma. Rituximab (100–500 mg/body) was administrated a week before surgery.
Results: A total of 25 ABO-incompatible transplants were analyzed. At the beginning of apheresis, the median titers of IgG and IgM antibodies were 64 (8~1024) and 24 (1~1024), respectively. The conditioning PE was performed on average 3.6 (2~8) times per patient. Transplants were suspended in 5 (20%) patients on account of sustained high titers of antibodies (>128) after a series of PE. Twenty (80%) patients achieved target titers less than 32 and received transplants just as planned. Among them, 14 (70%) patients had no episode of humoral rejection. The sign of rejection was observed in 6 (30%) patients, who were treated with a methylprednisolone pulse therapy and/or additional PE. Five (83%) patients were successfully rescued, but 1 (17%) with severe bacterial infection lost the graft. The complications associated with PE were minimal.
Conclusions: The conditioning PE therapy was performed safely, however 20% of patients were non-responder for PE. Thus, the more intensive PE schedule or the improved combination therapy especially concerning rituximab dosage or timing should be considered.

P-206

A COMPARATIVE STUDY FOR 'DOUBLE RBC' PHLEBOTOMY AND WHOLE BLOOD PHLEBOTOMY IN PATIENTS WITH ERYTHROCYTOSIS

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Background: Phlebotomy is a widely used treatment modality for erythrocytosis. Recently erythrocytapheresis has been introduced for phlebotomy. ALYX (Fenwal, DeerWeld, IL, USA) component collection system has been used for collecting double red blood cell (RBC) units from donors. We have employed this system for phlebotomy to treat the patients suffered from erythrocytosis.
Aims: The aim of this study was to evaluate the effectiveness of 'double RBC' phlebotomy (DRP) using ALYX in patients with erythrocytosis.
Methods: We performed DRP using ALYX to remove RBC mass from total of 20 patients with erythrocytosis. The effectiveness of this treatment was compared with 'whole blood' phlebotomy (WBP) which was performed for 87 patients. For 14 patients among them, we switched the procedure to DRP. The removed volume was 360–420 mL of RBCs in DRP and 400–600

mL of whole blood in WBP. Hematologic paramenters including hematocrit were measured before and after phlebotomies.
Results: The average delta hematocrit for each phlebotomy was 7.2 ± 2.5% in DRP and 3.2 ± 1.5% in WBP, and the difference was statistically significant ($P < 0.0001$). The average treatment intervals were 103.9 ± 70.9 days in DRP and 37.4 ± 22.5 days in WBP ($P < 0.0001$). Adverse reactions occurred in 6 patients (30%) during DRP and were limited to mild citrate toxicity.
Summary/Conclusions: The data showed that DRP was superior to WBP in terms of decreasing more RBC mass and reducing the frequency of hospital visit. DRP was a safe and effective technique for treatment of erythrocytosis.

6.3. Clinical Transfusion Evidence based transfusion medicine

P-207

THE STUDY OF DAMAGE OF THE RED BLOOD CELLS IRRADIATED BY G-RAYS IN DIFFERENT DOSES

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Aims: To study the changes of ultrastructure of the red blood cells(RBC) and the concentration change of electrolyte in plasma irradiated by different doses of γ-rays.
Methods: The blood samples were irradiated by γ-rays at different doses and stored at different time. Then, the RBC were fixed by 3% glutaraldehyde and 1% osmic acid, washed, dehydrated, freeze-dryed and plated with metal aurum ions. Next, we use scanning electron microscope to observe the RBC morphology. The same, we prepare the sample for transmission electron microscope by the following way: permeating and embedding with low viscosity embedding media spurr's kit, slicing and dyeing. Then, using transmission electron microscope to observe the RBC morphology. At the same time, the concentration of Na+, K+ and Cl- ions in the plasma and pH were detected before and after radiation respectively.
Result: The percentages of abnormal cells, for example, serrated RBC, thorn RBC and star RBC increased with increasing irradiation doses and storage time. And the normal biconcave disc-faced RBC reduced. The result showed that the concentration change of K+ ion is more significant than that of Na+ and Cl- ions after irradiation. A certian extent difference was found in pH with a dose of radiation and 4' storage condition.
Conclusion: The ultrastructure of RBC don't change obviously after irradiation by a certain extent doses of γ-rays and storage at a certain time. However, it will has some adverse effect on the RBC in a manner of irradiation dose-dependent and time-dependent.

P-208

THE STRATEGY OF PERIOPERATIVE BLOOD TRANSFUSION FOR CARDIOVASCULAR SURGERY

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Background: Although autologous transfusion is widely spreading in cardiovascular surgery which needs not to prepare allogeneic blood, allogeneic transfusion is still necessary in emergency operation due to consumptive coagulopathy and excessive bleeding. We investigated the present situation how much autologous and/or allogeneic blood is used to determine the appropriate preparation of perioperative transfusion in cardiovascular surgery.
Materials and methods: In our institution, 1457 cases of cardiovascular surgery were performed from September 2003 to June 2009. The usage of allogeneic and autologous transfusion were investigated in the following groups : (1) on pump coronary artery bypass grafting (CABG), (2) off-pump CABG (OPCAB), (3) valve replacement or plasty (VALVE), (4) graft replacement for thoracic aortic aneurysm (TAA), (5) graft replacement for acute aortic dissection (AAD), (6) graft replacement for abdominal aortic aneurysm (AAA), (7) ruptured- AAA. We also analyzed the appropriate dosage of blood transfusion including surgical blood order equation (SBOE) and a maximum surgical blood order schedule (MSBOS), which is efficient for blood order practice.
Results: The usage of red cells concentrates (RCC) was below 1.0 unit (140 ml) and the evasion rate of allogeneic transfusion was above 90% in CABG, OPCAB, VALVE and AAA. In these operations, the ratio of preoperative autologous blood donation (PABD) was below 1%, but that of acute normovolemic hemodilution (ANH) was above 80% except OPCAB and AAA. Especially, more than 80% of OPCAB and AAA were performed only with intraoperative blood collection (IBC) or without any blood transfusion including IBC. For TAA, the ratio of PABD and ANH was 50% and 84%, respectively, and the evasion rate of allogeneic transfusion was 81%. Usage of allogeneic transfusion in autologous group was significantly less as comparing with that of non autologous group ($P < 0.01$). AAD required 6.3 units of RCC, 11.9 units of fresh frozen plasma (FFP) and 15.9 units of platelet concentrate (PC). The ratio of allogeneic transfusion for AAD was 83%. Ruptured-AAA required 7.2 units of RCC, 10.0 units of FFP, 6.8 units of PC, and the ratio of allogeneic transfusion was 80%. The mean value of plasma hemoglobin level at the end of surgical operation was 9.9~10.9g/dl in each group. This conformed to guidelines for the use of blood products by the Japanese Health, Welfare and Labor Ministry. In the examination of SBOE, crossmatch to transfusion ratio (C/T) by using SBOE was below 1.0, inappropriate for AAD and ruptured-AAA. On the other hand, as for the MSBOS, C/T of that was 1.30 for AAD, 1.32 for ruptured-AAA. Both of which were within the ideal limit of less than 1.5, indicating appropriate blood usage.
Conclusion: We suggest the following transfusion strategy for cardiovascular surgery in our institution. For CABG and VALVE, only the preparation of ANH and IBC should be prepared. For OPCAB and AAA, only IBC is prepared. For TAA, it's possible to evade allogeneic transfusion with PABD and ANH. In the case of unsuitable for autologous blood collection, allogeneic blood is prepared. For AAD and ruptured-AAA, preparation would be done in accordance with MSBOS.

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CURRENT STATUS OF THE TYPE AND SCREEN SYSTEM IN AICHI MEDICAL UNIVERSITY HOSPITAL

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Background: The type and screen (T&S) system was initially introduced by Boral et al (Transfusion, 17:163–168, 1977) in the USA and became generally accepted in Japan in the mid-1980s. Having used this system for over a decade, we now overview its current status in our hospital. T&S has been applied to patients of elective surgery under the following conditions: (1) clear identification of ABO blood type, (2) positive Rh(D), (3) negative irregular antibodies, (4) possibility of blood transfusion below 30%, and (5) expected amount of bleeding under 600 ml. We reviewed T&S cases with specific reference to blood transfusion and bleeding.
Methods: Among cases of elective surgical operations performed during 2007, we examined the number of T&S cases, the proportions requiring blood transfusion, and the amount of bleeding.
Results: There were 5 494 cases of elective surgery, among which application for blood transfusion was accepted in 1 180 cases (21.5%). From these, 991 orders (84.0%) for T&S system were received. Twenty eight cases were excluded from analysis because of unmeasurable bleeding amounts due to contamination of urine, ascites, or pleural effusion, and 963 cases were subject for analysis. The majority (922 cases, 95.7%) did not require blood transfusion, and the average bleeding was 319 ml. Forty one cases (4.3%) received blood transfusion with 1 676 ml of bleeding on average. From the viewpoint of bleeding, the volume was below 600 ml in 772 cases (80.2%) and above it in 191 cases (19.8%). Most of the former cases (762, 98.7%) received no blood transfusion; however, ten cases (1.3%) required it. Average haemoglobin levels of the two subgroups were 12.7 g/dL and 9.9g/dL respectively, with a statistically significant difference ($P < 0.01$). Among the latter group, transfusion was performed in 31 cases (16.2%) but deemed unnecessary in 160 cases (83.8%). There was no significant difference in haemglobin levels between the two subgroups. Out of 963 T&S cases, autologous blood (average 800ml) was preserved in 228 gynecological cases (23.8%). Bleeding was below 600ml in 155 cases (68.0%) and above it in 73 cases (32.0%). One case of the former and two cases of the

latter group, totaling 1.3%, required additional allogeneic blood transfusion.

Conclusions: Among the cases ordered for T&S, only 4.3% involved blood transfusion, showing clearly that the application of the T&S system was reasonable, and that it saved work and time in cross-matching. On the other hand, taking into consideration the possibility of blood transfusion of below 30%, we can include other operations into the T&S system by reviewing its current status. Secondly, among those cases with bleeding of under 600ml, 10 needed blood transfusion. Their preoperational haemoglobin levels were significantly lower than the other group. Therefore, preoperational haemoglobin levels should be checked for cases using T&S. Thirdly, among the 228 cases with autologous blood preservation, additional allogeneic blood transfusion was performed in only three cases (1.3%). Assuming patients undergo ABO blood type and irregular antiobody screening, the T&S sytem is unnecessary for these types of operation.

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PREVALENCE OF BLOOD SCREENING MARKERS IN BLOOD DONOR POPULATION OF PAKISTAN AT HUSAINI HAEMATOLOGY AND ONCOLOGY TRUST.KHI. PAKISTAN

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Background: Blood transfusion services in Pakistan are not centralized. There are regulatory authorities present but there are no set standards implemented with monitoring and evaluation data. Blood Screening is a vital element and a backbone of safe blood supply. Transfusion Transmitted infections are a serious public health concern. Voluntary blood donors are considered to be the safest. The recruitment of blood donor and safest screening possible eliminates most of the risk of transfusion-transmitted infections.

Aims and objectives: The study was done from Jan to Dec 2008 at Husaini Haematology and Oncology Trust to find out the blood screening statistics (malarial parasite, HIV, HEP B SURF ANTIGEN, ANTI HCV, HIV ANTIBODY) of the blood donor population and frequency of voluntary non-renumerated blood donation.

Material and methods: The blood donor's history and screening markers data was evaluated on oracle database. The blood screening for Hep B surface Antigen and ANTI HCV and HIV Ag/Ab Combo and was done on ARCHITECT chemiluminescent micro particle immunoassay (CMIA) BY ABBOT. RPR Carbon, Non Treponemal test, screened syphilis for syphilis based on luetic regin detection and flocculation on slide. Malarial Parasite was screened by the Gold Standard, i.e, the microscopic examination of blood films.

Results: JAN TO DEC 2008

TOTAL NO OF BLOOD DONORS	98012
NO OF FAMILY REPLACEMENT DONORS	79380
NO OF VOLUNTARY NON RENUMERATED DONORS	18632
NO OF SCREENING NEGATIVE DONORS	87336
NO OF Hep B Surface Antigen positive samples	4119
NO OF ANTI HCV positive samples	5643
NO OF SYPHILIS RPR positive samples	896
NO OF SAMPLES POSITIVE FOR MP	6
NO OF SAMPLES POSITIVE FOR HIV	11

FREQUENCIES:

Voluntary blood donation is found to be nearly	20 %
Hep B surface antigen prevalence nearly	4%
Anti HCV prevalence	6%
Syphilis (RPR), frequency nearly	1%
Malaria Prevalence	0.00612%
HIV Prevalence	0.0112%

Fig 1: Karachi in Pakistan

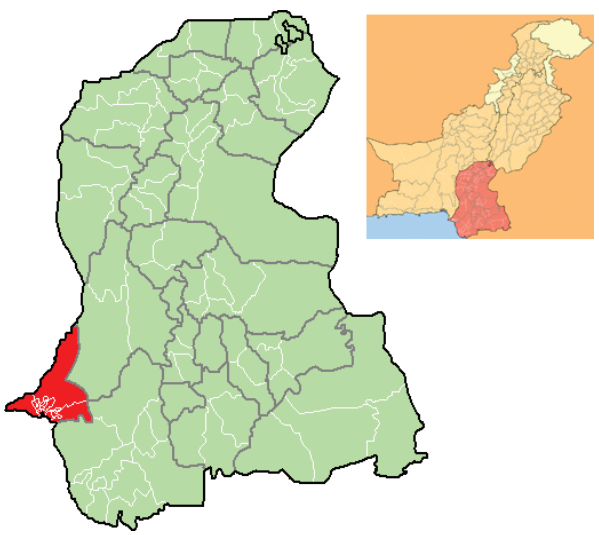
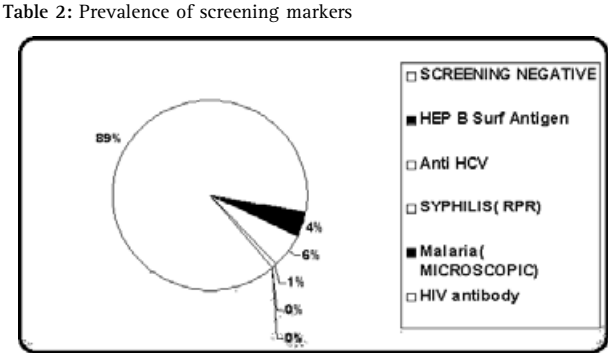


Table 2: Prevalence of screening markers



6.4. Clinical Transfusion Patient safety

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INTERACTION BETWEEN SERUM FERRITIN LEVELS AND OXIDATIVE STRESS IN MYELODYSPLASTIC SYNDROMES; EFFECTS OF ORAL IRON CHELATION

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Introduction: Oxidative stress induced by either high levels of iron or cytokines is considered as a modulator for pathophysiology of myelodysplastic syndromes (MDS). Increased cellular oxidative stress in red blood cells or platelets is also reported in MDS patients. The etiology of high levels of iron might be related to increased absorption and blood transfusion. We have studied about the relationship between iron levels and oxidative stress.
Methods: Eighty-three normal volunteers and 25 patients with MDS were nominated after obtaining written informed consents. Oxidative stress (dROM) was detected by FRAS 4 instrument (Wismerll) which measures serum peroxide levels using chromogenic procedure. Within-run precision study revealed the coefficients of variation as 6.9 % for a healthy volunteer. Complete blood count, and serum ferritin levels were also studied in clinical laboratories.
Results: The levels of dROM significantly correlated with age and LDL-cholesterol levels in the volunteers. No apparent relationship between dROM and BMI or dROM and hemoglobin was detected. In the patient group, dROM correlated with ferritin levels ($P = 0.011$) and negatively correlated with hemoglobin levels ($P = 0.036$). In one of the patients with MDS (75 yo, F, RCMD) who was treated with deferasirox (Exjade), dROM decreased in accordance with the decrease in ferritin levels. In this patient, transfusion requirement was not altered.
Discussion: Measurement of oxidative stress by FRAS 4 seems to be valuable to monitor the effects of iron chelation, and iron chelation might be necessary to reduce oxidative stress for the patients with high levels of ferritin. It will be necessary to study the clinical meanings of the evaluation of oxidative stress in MDS patients in combination with the factors interacting with iron metabolism including hepcidin and growth differentiation factor 15, and to establish an easier and more reliable method to detect oxidative stress clinically.

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THE ASSOCIATION OF FXIII VAL34LEU POLYMORPHISM WITH THROMBOTIC EVENTS IN PATIENTS REFERRING TO IRANIAN BLOOD TRANSFUSION ORGANIZATION

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Background and objectives: Replacement of Val34Leu polymorphism in subunit A of coagulation factor XIII results in the replacement of Valine with Leucine in amino acid 34. As a result of this substitution, FXIII Val34Leu polymorphism acts as a factor for individual protection against thrombosis (1). For the first time in Iran, the prevalence of this polymorphism in patients with thrombotic events and in healthy individuals was determined and studied.
Materials and methods: The study was performed as a retrospective case-control one.A total of 200 referral patients with thrombotic complications were admitted to IBTO Thrombosis and hemostasis Laboratory. One hundred healthy individuals were studied as control group. Their DNA was extracted using Qiagene kit. Using Polymerase Chain Reaction (PCR) and RFLP methods in the presence of restriction enzyme Cfo1, genotypes of FXIII Val34Leu polymorphism were identified. Statistical analysis was performed by SPSS software version 11.5 and confidence coefficient was 95%.
Results: The prevalence of FXIII Val34Leu polymorphism in patients was 23 % in patients and 36 % in healthy individuals. The allele frequencies of leucine in cases and controls were 13 % and 20 % respectively. These results showed significant differences between the two groups.
Conclusions: The present study demonstrates the association between FXIII Val34Leu polymorphism and protection against thrombotic disorders. The higher frequency of Leu allele and Val34Leu genotype in controls than in patients confirmed the results.

P-213

JAPAN'S GUIDELINES FOR RELIGIOUS OBJECTION TO BLOOD TRANSFUSION

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Background: Patients, or their legal guardians, may object to transfusion for religious belief. Although competent adults have the right to refuse blood transfusion, laws or judicial precedents have not totally established whether parents have the right to refuse necessary medical care and transfusion for their children. The United Nations Children's Fund found that almost 3500 children younger than 15 years die every year from child abuse and neglect in 27 developed countries.
Methods: The Japanese Joint Committee discussed and clearly defined when to accommodate the wishes of competent patients and when to protect younger children in situations of objection to blood transfusion.
Results: For Patients 18 years or Older and Competent to Make Decisions: The patient shall sign and submit a Certificate of Refusal of Blood Transfusion and Exemption From Liability. If medical provider cannot treat without transfusion, the provider shall recommend transfer to the care of another. For Patients Younger Than 15 Years or Not Competent to Make Decisions and A Guardian Objects: A) Medical provider shall report the situation as a case of child abuse to governing authority. The authority shall place the child under protective custody and petition a family court to suspend the rights of guardianship. Transfusion shall proceed with the consent of a court-appointed guardian. B) When one legal guardian consents to transfusion but the other objects, the provider shall administer blood transfusion with the consent of the guardian who consents. For Patients Between 15 and 17 Years Old and Competent to Make Decisions and A Guardian Objects: C) When the patient wishes to receive transfusion, the patient's informed consent shall be documented. D) When a legal guardian approves but the patient objects, the medical provider may transfuse according to medical necessity. The guardian's informed consent shall be documented. E) When all legal guardians and the patient object to blood transfusion, guidelines for patients 18 years or older shall apply.
Conclusion: Some parents/guardians may seek healing through religion rather than medical care. Medical neglect evaluations should focus on the child's needs rather than the caregiver's motivations or justifications. Religious objections should not be granted fundamentally different status from other types of objections. Health care providers should offer the best possible care that is consistent with a patient's age and competency.

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THE CONTRIBUTION OF INDIVIDUAL DONOR NUCLEIC ACID TESTING (ID-NAT) IN BLOOD TRANSFUSION SAFETY OF APOLLO GROUP OF HOSPITALS IN INDIA

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Background: Despite improvements in HIV, HCV and HBV serological tests in recent years, instances of viral transmission via transfusion

continue to occur because of preseroconversion window phase donation, immunovariant and nonseroconverting chronic carrier. Direct and sensitive detection of viral nucleic acid (NAT) could substantially decrease the incidence of transfusion-transmitted infections.
Aims: To analyze the effectiveness of NAT on the safety of our blood supply.
Method: Indraprastha Apollo Hospitals evaluated NAT with 12,224 samples using the Procleix Ultrio Assay (Chiron Corporation, Emeryville, CA) on semi-automated eSAS System in individual donation testing format (ID-NAT) in 2004–2005 and started routine ID-NAT screening from April, 2006. With the successful implementation of this technology, routine ID-NAT was also implemented in Apollo Hospitals Chennai in January 2008, which is a centralized NAT screening facility supporting Apollo Specialty Hospital and Apollo Hospital Bangalore. Till April 2009 Indraprastha Apollo Hospitals, New Delhi and Apollo Hospitals, Chennai have tested 59515 and 24,530 blood donors samples respectively. The Ultrio is a multiplex, highly sensitive NAT screening assay for simultaneous detection of HIV-1 RNA, HCV RNA and HBV DNA, in a single reaction tube. Reactive samples were discriminated tested using the same platform.
Results: During the evaluation phase, 12 224 unlinked donor samples from eight different blood banks were NAT tested, and eight yield cases were identified: 1 HIV-1, 1 HIV-1/HCV co-infection, and 6 HBV. The overall observed NAT yield was 1 in 1 528. Among 59 515 routine samples tested up to April 2009, 14 NAT yield cases were identified: 9 HBV, 3 HCV, 1 HBV/HCV co-infection, 1 HIV-1/HBV co-infection. The observed NAT yield during this routine screening phase for 3 viruses was 1 in 4 251. 24 530 routine samples were NAT tested at the Apollo Hospitals Chennai, and 9 HBV NAT yields cases were found. The observed NAT yield rate at this site was 1 in 2 726. Combining all 3 96269 donations have been tested, and the ID-NAT yield rate in our hospital group was 1 in 3 097. More importantly, since the implementation of ID-NAT, there have been no reported cases of transfusion-transmitted viral infections.
Conclusion: A total of 23 positive infectious donations since implementation of routine ID-NAT were detected and intercepted, leading to the prevention of approximately 46 cases of potential infections through different blood components. Experiences of other countries like Hong Kong, Singapore and South Africa confirm that ID-NAT is the best practice in preventing TTIs and maximizes the blood safety. Implementation of routine ID-NAT has not only improved the blood safety of Apollo Group of Hospitals, but also increased confidence in our blood supply amongst clinicians, patients, the general public and our blood bank staff.

P-215

THE EXPERIENCE ON USING BBI PERFORMANCE PANEL AND NIBSC LOW TITER WORKING STANDARD TO MONITOR THE VIRAL MARKER DETECTION KITS FOR BLOOD SCREEN IN KAOHSIUNG BLOOD CENTER IN TAIWAN

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Background: There has been performed four viral marks screen since 1996 for blood safety in Taiwan. They are HBsAg, Anti-HCV, Anti-HIV and Anti-HTLV. Hepatitis B is high prevalence in this area, so we don't perform Anti-HBc testing on blood screen. Now, we use Abbott/Murex ELISA kits on Tecan RMP processor since 2003. Even Though vendor say all released kits had been validated by QC/QA department, but we still concern the quality and the condition of shipping process from warehouse to customer.
Aims: For quality issue, we use BBI performance panels to validate those kits each lot on HBsAg, Anti-HCV, Anti-HIV and use NIBSC working standards to monitor the routine tests each run, too since 2003.
Methods: When use BBI performance panel and NIBSC standard serum to validate kits, the acceptance criteria are as follow: (1) Pass the performance panel test, (2) The result of PC and NC are in range which showed on COA, (3) The NIBSC working standard results are all positive when performed 10 times and (4) 200 negative samples should still keep negative result (the new criteria that the mean S/Co of 440 negative sample should below 0.7 since 2009). The acceptance criteria of the results of routine test are not only internal QC samples which provide in kit but also NIBSC working standard which extra added by us should pass.
Results: A total of 38 HBsAg lots and 40 Anti-HIV lots were passed in initial validation. Unfortunately, 3 Anti-HCV lots were failed in 42 lots. They were failed in BBI panel testing due to negative samples shift to positive result. We investigated the original sample which showed IND in RIBA test. We revised acceptance criteria and added more negative samples up to 440 to avoid poor specificity since 2009. In addition 2 anti-HCV lots were poor NC and PC result when started using in routine which had been approved in initial validation. We found a lot showed high NC result and found some fungus in NC sample bottle which had been verified by vendor later. The other lot showed poor PC and failed in NIBSC working standard particularly. They were presumed that those kits were stock in a high temperature environment in shipping process by vendor. We returned those 2 lots about 685 kits to vendor.
Conclusions: It may a reliable method to validate the kit by BBI performance panel for kit quality. But the validate result can't ensure kit when degenerate in routine use. It could be improved by extra low titer QC sample to monitor the routine process.

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AN ANALYSIS OF TRANSFUSION ERRORS IN A TERTIARY CARE HOSPITAL IN MALAYSIA

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Background: Human error remains a potential source of transfusion-related adverse effects. Incorrect blood components transfused is still the most common cause of transfusion related morbidity and mortality in most countries. This study was designed to investigate the sources and types of transfusion errors within a large tertiary hospital in an effort to understand its occurrence and reduce its incidence.
Methods: All transfusion related errors from 2006 to 2008 were recorded, investigated and analyzed using a standard reporting and investigation format.
Results: Eighty-six transfusion related errors were reported. 58.2% (50/86) of the errors originated from the wards and 25.6% (19/86) occurred within the transfusion laboratory. Thirteen (15.2%) errors were identified retrospectively when there was a discrepancy in blood groups between the current sample and historical records. One error originated from the microbiology testing laboratory while in another 3 cases, the cause and origin of error could not be identified.
Ninety three percent (80/86) of the errors were related to ABO grouping. The causes of the error were attributable to sample mislabeling (47), patient misidentification (5), testing errors (10), data entry errors (2), and mistakes in historical sample results (13). In 3 cases the cause could not be identified. Eighty percent (64/80) of these cases were near-miss events which were intercepted on review of historical patient records or checking of blood bags before blood transfusion.
Other events recorded unrelated to ABO grouping included registration of a patient under a wrong name almost resulting in a wrong transfusion, 4 blood packs being mislabeled by the component laboratory, a sero-positive blood unit reported as sero-negative, lack of monitoring of blood refrigerator temperature and a wrong sample used for compatibility testing resulting in a transfusion reaction.
In errors relating to mislabeling of samples and misidentification of patients, house officers accounted for 37 incidents and nurses 5. Mislabeling resulted when sample tubes were labeled by someone other than the phlebotomist, usually the nurse or when labeling was performed away from the patient. Errors in the transfusion laboratory occurred during manual and transcription processes. The errors were due to incorrect data entry (2), mistakes during manual blood grouping and compatibility testing (9), mislabeling of patient sample tubes (3) and blood packs (4) and lack of monitoring of equipment (1).

Five incidents of ABO incompatible transfusions occurred, 3 of which were from errors during blood sampling and 2 from errors in manual grouping and compatibility testing. None were fatal. Over the same period of three years, 114 915 compatibility tests were performed with 139 692 blood components transfused of which 74 337 were red cell units. The overall error rate was calculated at 6.2 per 10 000 blood components transfused.
Conclusion: Transfusion errors continue to be mainly caused by mistakes in patient identification and sampling. The ready availability of past patient records has been successful in intercepting these errors. Better patient identification processes need to be devised. In the laboratory, manual processes and transcription processes contribute to errors. The increased use of informatics and automation should address this problem.

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NAT EXPERIENCE AT THE ALL INDIA INSTITUTE OF MEDICAL SCIENCES (AIIMS), NEW DELHI-INDIA

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Background: Detection of viral nucleic acid using NAT technology is more sensitive in identification of infectious blood donated at the early stage of infection than antibody or antigen detection. The All India Institute of Medical Sciences (AIIMS) in New Delhi, India, took the initiative as the first in the country for NAT implementation in a government setting. In India, of the 5.59 millions annual donations, 55% are voluntary and 45% are replacement. The seroprevalence of HIV, HCV and HBV in general population is 0.36%, 0.9% and 2.5–4.0%, where among blood donors the prevalence is 0.3%, 0.7% and 1.4%, respectively.
Aim: To identify the NAT yield cases among our donor population using individual donation testing (ID-NAT) format.
Methods: The Procleix Ultrio Assay (Chiron-Novartis, Emeryville, CA) using TMA technology for simultaneous detection of HIV-1 RNA, HCV RNA, and HBV DNA, was implemented on the semi-automated Procleix eSAS system for routine blood screening. Initial reactive specimens were retested, and repeat reactive specimens were further tested with the discriminatory assays using the same platform to determine viral specific reactivity.Results of serologic tests were collected and compared with NAT results. Specimens with discordant results between NAT and serology were further evaluated with confirmatory serology and alternative NAT.
Results: By May 2009, a total of 5,818 linked blood donor specimens were tested in ID-NAT format. Of these, 107 (1.84%) were initially reactive and 76 (1.31%) were repeat reactive. After discriminatory testing, 70 specimens demonstrated viral specific reactivities. Of these 70, 65 specimens were also reactive in serologic testing, while the remaining 5 were seronegative (4 HBV with one also positive for anti-HCV, and one HCV) for a potential overall NAT yield rate of 1 in 1,164. No HIV-1 yield was identified. One of the 4 HBV yield specimens was positive for ant-HBc indicating occult hepatitis B infection (OBI), while the remaining 3 re were considered window period (WP) infections.
Conclusions: Implementation of Ultrio assay with semi-automated eSAS system for individual donation NAT testing is feasible in our laboratory. Identification of seronegative infectious donations were demonstrated among our donor population, particularly for HBV where the yield rate was found to be as high as 1 in 1,455. Our experience proves the value of routine NAT blood screening in enhancing the safety of our blood supply.

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REPORTED CASES OF TRANSFUSION-TRANSMITTED INFECTION IN KOREA JANUARY 2006 TO JUNE 2009

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Background: A report of a suspected transfusion-transmitted infection was submitted to a public health center by the clinic or hospital when a blood recipient asked them to report that they suspected his or her medical condition was the result of a viral infection or bacterial infection by blood transfusion, or the staff of a clinic or hospital recognized the suspected cases. The investigation was conducted by KCDC immediately after the report was received from public health center.
Aims: The purpose of this study is to evaluate the results of the investigations of suspected transfusion-transmitted infection occurred in Korea from January 2006 to June 2009.
Method: The investigation on the cases of suspected transmission of viral or bacterial infection by blood transfusion comprised of 5 steps:
- Investigating the records in relation to the transfusion and blood test of recipients,
- Investigating the donor's donation history and the results of donated blood test,
- Investigating the medical record of both the donor and the recipient,
- Testing blood donor samples,
- Visiting the donor and collecting blood from the donor and testing their blood.

Results: In total 105 cases of suspected cases were reported from January 2006 to June 2009: 52 cases in 2006, 28 cases in 2007, 16 cases in 2008 and 9 cases from January to June 2009. And there were 8 cases of HBV, 83 cases of HCV, 8 cases of HIV, 5 cases of syphilis and one case of malaria in the reported cases. Investigations were completed on 91 reported cases (86.7%) among them. According to the results of investigation, 4 cases (4.4%) of 91 were a transfusion-transmitted viral infection; one hepatitis B infection and 3 hepatitis C infection. Fifty-five cases? infections (60.4%) were not related to transfusion and for 32 cases (35.2%) it was impossible to finish the investigations. The investigations were not completed due to the following reasons: 6 cases of transfusion occurred prior to the introduction of blood screening assays, 1 case was related to an unknown number of the donor's blood, 25 cases were due to unknown identification number of donor or the rejection of donor's blood collection.
The period of transfusion of transfusion-transmitted viral infection was before 2004: 1 case in 1991, 2 cases in 2000, and 1 case in 2001. Two cases were related to blood product of packed red cell and 2 cases, leukocyte-reduced platelet concentrate.
Summary and conclusions: The goal of the investigation on suspected transfusion-transmitted infection is identification and preventing additional transfusion-transmitted infection by same donor?s blood. Considering about the verified viral infection by transfusion was before 2004, we supposed that blood safety is improved more and more by reinforcement of the blood donor history questionnaire and introduction of NAT.

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RESIDUAL RISK OF TRASMISSION OF HBV, HCV, AND HIV INFECTIONS IN JAPAN: A PROSPECTIVE STUDY

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Background: In Japan, all blood units are screened by tests for syphilis, HBV, HCV, HIV, HTLV-1, and human parvovirus B19, and serologically eligible blood units are then subjected to minipool nucleic acid amplification testing (NAT) for HBV, HCV, and HIV.
Aim: To investigate the safety of blood components in Japan.
Methods: We randomly selected five JRC blood centers (at Hokkaido, Iwate, Osaka, Ehime, and Fukuoka) and eight hospitals within the jurisdiction of these centers, and prospectively studied all patients who received blood transfusions in these hospitals between November 2003 and December 2006, in order to evaluate whether transmission HBV, HCV, or HBV infection occurred. The study design was as follows:
1. Pre-transfusion specimens were collected from patients and frozen after obtaining their informed consent.
2. Approximately three months after blood transfusions, post-transfusion specimens were collected from the patients and individually tested for HBV, HCV, and HIV by NAT at the JRC NAT centers. When the specimen

volume was not sufficient to perform NAT for all 3 viruses, HBV-NAT was prioritized over HCV-NAT, and HCV-NAT over HIV-NAT.
3. When the post-transfusion specimen was negative for all viruses, the study was terminated for the concerned patient: when positive, the patient's pre-transfusion frozen specimen was tested.
4. If the pre-transfusion specimen was positive, it was concluded that the patient was already infected before transfusion.
5. If the pre-transfusion specimen was negative, the concerned donors' repository specimens were tested. If their specimens were negative for HBV, the remaining pre-transfusion specimens were tested for HBcAb and HBsAb.
Results: We tested 2 139, 2 091, and 2 040 post-transfusion specimens HBV, HCV, and HIV, respectively, by NAT.
1. HBV DNA was detected in 73 (3.4%) of the 2 139 post-transfusion specimens;
a. HBV DNA was detected in the pre-transfusion specimens of 56 (2.3%) of these 73 patients, indicating that they were HBV carriers.
b. HBV DNA was not detected in the pre-transfusion specimens of the remaining 17. By a look-back study of the blood donors, it was estimated that 1 patient had transfusion-transmitted HBV infection.
c. 16 patients were at the late stage of HBV infection, but the HBsAg levels of two of these patients were quite low.
2. HCV RNA was detected in 150 (7.2%) of the 2 091 post-transfusion specimens. The pre-transfusion specimens were positive for HCV RNA in the case of all 150 patients, indicating that they were carriers.
3. HIV RNA was not detected in any of the 2,040 specimens.
4. In order to randomly select 165 patients who were negative for all viruses by NAT, 686 units were transfused. Of these, 291 donor repository specimens were positive for HBcAb and/or HBsAb, but none of them were positive for HBV DNA.
Conclusion: The current transfusion blood screening system in Japan meets the expected standards.

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CLINICAL RESEARCH OF URINE HUMAN IMMUNODEFICIENCY VIRUS (1/2) ANTIBODY DETECTION STRIPS (COLLOIDAL GOLD)

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Background: Acquired immunodeficiency syndrome (AIDS) is a serious infectious disease which caused by Human immunodeficiency virus (HIV). HIV antibody gradually appears several weeks after infection and could last for whole life. Serological test is the main method for HIV antibody testing, which consist of ELISA, colloidal gold as primary screening and Western Blot as ascertainment. It has been investigated that there are HIV antibodies in the saliva and urine of HIV infected. Comparing with traditional serological test, colloidal gold assay that tests HIV antibody in the urine supplies a simple, fast, accurate and safe method.
Aims: To investigate the sensitivity and specificity of our self-made colloidal gold strips for testing HIV antibody in urine by analysis the detect results of clinical samples.
Methods: Clinical samples consist of 671 HIV-negative samples, 266 HIV-positive samples. Each sample was tested by Western Blot and self-made colloidal gold strips. Analyze the results.
Results: There are 937 clinical samples for test, in which 671 are HIV-negative and 266 are HIV-positive confirmed by Western Blot. The results of test by strips were compared with Western Blot, and the specificity is 99.5%, sensitivity is 99.6%.
Conclusions: The self-made colloidal gold strips for testing HIV-1/2 antibody in urine has high sensitivity and high specificity.

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CLINICAL RESEARCH OF ORAL MUCOSAL TRANSUDATE HUMAN IMMUNODEFICIENCY VIRUS (1/2) ANTIBODY DETECTION KIT (COLLOIDAL GOLD)

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Background: Acquired immunodeficiency syndrome (AIDS) is a serious infectious disease which caused by Human immunodeficiency virus (HIV). HIV antibody gradually appears several weeks after infection and could last for whole life. Serological test is the main method for HIV antibody testing, which consist of ELISA, colloidal gold as primary screening and Western Blot as ascertainment. It has been investigated that there are HIV antibodies in the saliva and urine of HIV infected. Comparing with traditional serological test, colloidal gold assay that tests HIV antibody in the OMT (Oral Mucosal Transudate) supplies a simple, fast, accurate and safe method.
Aims: To investigate the sensitivity and specificity of our self-made colloidal gold strips for testing HIV antibody in OMT by analysis the detect results of clinical samples.
Methods: Clinical samples consist of 674 HIV-negative samples, 266 HIV-positive samples. Each sample was tested by Western Blot and self-made colloidal gold strips. Analyze the results.
Results: There are 940 clinical samples for test, in which 674 are HIV-negative and 266 are HIV-positive confirmed by Western Blot. The results of test by strips were compared with Western Blot, and the specificity is 99.8%, sensitivity is 99.9%.
Conclusions: The self-made colloidal gold strips for testing HIV-1/2 antibody in OMT has high sensitivity and high specificity.

P-222

ELEVEN MONTHS EXPERIENCE OF NAT BLOOD SCREENING IN NORTHERN THAILAND

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Background: Despite screening of blood donations with sensitive serological tests, there is still a risk of transfusion transmitted viral infections due to donations made during the serological window period. Testing by nucleic acid amplification technology (NAT) can narrow this window period and enhance the safety of the blood supply. NAT testing would greatly benefit Thailand where the prevalence of HIV-1, HCV and HBV is very high, especially in blood donors in northern Thailand.
Aims: The aim of this study was to evaluate the performance of two commercial multiplex NAT tests, the Chiron eSAS Procleix Ultrio test (Ultrio test) and the Roche cobas® s201 automated platform and the cobas TaqScreen MPX test (MPX test) for screening blood donations in northern Thailand.
Methods: The Chiron eSAS platform is a semi-automated platform for blood screening and the Ultrio test is a multiplex NAT test for the simultaneous detection of HBV, HCV and HIV-1. The Roche cobas s 201 platform is an automated platform for blood screening and the MPX test is a multiplex NAT test for the simultaneous detection of HBV, HCV, HIV-1 and HIV-2. The sensitivity and specificity of the tests were determined by testing 24 172 seronegative blood donations. On alternate day, all samples were tested either individually with the Ultrio test or in pool of 6 with the MPX test. A total of 12 138 samples were tested with Ultrio test and 12 034 samples were tested with the MPX test. Reactive samples were confirmed by duplicate testing, using samples taken from the plasma bag, to calculate the test specificity. The confirmed reactive samples were retested on the alternate NAT system and donors were followed up.
Results: One HIV-1 NAT- reactive donor was detected by the cobas MPX test in pools of 6. This sample was also detected when tested individually with the Ultrio test. No HCV NAT only yield cases were detected while 10 and 17 cases for HBV were detected by the Ultrio and MPX tests respectively. One of the Ultrio NAT yield samples was not detected in pools of 6

with the MPX test. Of the 17 samples detected by the MPX test, only eight were detected when tested individually with the Ultrio test. The NAT yield rate for HIV-1 and HBV were 1:24 000 and 1:900, respectively. Follow up of the donors showed that several of the HBV yield samples were from donors with occult HBV.
Conclusions: Both NAT assays are able to detect infectious samples that were missed by routine EIA assays at Maharaj Nakorn Chiang Mai hospital, Chiang Mai University. Window period HIV-1 and HBV donations, as well as donations from donors with occult HBV were detected by both tests. However, the MPX test seems to have any increased rate of detection for HBV. The assay features of multiple target detection coupled with automation greatly improves both blood safety and efficiency of testing, both imperative aspects of blood transfusion.

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IS THE 10% BELOW CUT OFF CRITERION TO DEFINE THE GREY ZONE FOR ELISA ASSAYS ADEQUATE TO ENSURE BLOOD SAFETY?

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Background: The sensitivity of the screening tests for Transfusion Transmissible Infections is an important factor in ensuring blood safety. For ELISA based assays, as per kit criteria, the Initially Reactive (IR) samples, i.e. with Optical Density (OD) greater than Cut Off (CO), and those less than CO but within the 10% 'Grey Zone' (GZ), are to be retested in duplicate. All others are considered Non Reactive (NR) and fit for transfuse.
We observed that some samples had an OD less than GZ, but much higher than the other non-reactive samples of that run. These samples are considered negative as per kit criteria. However, we termed the samples in the 30% range below CO as ''Extended Grey Zone''(EGZ) and retested them in duplicate. A few of these EGZ samples tested repeat reactive. If these EGZ are not retested then false negative blood units can be transfused with serious consequences to the patient.
Aim: To evaluate EGZ samples to improve blood safety.
Methods: The study was conducted at Rotary Bangalore-TTK Blood Bank, Bangalore Medical Services Trust, Bangalore during 2007 – 2008.
All blood samples at our centre are tested with the 3rd / 4th generation EIA kits for HBs Ag, anti HIV 1 and 2 and anti HCV. In addition to the IR samples, EGZ samples were also retested in duplicate. All Repeat Reactive (RR) units were considered not fit for transfusion and discarded.
Results: The number of blood units screened by ELISA from January 2007 to December 2008 was 44179.
Of the 44 EGZ samples for anti HIV, 2 were RR. Of the 40 EGZ samples for HBsAg, 3 were RR and of the 67 EGZ samples for anti HCV, 2 were RR.
Conclusion: There may be several reasons that could give rise to this phenomenon including technical discrepancies, or low antibody titer. The kits used are manufactured in countries outside India and therefore may not detect viral variants prevalent in the Indian population.
This was a retrospective study and the 7 EGZ samples, which were RR, were not issued to patients. Due to resource constraints, confirmatory tests could not be performed on these samples hence we do not know if these samples were False Reactive. We hope to continue the study and do confirmatory testing to test the validity of the EGZ phenomenon. Nevertheless, if indeed such Extended Grey Zone samples were confirmed positive, it would mean that if the 10% GZ criteria alone are followed, False Negative units could be issued to patients.
In developing countries where Nucleic Acid Testing is not mandatory and high prevalence of hepatitis is there, this extended grey zone criteria can be an additional layer of safety in blood transfusion centers.

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IMPORTANCE OF COMPLETE BLOOD REQUEST FORMS AND SAMPLE IDENTIFICATION AS AN IMPORTANT STEP IN PRE-TRANSFUSION NON-SEROLOGICAL TESTING

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Background: The first step in successful pre-transfusion testing is receipt of timely requests for blood with adequate clinical details & correctly labeled samples. It was noticed that many incomplete blood request forms were directed to the NBTC that was mandatory to be accepted either because it is an emergency case or the patient is coming from a far distance. So by the year 2005 the decision was taken for implementation of standardized blood request form and instructions for sample identification through the co-operation between hospitals or institutes referring patients and blood transfusion centers to ensure safety of blood transfusion.
Aim: Implementation of standardized blood request form & sample collection by highlighting causes of deferral of blood requests or samples directed to the issuing department in the NBTC due to the importance of these items to ensure the receiving of the patients safely prepared blood or one of its components with correct specifications in appropriate time.
Methods: - standardization of blood request form and clear instructions for sample identification
- Distributing these forms to all hospitals the NBTC is dealing with.
- Supplementation and distributing of these forms in the waiting area of the patients and hospital representatives.
- refusing the acceptance of incomplete forms.
- Collecting data from issuing laboratory in the NBTC about number of blood requests accepted & deferred and the causes of their deferral.
Results: Statistics performed in the last six months the period between 1st Janury09-1st July 09 revealed that the issuing laboratory receives nearly 3000 blood request and sample/month. around 450 samples are deferred scoring 15% classified as
Samples: - Absence of patient name on the sample: 33%
- Clotted sample: 28%
- inadequate sample for investigations : 1%
Blood request:
- Incomplete patient identification concerning age, weight and, last transfusion: 20%
- Absence of patient name from the blood request: 10%
- Request not directed from an authorized institute for blood transfusion: 8%.
Summary: Complete blood request form, sample labeling and identification is one of the most important steps in pre-transfusion non-serological testing.

P-225

A FATAL CASE OF DELAYED HEMOLYTIC TRANSFUSION REACTION ASSOCIATED WITH ANTI-DIEGO B AND ANTI-E ANTIBODIES

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Background: An unexpected red cell antibody, anti-Diego b is concerned in some ethnic groups such as Mongolian and Indio populations, however, the clinical aspects of this alloantibody is not yet well established. Di b negative frequency is 0.2% in Japanese.
Method: 61 years old Japanese male had IAT incompatible but ABO and D types identical blood transfusion, 20 units of RCC-LR equal to 4,000 ml, during urgent cardiovascular surgery for repair of acute aortic dissection, Stanford A type, causing severe aortic valvular regurgitation. His transfusion history was unknown, but past history of cerebral injury at 24 years. o. and operational scars in the abdomen suggested the previous transfusion exposures. The patient was later revealed to have alloantibodies to

antigens, i.e. Di b and Rh-E, with the phenotype frequency 99.8 and 50.0 % in Japanese, respectively, so that the titers elevated rapidly in a week after transfusion; the former 128 to 1024, the latter 32 to 512, respectively.
Results: The postoperative course was quite well. However, hemolysis occurred suddenly on Day 7 with hemoglobinuria; Hb decreased from 11.2 to 5.6 g/dL, LDH increased 357 to 1524 IU/L, and T.Bil 1.5 to 7.6 mg/dL. He died on Day 8 during plasma exchange and hemodialysis with renal failure, K+ 7.4 mEq/L, and LDH 3250 IU /L. The autopsy revealed severe systemic atherosclerosis, icterus, DIC, and splenomegaly (190 g) with erythrophagocytosis of macrophages, being compatible with delayed hemolytic transfusion reaction.
Conclusion: This case and other Japanese reports suggest that anti-Di b antibody is important especially in Mongolian. di(a+b-) phenotype of red cells should be included for commercial antibody screening panels to identify the specificity of pan-reactive alloantibody was found.

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CASE STUDY OF A THALASSAEMIA MAJOR CHILD WITH UNIDENTIFIED MUTATION AND CARRYING BLOOD GROUP BOMBAY AT HUSAINI HAEMATOLOGY AND ONCOLOGY TRUST, KHI, PAKISTAN. YEAR 2008

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Background: Beta Thalassaemia is a prevalent genetic hemoglobin disorder with a carrier rate of more than five percent in Pakistan. Approximately 70 000 patients are registered with thalassaemia major in multiple transfusion centres of the country, in addition to that there are many thousands still unregistered and looking for facilities, which are scarce in the country. Bombay Blood group is the rarest found till now in Pakistan and there are 14 people till now identified in the country carrying Bombay blood group at Husaini Haematology and Oncology Trust including one thalassaemia major patient.
Aims and objectives: Mutation analysis of the thalassaemia major carrying the blood group Bombay with analysis of the mutations of her parents to find out the characteristics mutation might be related to blood group serology.
Material and methods: The child was completely diagnosed and managed at the thalassaemia comprehensive management center of Husaini Haematology and Oncology Trust and parental counselling was advised.
- Blood group serology with red cell antibody screening and identification performed by using commercially prepared reagents.
- DNA analysis of the child and her parents was outsourced and coordinately done by isolation with proteins K/Rapid salting extraction method and tested for the following prevalent mutations of the disease in Pakistan.
IVS 1-5(G-C), IVS 1-1(G-T),FRAME SHIFT 8/9,FRAME SHIFT 41/42, 619 BASE PAIR DELETION,CODON 5(-CT),CODON 15 (G-A),CODON 30(G-C).CODON 30(G-A), FR16 (-16), Cap +1 (A-C),IVS 11-1 (G-A), IVS 1-25,DELTA BETA THALASSAEMIA, HB LEPORE,HBS AND HBE.
Beta Thalassaemia mutations are characterized by PCR based on allele specific priming known as Amplification Refractory Mutation System (ARMS).
Bombay Blood Group donors were enrolled and identified by various media campaigns and a register of donors was revised.
Results: - Total 13 people, 10 males and 3 females were known and identified with the blood group Bombay in Pakistan.
- Out of 13, two are not permanent residents, three are underage, one female is not agreed to donate and three are not eligible due to medical reasons.
Results of thalassaemia major patient: Blood group Bombay Rh Positive by forward and reverse methods.
Additional Testing Results: - Grouping performed after incubation of 15 minutes, 30 minutes, and 60 minutes at 37degrees to rule out cold antibodies but same reaction was observed as above.
- Direct Coomb [simple quote]s Test: Negative.
- Auto Control: Negative.
- Red Cell Antibody Screening on 3-cell panel: Positive.
- Red Cell Phenotype for H antigen: Negative.
Mutation analysis: - No mutations identified from the tested Panel the thalassaemia major child.
- No Mutations were identified from the same panel tested in both parents.
(technical rate for all kinds of DNA analysis is approximately 0.5%, so the negative result does not eliminate the possibility that the child carries a beta thalassaemia mutation that is still unidentified).

6.5. Clinical Transfusion Blood utilization

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INAPPROPRIATE USE OF BLOOD FOR MALARIA: CHANGING TRENDS TOWARDS BLOOD COMPONENT USE

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Background: Gujarat (West India) is the high malaria endemic zone. In spite of prevention and control programs infection still persists. Besides severe anemia these patients also have bleeding episodes. In India clinicians still have tendency to transfuse whole blood (WB) to correct anemia or for managing bleeding episodes.
Aims: The aim is to assess the appropriateness of utilization of blood for Malaria infection during the years 2004 to 2008.
Methods: Five years' data of malaria cases was analyzed in Microsoft excel on the basis of parasite species, Hb, age, sex and components transfused.
Results: A total of 3636 malaria patients in the age group 1 to 99 years (Mean 23 ± 21 years) received 927 (10.9%) units of WB, 4354 (51%) red cell concentrates (RCC), 1238 (14.5%) fresh frozen plasma (FFP), 1993 (23.3%) platelets and 29 (0.3%) units of cryoprecipitate. The year wise analysis of utilization of blood suggested the significant reduction ($P < 0.005$) in WB use from 16.7% in 2004 to 6.2% in 2008. Further analysis of 3087 cases receiving WB and/or RCC showed that for cases having < 5g/dL Hb, 19.8% WB units were used in 2004 and 6.8% in 2008. Thus showing significant reduction in use of WB for anemia ($p < 0.005$). About 90% malaria cases were due to *P. falciparum* and 10% *P. vivax*. Only 15 cases had both the parasites. Mean Hb concentration was comparable in both the types of malaria and component usage was also comparable except for the fact that single donor platelets were used in 43 cases of *P. falciparum* malaria in addition to random donor plateles (RDP) while in *P. vivax* maleria only RDP were used. Some malaria cases were associated with complications like Disseminated intravascular coagulation (DIC) in 0.88% cases, cerebral malaria (1.8%), Dengue (0.44%) and hepatitis (1.4%). Majority of these cases were of *P. falciparum* type. Maximum units/case was utilized for DIC (17 units/ case). Single donor platelets and cryoprecipitate were more frequently used for DIC cases.
Summary and conclusion: Even though prevalence of P. falciparum and P. Vivax malaria is equal in malaria cases, in the series of transfused malaria cases 90% cases are of P. falciparum type. Thus severe malaria is more frequently due to P. falciparum. Use of Whole Blood is significantly reduced over last five years and component use has increased, mainly due CME programs on ''Rational use of blood''organized for clinicians. Earlier the Blood Centers were supplying only red cells for correction of anemia in malaria hence there were no emergencies. But now since severity of malaria infection has increased and there are Cerebral malaria and DIC cases, in addition to red cells, products like plasma, cryoprecipitate and platelets are necessary and Blood Centers should be prepared to handle emergency situations.

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BETTER ORGANIZATION OF CLINICAL BLOOD UTILIZATION BY APPLYING FOCUS-PDCA AND C/T RATIO

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Background: Blood is a limited resource which should be conserved and used properly. In the setting of an elective surgery there is usually no need for blood transfusion (Abdelhadi, 2001). However, in some circumstances, excessive ordering of blood could be brought about as a precautionary measure, which might create a false sense of security to the doctor involved. Such practices place blood banks under enormous amounts of stress especially if it is difficult to recruit more donors. Whether this excessive ordering is caused by the fear of inadequate supply or simple ignorance the result is the same, depletion of blood supply and worsening the situation. Before blood can be transfused, benefits to risks should be weighed because of the many infections and complications that are related to transfusion. (Muizuddin, et al., 2007).
Aim: Find a way to minimize blood usage through rationalizing blood order.
Methods: The project used FOCUS-PDCA and C/T ratio. The project took place at ALWASL hospital one of Dubai Health Authority hospitals. ALWASL hospital is 340 beds maternity and paediatric hospital. Its blood bank issues more than 100 units of blood units per day. AWH blood bank supplies the needs of Thalassaemia center along with the AWH itself. After blood transfusion policy implementation the project used 2004 data as pre implementation and 2005, 2006, 2007 as post implementation and it used 2008 for auditing.
Results: C/T ratio has been reduced from;
16/1 in 2004
5 /1 in 2005
4 /1 in 2006
2/1 in 2007
3/1 in 2008
Discussion and conclusion: Blood Transfusion policy implementation succeeded in tremendously minimizing blood ordering pattern. C/T ratio has come down to 2 /1 by the end 2007. Project's idea was applied in the other two hospitals belong to Dubai Health Authority. The project helps in tremendously minimizing the problem of shortage of blood in DHA.C/T ratio monitoring should be a continuing process. Continues educational/ orientation programs for Medical staff needs to be in action and an ongoing process. The idea of the project is not new but it was applied in this place for the first time.
References:
Muizuddin M., Jawaid M., Alam S.N., Sadia Saewas Soomro S.S., Manzar S. (2007) UTILIZATION OF BLOOD IN ELECTIVE CHOLECYSTECTOMY. Pak J Med Sci 2007 Vol. 23 No. 3
Abdelhadi M. Blood Conservation in Elective Surgery. Kuwait Med J 2001;33(3):232-4.

Table 1: C/T ratio 04-08 line graph

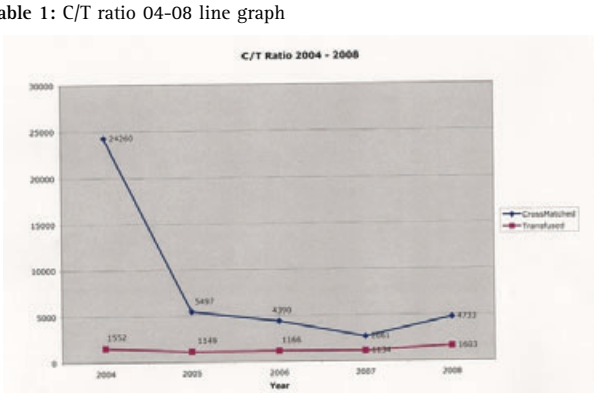

Table 2: C/T Tatio Table

	Jan-04	Feb-04	Mar-04	Apr-04	May-04	Jun-04	Jul-04	Aug-04	Sep-04	Oct-04	Nov-04	Dec-04	YEAR 2004
CrossMatched	2160	1740	2088	2035	2224	1836	3340	2739	2063	768	1940	830	24290
Transfused	130	144	134	145	95	110	163	148	134	73	111	136	1662
RATIO	16:1	12:1	15:1	14:1	23:1	17:1	17:1	19:1	15:1	11:1	18:1	7:1	15:1
	Jan-05	Feb-05	Mar-05	Apr-05	May-05	Jun-05	Jul-05	Aug-05	Sep-05	Oct-05	Nov-05	Dec-05	YEAR 2005
CrossMatched	764	423	305	628	680	383	384	657	654	567	258	289	5497
Transfused	149	80	65	158	114	74	80	130	115	115	66	88	1146
RATIO	5:1	5:1	5:1	4:1	6:1	5:1	5:1	5:1	6:1	5:1	4:1	3:1	5:1
	Jan-06	Feb-06	Mar-06	Apr-06	May-06	Jun-06	Jul-06	Aug-06	Sep-06	Oct-06	Nov-06	Dec-06	YEAR 2006
CrossMatched	607	659	702	390	372	387	286	179	128	189	188	279	4385
Transfused	155	199	196	73	74	119	89	80	69	79	80	74	1188
RATIO	4:1	4:1	5:1	5:1	5:1	3:1	3:1	2:1	2:1	2:1	2:1	4:1	4:1
	Jan-07	Feb-07	Mar-07	Apr-07	May-07	Jun-07	Jul-07	Aug-07	Sep-07	Oct-07	Nov-07	Dec-07	YEAR 2007
CrossMatched	208	237	316	178	236	166	189	206	260	268	227	347	2661
Transfused	88	104	62	69	122	64	72	107	100	97	120	145	1134
RATIO	2:1	2:1	5:1	3:1	2:1	3:1	3:1	2:1	3:1	3:1	2:1	2:1	2:1
	Jan-08	Feb-08	Mar-08	Apr-08	May-08	Jun-08	Jul-08	Aug-08	Sep-08	Oct-08	Nov-08	Dec-08	YEAR 2008
CrossMatched	184	237	314	304	329	428	224	575	455	631	508	489	4733
Transfused	90	121	120	110	135	149	85	146	126	190	156	121	1603
RATIO	2:1	2:1	2:1	3:1	2:1	3:1	3:1	3:1	4:1	3:1	3:1	4:1	3:1

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ANALYSIS OF THE USE OF FRESH FROZEN PLASMA IN PATIENTS TRETAED AT THE CLINIC OF INTERNAL DISEASES OF THE CLINICAL CENTER DR DRAGISA MISOVIC - DEDINJE

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Introduction: Currently available literature data demonstrate that in some places, the clinical use of FFP is still massive and unjustified.
Objective: Comparative survey and analysis of the consumption of FFP in two different time intervals in patients treated at the Internal Diseases Clinic and the assessment of the justification of its use.
Material and methods: Retrospective processing of collected data regarding the use of FFP at the Internal Diseases Clinic of the CHC Dr Dragisa Misovic-Dedinje in two consecutive two-years intervals (the first: 2005–2006, and the second: 2007–2008).
Results: In the first time interval, total of 4598 units of FFP were administered throughout the whole CHC Dr Dragisa Misovic-Dedinje, out of which total of 266 (5,78%) was transfuzed at the Clinic of Internal Diseases with 127 hospital beds, in 83 patients (3,2 in the average). Among them, 51 (61%) were male and 32 (39%) were female patients. The highest number of FFP units, i.e. 95 (2,9 in the average), were used in the treatment of 32 patients with bleeding episodes resulting from the oral anticoagulant drugs overdose (Warfarine), 84 (3,5 in the average) were administered to 24 patients with the diagnosis of liver cirrhosis and insuffitientio hepatis (INR>2, without bleeding), 55 FFP units (2,8 in the average) were administered to 19 patients with hypoproteinemia and hypoalbuminemia, 11 units of FFP were administered to one patient with accute DIC, while 21 (3,0 in the average) were administered to seven patients diagnosed with Cahexia, Anorexia, Malnutrition, Ascites, etc.
In the second time interval, total of 5807 units of FFP were used throughout the whole Clinical center Dr Dragisa Misovic-Dedinje, out of which 487 (8,38 %) units of FFP were administered at the Internal Diseases Clinic, with the same number of hospital beds, for the treatment of 140 (3,5 in the average) patients, among which 71 (51%) were male and 69 (49%) female patients. The highest number of FFP units, i.e. 181 (3,6 in the average) were administered to 50 patients with bleeding episodes caused by oral anticoagulant therapy overdose (Wrfarine), 50 (3,2 in the average), another 50 (3,2 in the average) units of FFP were administered to16 patients with liver cirrhosis and insuffitientio hepatis (INR > 2, without bleeding); 174 (3,7 in the average) units of FFP were administered to 51 patients with hypoproteinemia and hypoalbuminemia, while 82 (3,6 in the average) units of FFP were used for the treatment of patients with Cahexia, Anorexia, Malnutrition, Ascites, etc.
Conclusion: In spite of the efforts to adhere to the recommendations stated in the National Guidelines for the Use of FFP, the use of FFP in all non-surgically tretaed patients increased by 83,1% compared with its use in the previous time interval. Increased consumption of FFP in patients overdosed with oral anticoagulant drugs, as well as in patients with severe forms of hypoproteinemia nad hypoalbuminemia can be justified, to a certain extent, by the fact that the lack of financial resources prevented the purchase, i.e. the administration of the costly coagulation factor concentrates and colloid solutions, i.e. volume expanders.

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PREPARATION AND ADSORPTION PROPERTIES OF POLYACRYLIC ACID-GRAFTED POLYBUTYLENE TEREPHTHALATE NONWOVEN FABRIC FOR LOW-DENSITY LIPOPROTEIN-CHOLESTEROL FROM HYPERLIPEMIA PLASMA

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Background: Elevated levels of low density lipoprotein-cholesterol (LDL-C) are associated with an increased incidence of arteriosclerosis. There are about 3%~5% plasma from blood donors which cannot be used because of high LDL-C, total cholesterol(TC) and total triglyceride (TG). High-density lipoprotein cholesterol (HDL-C) is positively associated with a decreased risk of coronary heart disease (CHD). Lowering plasma concentrations of LDL-C can delay the progression of atherosclerosis. In the past decade, several research groups have developed various adsorbents to remove LDL-C by using various polyanion compounds, such as sulfonic groups, cholesterol, IgG, polyacrylic acid (PAA), and Polybutylene Terephthalate nonwoven fabric (PBTNF) is widely used in blood filter and other biomedical fields. However, original PBTNF is hydrophobicity and its blood compatibility cannot be satisfied with blood. So ultraviolet surface modification method was used to modify the surface of PBTNF. After the treatment, the hydrophilicity and adsorption capacity of LDL was greatly improved.
Aims: The aim of the present work is to selectivity remove LDL-C, TC and TG from hyperlipemia plasma by PAA grafted PBTNF. In addition, the blood compatibility of the modified PBTNF was evaluated.
Methods: The ultraviolet surface modification method is an effective way to increase the surface concentration of an immobilizing site. The acrylic acid (AAC) was introduced to the PBTNF surface first by UV in the presence of diphenylmethanone. And then we prepared five kinds grafting degree PAA-PBTNF. The modified PBT was characterized by X-ray photoelectron spectroscopy (XPS). The amount of grafted PAA was determined by titration method. The water contact angle of the PBTNF was also characterized by contact angle goniometer. Platelet adhesion test was done in order to evaluate the blood compatibility in vitro.
Results: The XPS result confirmed showed that PAA was successfully grafted onto PBTNF. The water contact angle of the PBTNF was decreased by PAA grafting. And the five kinds grafting degree of PAA-PBTNF were 11%(P1), 22%(P2), 29%(P3), 35%(P4), 50%(P5), the amount of PAA on PBTNF were about 20.1, 42.4, 53.2, 61.6, 72.5 μmol/cm^2 by titration method. Adsorption capacity of the five kinds PAA-PBTNF were studied which showed the adsorption percentage of LDL-C, TC, TG and HDL-C to be 24% vs 48% vs 70% vs 83% vs 84%, 18% vs 26%vs42% vs 50% vs 52%, 26% vs 36% vs 34% vs 40% vs 41% and 1% vs 2% vs 14% vs 15% vs 35%, respectively. Although P5 had the largest adsorption capacity, P5 had obvious adverse effects on HDL. The adsorption capacity of P4 was considerable compared to P1, P2 and P3. Moreover, P4 had better selectivity in removing LDL-C, TC and TG and without significantly affecting total protein levels in the plasma. The platelet adherent destiny on the five kinds grafting degree PAA-PBTNF was showed as follows, P1[(8.09 ± 1.05)-106 platelets/cm2], P2[(7.22 ± 0.88)-106 platelets/cm2], P3[(3.47 ± 0.72)-106 platelets/cm2], P4[(1.62 ± 0.33)-106 platelets/cm2], P5[(2.93 ± 0.36)-106 platelets/cm2].
Conclusions: PBTNF grafted with polyacylic acid exhibited an excellent selectivity in removing LDL, TC and TG without significantly loss of HDL and total protein making it attractive for hemopurification applications.

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THE SURVEY OF BLOOD TRANSFUSION CAUSES AND CROSSMATCH TO TRANSFUSION RATIO IN AMIR-ALMOEMENIN AHWAZ HOSPITAL DURING MARCH-JUNE 2008

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Blood transfusion has the potential for acute or delayed complications and the transmission of infection. The risks associated with transfusion can be reduced by minimizing unnecessary transfusions through the effective clinical use of blood and blood products and the appropriate use of simple alternatives to transfusion which are safer and more cost effective. The study of blood transfusion causes and crossmatch to transfusion ratio helps us to recognize unnecessary blood transfusion and plan for effective educational programs.
In this cross sectional and prospective survey, we studied all the blood transfusion requests referred to Amir almoemenin blood bank hospital in

Ahwaz (Iran) during March-June 2008(3 months experience). All data analyzed by SPSS 16.
We found that 124 patients received 252 blood component units during this period (2.03 units per patients; 12.9 %FFP, 84.7% Packed RBC and 2.4 % platelet) and the cross match to transfusion ratio was 1.04. The 29% of transfused cases was males and 79% female. The age was of patients 5 days to 91 years age. The main of transfusion requests was anemia (38/124; 30.6 %), Cesarean surgery (18/124; 14.5%) and bleeding (15/124;12.1 %).
Our findings showed good cross match/ transfusion ratio, but about the requests of blood, we found the request wasn't precise and the anemia was the highest blood transfusion request, on of reason may be the high number of thalasemic and hemoglubinopathic patients but we should find simple alternative for an other situations. The high number of blood transfusion in seizures surgeries should be revised and corrected by educational programs. The activation of blood bank committee in all hospital, and continues educational programs about transfusion medicine recommended.

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THE APPROPRIATENESS AND PHYSICIAN COMPLIANCE OF PLATELET USAGE BY COMPUTERIZED TRANSFUSION DECISION SUPPORT SYSTEM IN A MEDICAL CENTER IN TAIWAN

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Background: Platelet transfusions are used to treat thrombocytopenic patients, but the prescribing of inappropriate platelet transfusions is always a significant problem. Several studies demonstrated that computer-based clinical decision support systems could improve physician performance and improve outcomes for inpatients. Since September 2004, a computerized transfusion decision support system (CTDSS) has been used in Kaohsiung Medical University Hospital, an academic medical center with 1 400 beds in Taiwan. The computerized transfusion requisition form would show the criteria of ordered blood component for the physician to choose before completing the transfusion order.
Aims: We investigate the physician compliance and the appropriateness of platelet usage in our institution for evaluating the performance of CTDSS and for the evidence-base of current platelet transfusion practice in a medical center in Taiwan.
Methods: Total 5 887 platelet transfusion episodes between January and December 2008 were reviewed. The demographic data and the ''true reason''for each transfusion practice were obtained from the computerized hospital information system. The pre- and post- transfusion platelet counts were retrieved from the laboratory information system. The physician compliance and the appropriateness of platelet usage, the transfusion episode characteristics, the possible factors associated with the appropriateness and the platelet increments after transfusion were investigated. One hundred and thirty-three transfusion episodes without pre-transfusions platelet count data were excluded to evaluate the appropriateness of platelet usage in our study.
Results: The physician compliance in our study is 85.4%, and the total appropriateness of platelet usage is 69.6%. Of all the transfusion episodes, two thirds ordered single-donor platelet concentrates. By admission type of service, 82.8% episodes are from inpatient department, and more then half (51.6%) are from internal medicine unit. The most common indication for platelet usage chosen from CTDSS is ''prophylactic use, platelet count ? $20 \times 103/\mu L$'', and the second is ''patients with bleeding and platelet count ? $50 \times 103/\mu L$''. There are 539 (9.2%) episodes ordered platelet transfusions even pre-transfusion platelet counts more than 100 000/μL. The highest inappropriate order rate is from emergency department, and more than half inappropriate episodes are from the surgery unit. Almost all inappropriate orders from surgery unit are due to the lower threshold than the guideline for prophylactic platelet transfusion before surgery. The post-transfusion platelet counts increased in about two thirds platelet transfusion episodes, and most of them are with the pre-transfusion platelet counts less then 50 000 /μL.
Conclusions: The CTDSS in our institution could help the ordering physician to remind of the transfusion appropriateness, but it has no power to reject the inappropriate transfusion requisition. The education of transfusion medicine should be arranged for the medical staff of all grades responsible for prescribing platelet transfusion, and the CTDSS should set several criteria to intervene in the appropriateness of transfusion practice to reduce the waste of blood component, avoid transfusion risks, and decrease hospital costs.

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TRANSFUSION CARE FOR TRAUMA

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Background: Ten to fifteen percent of red cells are used for treating injured patients with varied practice in their treatment. Patterns of blood product usage may be useful for transfusion planning, audits and blood supply management in case of shortages and disasters.
Aims: The aim of the study was to establish baseline information on blood components used in trauma care in relation to injury severity, location and time of injury in a predominantly blunt trauma population.
Methods: A linked electronic database was developed using trauma, clinical, epidemiological and transfusion data. All trauma patients admitted in the period 2006 to 2007, identified from the trauma database were linked by hospital medical number. Patients with an injury severity score (ISS) > 8 and patients transfused in the first 24 hour of injury were included.
Results: Five hundred and thirty nine trauma patients were identified over the two year period. The median age was 35 years. Ninety seven percent of the trauma patients presented with blunt injury. Fifty eight (11%) patients received 372 units of red cells utilising fifty eight percent of total red cells in the first 24 hours of injury. Three percent of the patients received ten or more red cell units (massive transfusion, MT) utilising 92% of the total red cells in first 24 hours of injury. All MT patients received plasma, 88% received platelets and 41% received cryoprecipitate. One hundred and sixty units (43%) were transfused in the operating room, one hundred and seven (29%) in the intensive care unit and one hundred and five (28%) in the retrieval and emergency room during the first 24 hours of injury. Forty nine percent of the patients (28/58) received red cell transfusion in the first week of injury. Transfusion correlated with injury severity score (ISS); fifty three (26%) of the patients with ISS≥15 received transfusion in the first 24 hours of injury compared to nine (3%) of patients with ISS <15. The mortality in transfused patients was 17% and 23% in the massive transfusion group.
Conclusion: Two third of the total red cells transfused were given in the first 24 hours of injury and the remaining one third in the first two weeks of injury. A small group of severely injured patients consume large numbers of products and identification of those patients in need of massive transfusion using specific indicators may be helpful in mobilising resources and improving outcomes.

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EVALUATION OF HB VALUES AND NUMBER OF RCC UNITS TRANSFUSED TO PATIENTS IN A CRITICAL CARE UNIT

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Background: National Hospital of Sri Lanka is the largest hospital and the only tertiary referral center for all the hospitals in Sri Lanka. The bed strength of the hospital is 3 600 and bed occupancy in the main units is 100% – 150%. At most of the times critically ill patients from these units are admitted to the Intensive Care Units (ICU). ICUs are allocated to medical, neurological, surgical, coronary care, accident and trauma.
In the Medical ICU there are 12 beds.14 medical units in the hospital transfer their critically ill patients to this ICU.

Aim: To evaluate the Pre-Transfusion Hb and number of units of Red Cell Concentrates (RCC) transfused to the critically ill patients during their stay at MICU within a period of one year and to assess the appropriateness of recommending RCC transfuse.
Method: Retrospective analysis of RCC transfusions issued for the requests for critically ill patients of medical ICU in NHSL within the period of year 2005 January to 2005 December.
Results: Total of 604 patients were admitted to the ICU during this period. 108 patients were transfused with 194 units of RCC during their stay.
1 unit of RCC for 66 (61%)patients,2 units for 20(18.5%),3 units for 12(11.5%),4 units for 4(3.7%),5 units for 4(3.7%),and more than 5 units for 2(1.9%)patients.
Pre-Transfusion Hb percentage in the study population was in 58 (53.7%) patients Hb was lower than 7g/dl, in 32(29.6%) patients Hb stands between 7 and 10 g/dl, in 18(16.6%) patients Hb was more than 10 g/dl.
Number of units transfused for Hb levels < 7g/dl was 73, for 7–9 g 48,for more than 10 g/dl 32. Number of units unused and returned was 53.
Conclusion: The obtained results showed that majority of patients (61%) were transfused with 1 unit of RCC during their stay at ICU, which could have being avoided with other measures. Also 16.6% of the total transfused RCC units were transfused to patients who have Hb more than 10 g/dl, which was also unnecessary. It will be better if the patients were assessed individually and transfusion decisions should be taken after evaluating the patient's condition carefully.

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ASSESSING THE RISK FACTORS FOR BLOOD TRANSFUSION DURING CESAREAN SECTIONS TO MINIMIZE UNNECESSARY CROSS MATCHES IN A TERTIARY CARE MATERNITY HOSPITAL IN SRI LANKA

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Background: Rational utilization of blood products is imperative as supply of safe blood products are scarce. Reservation of blood for elective and emergency surgeries comprises a large proportion of blood utilization in any health care system. In many Sri Lankan hospitals reservation of blood for elective and emergency caesarian sections is done irrespective of the indication and the patients' clinical condition leading to waste of resources.
Aims: Objective of the study was to determine the incidence of blood transfusion in cesarean sections identifying factors predictive of the need for transfusion and evaluate the feasibility of type and screening method replacing crossmatching in De Soyza Maternity Hospital, Sri Lanka.
Methods: This retrospective study reviewed 2721 cesarean sections done over a period of 6 months. Demographic characteristics, indication and the urgency of the caesarian section and the number of units cross matched and transfused were recorded. Crossmatch to transfusion ratio (C/T ratio) and the transfusion index (TI) were calculated for each indication.
Results: A total of 3326 blood packs were reserved for the 2721 cesarean sections performed within the study period. Out of the 3326 packs only 217 packs had been transfused intraoperatively or postoperatively for 126 patients (4.63%). An unbooked patient was four times more likely to receive a blood transfusion during cesarean section than women who had regular antenatal care ($p<0.05$). There was no increased risk of blood transfusion in emergency caesarian sections [n = 1803(66.26%); ($P > 0.05$)]. Placenta previa was significantly associated with transfusion during cesarean section (C/T ratio = 12.66 = .23 $P < 0.05$). Pre existing medical disorders including anaemia, conditions with high risk for post partum haemorrhage and twin pregnancy were also associated with increased red cell transfusion.
Conclusion: Based on these results, a type and screen method for preoperative blood ordering is recommended for most patients other than pregnancies complicated by placenta previa, medical disorders including anaemia, conditions with high risk for post partum haemorrhage and twin pregnancy, with substantial savings in money and laboratory personnel time without compromising patient care.

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SAFETY OF GYNECOLOGICAL SURGERIES IN TRANSFUSION MEDICINE POINT OF VIEW: A STUDY ON BLOOD ORDERING AND TRANSFUSION HABITS OF ELECTIVE GYNECOLOGICAL SURGERIES IN A TERTIARY CARE HOSPITAL IN SRI LANKA

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Background: Despite all its potential benefits blood transfusion in elective gynecological surgeries is expensive and is associated with risk of serious adverse reactions and disease transmission. The need to reserve a large number of blood units for these elective surgeries is an additional burden on the blood banks as there is always a shortage of supply of safe blood. Blood typing and antibody screening procedure in place of cross matching is an effective method to lower costs with no compromise in patient care.
Aims: To evaluate the current blood ordering and transfusion practices of elective gynecological surgeries at the De Soysa Hospital for Women, Sri Lanka.
Method: A retrospective study of blood ordering routines for 1140 elective gynecological surgeries was carried out for 10 months. The number of blood units crossmatched (C) for each category of gynecological surgery and the number transfused (T) were recorded and the crossmatch to transfusion (C/T) ratio and the transfusion index (TI) was calculated.
Results: For the 1140 gynaecological surgeries 1556 blood packs were crossmatched and 151 were transfused in 81 transfusion events. Overall C/T ratios were high with lowest ratios in Dilatation & Curettage (3.8), Myomectomy (4.9) and exploratory laparatomy (5.7). Surgeries with the highest transfusion indexes were Myomectomy (0.36), Dilatation & Curettage (0.34) and exploratory laparatomy (0.25). More than 65% of all surgeries had C/T ratios over 10 and 83% of surgeries had a transfusion index less than 0.25.
Conclusion: There is an excessive over crossmatching of blood and unnecessary preoperative reservation for all the gynaecological surgeries. Preoperative cross matching of blood can be suggested only for Dilatation & Curettage, myomectomy, exploratory laparatomy and ectopic pregnancies. Grouping and screening for the other surgeries could save a lot of resources and manpower. Also comparison of the data to other countries shows remarkable safety in gynecological surgeries with low risks of red cell transfusion.

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INTRAINSTITUTIONAL EFFORTS AND THE INSPECTION AND ACCREDITATION (I&A) PROGRAM EFFICIENTLY PROMOTE APPROPRIATE USE OF BLOOD AND BLOOD COMPONENTS

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Background: In Japan, the guidelines for blood transfusion practice and the guidelines for use of blood products were issued by the Ministry of Health, Labor and Welfare in 1999 and revised several times afterward. All the physicians and healthcare professionals involved in transfusion therapy in Osaka Medical College Hospital (OMCH) have studied these guidelines and promoted the appropriate use of blood and blood components.
Aim: The aim of this study is to evaluate the efficacy of the efforts to improve transfusion practice by analyzing efforts to reduce the discard rate (DR) of blood components.

Methods: We examined the number of transfused and discarded bags of blood components that were recorded in the blood transfusion database (BTD®, Ortho-Clinical Diagnostics, Tokyo, Japan) from 2000 to 2008. The DR was annually calculated by dividing the number of discarded bags by that of discarded and transfused bags. The efforts carried out to promote the appropriate use of blood components were annually abstracted from the minutes of the hospital transfusion committee (HTC) in OMCH.

Results: In 2000, the DR was 36.6%. In 2001, when the HTC was established, the DR was 24.6%. In 2002, when a type and screen (T&S) policy was introduced, the DR was 10.4%. In 2003, when compulsory documentation of blood discarded by clinicians was initiated, the DR was 3.6%. In 2004, the inspection and accreditation (I&A) program on blood transfusion practice was introduced by the I&A committee of the Japan Society of Transfusion Medicine and Cell Therapy. Conforming to a recommendation in a report by the I&A committee, we formulated a standard operating procedure (SOP) manual of transfusion therapy and introduced it throughout the hospital. A monthly audit of transfusion therapies by the members of the HTC was also initiated. The DR in this year was 2.4%. In 2005, when the transfusion-related guidelines were posted on the intranet of the hospital, the DR was 2.0%. In 2006, although a monthly report on blood utilization of each clinical department was initiated, the DR went up to 2.8%. Therefore, a computer-based medical order system for transfusion was established. With this system, physicians must make a medical record consisting of the purpose of treatment of a patient, changes in clinical evidence between pre- and post-transfusion, and an assessment of the advantages of the transfusion therapy. The DR of the following year, 2007, improved to 1.6%. In 2008, the DR was 1.9%, similar to that of the previous year.

Summary and conclusions: Almost all the efforts carried out to promote the appropriate use of blood components proved to be efficient for reducing the DR. Continuing efforts by the HTC are indispensable; for example, the SOPs need to be reviewed regularly to ensure that standard procedures are kept up to date. Audits should be conducted monthly and the results reported to all the members of the HTC. Evaluation by a third party, such as the I&A committee, is also necessary to identify some problems that may be overlooked by the intrainstitutional commission.

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AIMING TOWARDS PROPER STOCK MANAGEMENT IN A HOSPITAL BLOOD BANK

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Introduction: Goal of blood stock management is to ensure effective use of limited blood resource. Among factors influencing stock management, demand of the hospital service plays a key role. National Hospital of Sri Lanka (NHSL) is the main tertiary care hospital of the country, with medical, acute trauma management and surgical units where complicated surgeries are performed. Analysis of past requests and issues of blood and blood components will guide to predict the future stock requirements, adequate enough to maintain appropriate high quality products given at correct times with minimal wastage.

Aim:

To evaluate, 1. The number of daily, monthly and annual requests and usage of red cell concentrates (RCC), fresh frozen plasma (FFP) and platelet concentrate (PC).

2. The annual trend of demand and the monthly variations of demand over a year.

Methods: Retrospective analysis of requests and usage of blood and blood components in 2006, 2007 and 2008 was done at blood bank, NHSL. Registers and records maintained at request acceptance counter and blood and blood component issue counter were used to collect data.

Results: - Annual requests of RCC were 69345, 59143, 61000 in 2006, 2007, 2008 respectively. Calculated mean monthly requests were 5778, 4928, 5083 and mean daily requests were 192,154,169.

- Annual issues of RCC were 17985, 18368, 19111 in 2006, 2007, 2008 respectively. Calculated mean monthly issues were 1498, 1538, 1584 and mean daily issues were 50, 51, 53.
- Annual requests of FFP were 23522, 26153, 25920 in 2006, 2007, 2008 respectively. Calculated mean monthly requests were 1960, 2197, 2160 and mean daily requests were 65, 73, 72.
- Annual issues of FFP were 22333, 21976 and 20957 in 2006,2007,2008 respectively. Calculated mean monthly issues were 1861,1831,1746 and mean daily issues were 62,61,59.
- Annual requests of PC were 15908,17697,17665 in 2006,2007,2008 respectively. Calculated mean monthly requests were1242,1474,1472 and mean daily requests were 41,49,49.
- Annual issues of PC were 13662,12336, 12639 in 2006,2007,2008 respectively. Calculated mean monthly issues were 1138,1028,1053 and daily issues were 38,34,35.

Reduction in requests and issues of all components was observed in April.

Conclusions: The trend of RCC requests was decreasing and issues was increasing, but still request to issue ratio 3.84,3.01 and 3.i8 in 2006,2007 and 2008 respectively. The higher the value the more blood that was being requested unnecessarily leading to increased past expiry rates in RCC stocks. Therefore it is essential implement maximum surgical blood order schedules and guidelines on RCC indications to the clinical staff. The trend of FFP requests was increasing and the trend of issues was decreasing. That is because some requests were given cryo supernatant plasma instead of FFP where indicated. The trend of PC requests was increasing and the trend of issues was decreasing. In this case blood bank should aim towards an increase in collection while educating the clinical staff about appropriate use.

Table 1: Analysis of request & issues

		RCC				FFP			PC		
Year	Type	Annual	Monthly	Daily	R: I	Annual	Monthly	Daily	Annual	Monthly	Daily
2006	Requests	69345	5778	192	3.8	23522	1960	65	15908	1242	41
2006	Issues	17985	1498	50		22333	1861	62	13662	1138	38
2007	Requests	59149	4928	154	3.0	26153	2197	73	17697	1474	49
2007	Issues	18368	1538	51		21976	1831	61	12336	1028	34
2008	Requests	61000	5083	168	3.1	25920	2160	72	17665	1472	49
2008	Issues	19111	1584	53		20957	1746	59	12639	1053	35

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APPROPRIATENESS OF THE USE OF ALLOGENIC RED CELL TRANSFUSION AMONG PATIENTS ADMITTED TO GYNECOLOGY UNITS AT TEACHING HOSPITAL ANURADHAPURA

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Introduction: Millions of lives are saved each year through blood transfusions. Blood transfusion has become an essential part of modern healthcare. Used correctly, it can save life and improve health. However, as with any therapeutic interventions, it may result in acute or delayed complications.

National blood transfusion service in Sri Lanka is a non-fragmented centralized service. It includes a national blood centre and 74 hospital based blood banks. Five out of 74 hospital - based blood banks function as provincial blood centers. Most blood products are usually transfused to patients admitted to gynecology units either to replace acute blood loss or to correct pre and post operative anaemia. Anaemia is a common problem among the elderly women with gynecological problems. In this study provincial blood centre situated at the Teaching Hospital in the district of Anuradhapura was taken as the study focal point.

Aim: Aim is to determine the use of allogenic red cell transfusion among patients admitted to gynecology unit at Teaching Hospital Anuradhapura.

Methodology: Prospective study was conducted for the period of August 2007 to March 2008 using all patients admitted to gynecology units and who needed red cell transfusion were taken as the study sample. Total

number of subjects studied was 1130. Data was collected using blood bank red cell request forms, patients Bed head tickets, Anaesthesia record sheets, patient's other investigation reports, Red cell issue registers and return register, and bed site questioning for other specific data. Data analysis done manually. Appropriateness of the use of allogenic red cell transfusion assed by using BCSH - 2001 guidelines

Pre-operative, per- operative and post operative indications were classified as appropriate, probably appropriate, or inappropriate as mention below. appropriate - if the criteria met according to the BCSH guidelines.

Results: Out of 1130 patients, 225 patients had been RBC Transfusion during Pre-operative period. So Appropriateness of RBC Transfusion is 78.22%, probably appropriate 16% and in appropriate 5.78%. 30 patients had been transfused during per- operative period. out of 30 16.7% Transfusion are appropriate, 16.7% probably appropriate and 66.6% Transfusion are inappropriate. A total of 45 patients had been transfused during post operative period. 24.4% Transfusion are appropriate, 11.1% Transfusion are probably appropriate. 64.5% are inappropriate. Cross match to Transfusion ratio (C:T) is 3.8:1 In this study, most of the red cell usage were appropriate (65%), 15.3% were probably appropriate, and 20.6% were inappropriate Transfusion based on BCSH guidelines 2001.

Conclusion: 1. Need formulation of MSBOS because C:T ratio > 2

2. RBC Transfusion guidelines should be made available and implementation of these guidelines should be monitored.

3. Need continuing medical education programme on guidelines and quality transfusion practice for the clinical staff.

4. Hospital transfusion committee (HTC) should be established and they should be involved in implementation of National guidelines in order to ensure safe and good transfusion practice.

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RETROSPECTIVE ANALYSIS OF CROSS MACH TO ISSUE RATIO OF ROUTINE SURGERIES OF LARGEST PEDIATRIC HOSPITAL IN SRI LANKA

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Introduction: Lady Ridgeway Hospital for Children is the largest pediatric hospital in Sri Lanka providing tertiary care. Wide numbers of surgeries including several complex procedures are carried out in seven surgical units both on routine and emergency basis. Blood cross mach requirement for these units comprise significant portion of workload of blood bank and therefore this study was planed to assess the cross mach to issue ratio in view of introducing maximum surgical blood ordering schedule which is relatively new concept for pediatric surgeries in Sri Lanka.

Methodology: Elective surgical procedures for whom blood cross mach were requested, from 1st January to 30th of June 2009 were traced from the theater records and matched against issues recorded in the blood bank. The cross mach to issue ratio were tabulated under the surgeries which were categorized according to the site, time duration taken for the procedure, and the vascularity of the site.

Analysis: Total number of 996 surgeries for which cross mach of blood needed, were done and 254 units were transfused. Complex cardiac bypass surgeries had highest cross mach to issue ratio (77%) followed by skin grafting procedures for burn patients and open abdominal surgeries. On the other end of the list were many ENT surgeries, facial reconstruction surgeries and cardiac catheterizations which needed least number of blood transfusions.

Recommendations: Cross mach request for elective surgical procedures consist significant portion of total number of requests, of which many surgeries had higher ratio of issues needing prompt blood supply and some other surgeries had low issue ratio and rarely needed blood. In this context, implementation of maximum surgical blood ordering schedule will, undoubtedly reduce the work load of the blood bank staff where by improving the quality of work as well as the blood availability.

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A STUDY OF PLATELET CONCENTRATE USAGE AT A TERTIARY CARE HOSPITAL IN SRI LANKA

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Background: Colombo South Teaching hospital (CSTH) is in the southern part of Western Province Sri Lanka with bed strength of 1093. Inappropriate use and inadequate dosage of platelet is very common in Sri Lanka although guidelines for platelet transfusion are distributed among all the hospitals. Data of platelet transfusion within six months were analysed retrospectively from September 2008 to February 2009 in CSTH Sri Lanka. Patients from all specialities namely medical, surgical, paediatrics & obstetric were included in the study.

Aim: To study the pattern of platelet transfusions in relation to diagnoses and to study whether the dose prescribed is adequate or not.

Method: Data such as clinical indication, number of units requested, age / weight of the patient and requesting speciality were collected from laboratory request form for platelet transfusions. Number of units issued collected from the blood bank platelet issue register. Timing of the transfusions and the adequacy of the dose of platelets transfused were traced from patients' records (Bed head tickets).

Results: There were total of 288 episodes of platelet transfusions where 948 units were used. At the medical department 72% of platelet transfusions were used (203 episodes 668 units, an average 3.3 units per episodes),at the paediatric department 9% (46 episodes, 107 units an average of 2.2 units per episode)were used. For the patients in Surgical and Accident department 15% (93 episodes, 136 units an average of 4.1 units per episode) and in the Gynaecology and obstetrics' department 4% were used (6 episodes, 37 units, 6.1 units per episodes).

Indications for platelet transfusions were Dengue haemorrhagic fever (70%), massive transfusions (13%) Disseminated intra vascular coagulation (8%) major obstetric haemorrhage (6%), conditions were there is reduce platelet production - Aplastic anaemia, Myelodysplasia (2%) and Immune Thrombocytopenia with active bleeding (1%) There were 49 episodes where one platelet concentrate was transfused and there were 69 episodes where 2 platelet concentrates were transfused. In the majority of episodes (136) 4 platelet concentrates were transfused. There were 30 episodes where 5 platelet concentrates were transfused in each episode and there were 13 episodes where 6 platelet concentrates which contained the adult therapeutic dose were transfused. (1 unit of platelet contains approximately 55 x 10^9 platelets). Out of the patients transfused 75% had the post transfusion platelet count recorded (one hour and 24 hour) and 68.5% patients had the target required level while 6.5% patients couldn't achieve the target expected.

Conclusion: Analysis of indications of platelet transfusions and the dosage prescribed reveals that the results are satisfactory except in few occasions. Majority of one unit of platelet transfusions were in the paediatric wards. This evidently shows the better transfusion practices established in Colombo South Teaching Hospital Sri Lanka implemented through the hospital transfusion committee and continuous awareness programmes on appropriate use of blood and blood products by the Department of Transfusion Medicine in the hospital.

6.6. Clinical Transfusion

Hereditary bleeding disorders

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ANALYSIS OF BLEEDING DISORDER PATIENT'S REGISTRY AT HUSAINI HAEMATOLOGY AND ONCOLOGY TRUST, KARACHI, PAKISTAN FROM JUNE 2006 TILL JUNE 2009

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Objective: Analysis of bleeding disorder patients according to age, severity, type and socioeconomic status at Husaini Haematology and Oncology Trust, Karachi, Pakistan from June 2006 till June 2009.

Background: Hemophilia A and B are x-linked bleeding disorders. The incidence of hemophilia is nearly 1 in 5000 males. Many children with hemophilia are born each year and there are nearly 7000 patients with severe hemophilia A at present. There is no standard data available though hemophilia federation of Pakistan has kept a disease registry but even then the data analysis is not done on regular basis. The greater availability and use of cryoprecipitates and factor concentrates since 1970s has greatly improved the management of hemophilia with an overall reduction in the morbidity and mortality.

Aim and objectives: Husaini Haematology and Oncology Trust has registered the patients of hemophilia and von willebrand disease since last three years and our aim is to collect an initial data of disease prevalence, severity, age distribution and socioeconomic status of patients to further enhance our services and maintain proper data base. We are providing Cryoprecipiates, fresh frozen plasma, and factor replacement therapy in addition to physiotherapy, blood screening,dental, joint care and genetic counseling of the disease free of cost as an NGO working since last more than three decades.

Material and methods: All data is collected in Microsoft office access specially designed to main individual permanent register and followup register of a patient.

Results: Disease population statistics

- no of registered patients with hemophilia a and b = 352
- no of registered patients with vonnwillebrand disease = 37
- no of patients with rare factor deficiencies and other platelet inherited disorders =11

Age distribution: - age group 0–13 years hemophilia a 31, hemophilia b 24, vwillebrand disease 12.

- age group 14 - 18 years hemophilia a 94, hemophilia b 19, v willebrand disease 18.
- age group 19 years and plus, hemophilia a 168, hemophilia b 16, v willebrand disease 7.

Total patients according to type of disease.

- hemophilia a males 303, females 0.
- hemophilia b males 49, females 0
- von willebrand disease males 9 females 29
- factor 1 deficiency males 2
- factor 7 deficiency males 5
- factor 13 deficiency males 2
- combined factor deficiency males 2

Socioeconomic status in terms of percentage in total patients.

- type 1 (monthly income between 3 to 5 thousand pak rupees)
- 54 percent.
- type 2 (monthly income between 5 to 15 thousand pak rupees)

21 percent.

- type 3 (monthly income below 5 thousand or not earning and dependent on someone else)

22 percent

- type 4 (monthly income more than 15 thousand pak rupees)

3 percent.

Hemophilia and other bleeding disorder patiets in pakistan needs extensive followup and care in terms of factor replacement therapy, prophylaxis, disease followup and laboratories as it is an extremely expensive burden on the whole society particularly in pakistan where there is no national followup and registry well maintained for the disease and no govt support is provided to the patients on regular basis.

6.7. Clinical Transfusion Haemovigilance

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OUR EXPERIENCE IN TRANSFUSION REACTIONS MONITORING

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Introduction: Despite all lately applied procedures performed in order to provide as safe as possible transfusion tretament of patients, each transfusion of blood/blood comoponent still carries certain risks.
Objective: Survey of the number and type of reported transfusion reactions (TR) in two different time intervals, analysis of obtained results and their comparison with similar data from literature, were performed in order to see if there was any significant improvement in the process of monitoring and reporting of TRs in our Clinical Center.
Material and methods: Retrospective analysis of the number and type of TRs reported in the Blood Transfusion Service of the Clinical Center ''Dr Dragiša Mišović-Dedinje''was performed in two different time intervals: the first (13 years) from 1985 till 1997, and the second (11 years) from 1998 till 2008. Results:In the first time interval, 222 immediate TRs were reported, 17,1 in the average on the annual basis, i.e. 43 per 10 000 administered blood/RBC units (total of 51 753 blood/RBC units). In the second time interval, 71 immediate TRs were reported, 6,4 in the average on the annual basis, i.e. 20 per 10 000 administered blood/RBC units (total of 36 760 blood/RBC units). Based on data stated in the TR report signed by the medical doctor in charge and after having performed blood transfusion and all other necessary analyses, it was concluded that in the first time interval there were:153(68,9%) FNHTRs, 68(30,6%) alergic and 1(0,5%) HTRs. In the second time interval, majority consisted of FNHTRs: 40(56,4%), followed by alergic TRs: 28(39,4%), while in 3(4,2%) the cause of TRs was undetermined.
Discussion: Analysis of the above stated data shows that in the more recent yearas the number of reported TRs is considerably lower. As of 1991, we have not had any reported case of HTR. The question is why we have not had reports of platelet and FFP TRs (in the second observed time interval, a total of 26 144 units of FFP and 3 830 platelet concentrates were used). It cannot be said with certainty that the above stated data are the result of the improved quality of work or that perhaps they are the consequence of an inadequate follow up of patients during and after blood transfusion, or the lack of TRs symptoms recognition in patients having poor general health condition due to the underlying illness, and non-reporting of some milder forms of TRs andthelike.
Conclusion: In order to apply maximum safe blood, it is necessary to provide and monitor the quality of blood/blood components administration, to educate hospital staff regarding all aspects of safe administration of blood/blood component, timely recognition of the symptoms and causes of TRs and to report them immediately (precondition of that is a well organized haemovigilance system on the local, hospital level), and last but not least to establish hospital transfusion committees in charge of the realization and follow up of recommended measures.

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AN ACTIVE HEMOVIGILANCE PROGRAM TO CHARACTERIZE THE SAFETY PROFILE OF 16,631 PLATELET COMPONENTS PREPARED WITH INTERCEPT BLOOD SYSTEM™ TRANSFUSED IN ROUTINE CLINICAL PRACTICE

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Background: Since implementation of INTERCEPT™platelet components in clinical use, an active hemovigilance (HV) program has been on-going for five years to characterize and extend the safety profile of INTERCEPT INTERCEPT platelets (I-PLT) in routine use.
Aims: This report summarizes 16,631 I-PLT txn administered to 3 274 pts at 17 different sites in eight European countries and provides a safety profile of I-PLT transfused in routine practice to a broad patient population.
Methods: Apheresis or pooled buffy-coat platelet components were leukoreduced, suspended in ~35% plasma and 65% InterSol', treated with the INTERCEPT and stored for up to seven days. INTERCEPT treatment replaced bacterial screening at all sites and gamma irradiation for 97.5% (16 325/ 16 631) of I-PLT txn. Blood centers using INTERCEPT platelets completed a data form after each txn regardless of whether a reaction occurred. The focus was the response to txn within the first 24 hours regardless of outcome. A common INTERCEPT Hemovigilance Transfusion Report Form was utilized. Investigators recorded: patient (pt) demographics, primary diagnosis and indication for transfusion, and type of I-PLT product. For each occurrence of an adverse event, the following data were collected: time of adverse event following txn, clinical description of event, vital signs, clinical and laboratory data (radiographs, bacterial cultures), event severity (grade 0–4), serious or non-serious classification, and causal relationship to txn (unrelated, probably unrelated, possibly related, probably related, or related).
Results: From October 2003 to the present, data from 16,631 I-PLT txn administered to 3 274 pts (60.3% males/ 39.7% females) have been collected. The majority of the recipients were hematology/oncology patients (1 643, 50.2%) many of whom received hematopoietic stem cell transplants s (n = 307). The majority of I-PLT were administered in non-intensive care hospital units (13 152, 79.1%), and the remainder were transfused in intensive care units (2 496, 15%) and outpatient clinics (981, 5.9%). Transfusions associated with ''related''(possibly related, probably related, or related) adverse events following I-PLT txn were infrequent (110/ 16,631 =0.66%). Eighty-two pts (2.5%) experienced at least one related adverse event following one or more INTERCEPT txn. Most reactions were mild and of grade 1 severity and were representative of the events expected with conventional PLT txn. The most frequently reported signs/symptoms were chills, fever, and urticaria. Eleven SAE's were reported, with one having causal relationship (hypotension possibly related) to I-PLT txn. No cases of Transfusion Related Acute Lung Injury (TRALI), TA-GVHD, transfusion related sepsis or death due to an INTERCEPT txn were reported.
Conclusions: In this program, 99.34% of I-PLT administrations were without a related txn reactions. Adverse events following I-PLT txn classified as related to txn were infrequent, mild in severity, and representative of the events expected with PLT txn. The use of an HV program to capture ongoing safety information is a valuable tool to characterize the safety of I-PLT.

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ACTIVE HEMOVIGILANCE OF PEDIATRIC PATIENTS SUPPORTED WITH PLASMA COMPONENTS PREPARED WITH PHOTOCHEMICAL TREATMENT (INTERCEPT™)

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Background: During the fivr years since introduction of INTERCEPT platelet components into routine clinical use, transfusion of INTERCEPT platelets to pediatric hematology-oncology patients has been shown to be safe and efficacious. Subsequently INTERCEPT plasma components also received CE Mark registration in 2006. An active hemovigilance program was implemented to gather safety information on routine transfusion of INTERCEPT plasma components (IPL).
Aims: This study analyzed the transfusion safety profile in 348 pediatric patients receiving 1,029 transfusions.
Methods: IPL produced in routine practice had a mean volume of 200 mL. Plasma transfusions were ordered by primary care physicians per standard of care. This study assessed the response to all study IPL transfusions within the first 24 hours. The primary endpoint was the incidence of acute

transfusion reactions (ATR) defined as an adverse event (AE) possibly related, probably related, or related to the plasma transfusion. For each transfusion, patient demographics and primary diagnosis and indication for transfusion were recorded regardless of whether an AE was observed. For AEs, the following data were collected: time of event following transfusion, clinical description, vital signs, results from clinical and laboratory tests (radiographs, bacterial cultures), severity (grade 0–4), serious or non-serious nature, and causal relationship (unrelated, probably unrelated, possibly related, probably related, or related).

Results: To date 1 029 transfusions, comprised of 1 641 units of IPL, have been administered to 348 pediatric patients (61.8% male, 38.2% female). The mean age was 4.2 years (160 children between 1–18 years, 188 infants <1 year). Of the 348 patients, 109 (31.3%) received IPL transfusion for a hematologic disorder (including 3 with congenital coagulation deficiency, 101 with acquired coagulopathy, and 2 with TTP), 83 (23.9%) for surgery, 156 (44.8%) with another diagnosis as an indication for plasma transfusion. Average number of transfusions (txn) per patient was 3.0 txn (range 1–55, median 2.0). Each patient received a mean of 4.7 units of IPL (range 1–99, median 2.0). Compared to frequency of transfusions in infants (< 1 year), older pediatric patients (1–18 years) received higher numbers of transfusions and plasma components (3.4 txn/7.0 IPL vs. 2.6 txn/2.7 IPL). Patients 1–18 years old with hematology diseases received more transfusions and plasma products (mean 4.7 txn/7.5 IPL) than other diseases. One patient with congenital coagulation deficiency and two patients with TTP received the largest number of transfusions and IPL products. In all patients, 153 (44%) patients had previous transfusions, of which 63 (18.1%) patients received INTERCEPT platelets. Among the 1,029 transfusions, no AE, ATR, SAE, deaths or episodes of TRALI due to an IPL transfusion were reported.

Conclusions: To date no ATR with IPL transfusions were reported in pediatric patients including infants. These results provide additional indication that routine IPL transfusion is safe and well tolerated in this patient population.

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THE IMPORTANCE OF THE EFFECTIVE INTERNAL REPORTING SYSTEM OF ADVERSE REACTIONS OF BLOOD TRANSFUSION IN HOSPITALS

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The report of the adverse effects of blood transfusion to the Ministry of Health, Labour and Welfare (Pharmaceutical and Medical Devices Agency, PMDA), which was previously conducted as an operational regulation, became an obligation of the medical personnel by the amendments of The Pharmaceutical Affairs Law and the Law to Assure the Stable Supply and Safety of Blood Products (The New Blood Law) of July 2003. Thus, any information concerning adverse effects, infectious agent transmission or troubles due to use of medical products (including blood products) or medical devices must be immediately reported, and based on this information, the Ministry of Health, Labour and Welfare (PMDA) must take safety measures for their appropriate use. Concerning the adverse reactions of blood products, the blood transfusion services must centralize the information and report them to the Japanese Red Cross Blood Center (JRCBC), which investigate on the causes of adverse effects and report them to the Ministry of Health, Labour and Welfare (PMDA).

In our university hospital, previously, the reporting system of adverse effects of transfusion was based on the checking in the check box, in the label for cross-checking send together with the blood bags, for the presence or absence of adverse effects and making a brief description of the symptoms or signs in case they were observed. At that time, the frequency of adverse effects was very low, and in some cases, the label was returned checked for the absence of adverse effects even when the blood product was not used.

Taking these facts, we decided to take measures for the appropriate reporting of the adverse effects of blood transfusion. First, the members of the Transfusion Committee of the hospital, established in May 2003, were made aware of the obligation of the reporting by Law, and subsequently announced to the whole hospital staff by means of e-mailing system. Next, the reporting system was changed to a more detailed and specific one, where the most frequently observed symptoms and signs are described, and the medical personnel just check the one observed, and describe the details requested. The reporting form can be downloaded from the internet site of the blood transfusion service in the hospital system, and after filled, is send to the blood transfusion service. When necessary, the blood transfusion service give instructions to the doctor in charge for the appropriate manipulation of the causative blood product, the patient's sample collection and the laboratory tests to be performed. And the patient's sample is sent to the JRCBC, together with the report, for further testing.

After implementation of the above measures, the incidence of reported adverse effects increased, and also the severe ones such as anaphylactic shock and TRALI have been reported. Previously, no cases of TRALI had been reported. Therefore, we concluded that providing adequate information to the whole staff of the hospital and the implementation of an easy and appropriate reporting system are essential for the accurate knowledge of the adverse effects of blood transfusion, including the autologous one.

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ELEVATED CA2+ INFLUX-INDUCING ACTIVITY TOWARD MAST CELLS IN PRETRANSFUSION SERA FROM PATIENTS WHO DEVELOPED TRANSFUSION-RELATED ADVERSE REACTIONS

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Background: Type I allergic reactions such as urticaria-like manifestations constitute a large percentage of transfusion-related adverse events. Along with donor factors, patient factors might be involved in these reactions. In fact, sera from some patients with chronic idiopathic urticaria are reported to show histamine-releasing activity. It is possible that sera from patients who develop transfusion-related Type I allergic reaction may originally possess histamine-releasing activity.

Aims: An influx of Ca2+ precedes the degradation of mast cells. Therefore, the hypothesis that pretransfusion serum samples from patients who developed transfusion-related adverse events have Ca2+ in?ux-inducing activity and histamine-releasing activity in mast cells was examined.

Methods: Pretransfusion sera derived from 145 patients who reportedly developed febrile nonhemolytic type adverse reactions after allogeneic blood transfusions were collected. Sera derived from 54 patients who did not react adversely to transfusions were used as control. In addition, sera derived from 107 blood donors were used as another control. Mast cells were obtained by culturing peripheral blood CD34+ cells and mixed with the serum samples. Cells with elevated intracytoplasmic Ca2+ concentrations were monitored with indo-1 acetoxylmethylester using ?ow cytometry to evaluate Ca2+ in?ux-inducing activity in serum. The amount of histamine released into the supernatant was measured using an enzyme immunoassay kit to evaluate histamine-releasing activity. In some assays, cells were incubated with pertussis toxin, a Gi-protein blocker. Some sera were treated with 56°C for 30 min for complement inactivation or treated with protein L beads to remove immunoglobulin.

Results: Values of Ca2+ in?ux-inducing activity were higher ($P < 0.05$) in sera from patients with reactions (27.68 ± 20.38 [n = 145]; range, 0.49-84.90) than in sera of patients without reactions (10. ± 6.50 [n = 54]; range, 2.67–36.97) and control sera (9.67 ± 5.88 [n = 107]; range, 1.11–25.68). Ca2+ in?ux-inducing activity was higher ($P < 0.05$) in patients with pure urticaria (mean, 30.58 ± 21.3 [n = 30]; range, 70.43-4.1) than in patients with fever alone (mean, 18.61 ± 14.71 [n = 18]; range,

45.94–3.19). Levels of histamine-releasing activity were higher (P < 0.001) in Ca2+ in?ux-inducing activity-positive sera than in Ca2+ in?ux-inducing activity-negative sera. Both Ca2+ in?ux-inducing activity and histamine-releasing activity were blocked by pertussis toxin. Ca2+ influx-inducing activities of pre-transfusion serum samples with reactions remained substantially after the treatment with protein L beads. In addition, heat treatment did not affect the elevated activity of Ca2+ influx-inducing activity of pretransfusion serum samples with reactions.
Conclusions: Pretransfusion sera from some patients who reacted adversely to transfusions exhibited elevated levels of Ca2+ in?ux-inducing activity and histamine-releasing activity in mast cells. These activities might be attributable to adverse reactions, especially to urticaria-like manifestation. The Gi protein-coupled receptor complexes on mast cells and its ligand must be involved in this activity.

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A REPORTING SYSTEM FOR BLOOD COMPONENTS IN TRANSFUSION REACTIONS IN A UNIVERSITY HOSPITAL: THE NATIONAL HEMOVIGILANCE SYSTEM

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Background: The revised guidelines for blood transfusion include information on safekeeping of the empty bag and collection of data regarding transfusion reactions. After white blood cell removal before manufacturing was introduced in Japan in November 2007, the expiration date of platelet concentrates was extended to four days. Accurate quantification of the bacterial contamination of blood components is difficult, and use of a transfusion reaction monitoring system in each hospital is important for exclusion of this risk.
Methods: The National Hemovigilance System is under development in Japan as a reporting system for all reactions due to transfusion, and has been used online in our hospital since July 2003. Empty transfused bags are returned to the division of blood transfusion and are stored for further investigation of reactions. When a serious effect has occurred, the residual blood component is collected and a bacterial culture of this blood and the patient's blood is performed. The lot is also transferred to the Japan Red Cross Blood Center for inspection.
Results: No hemolytic side effects were observed from January 2003 to December 2007, but non-hemolytic transfusion reactions occurred in 237 of 2 743 patients (6.3%). The frequency of non-hemolytic transfusion reactions was 524 in 52 772 bags (1.0%), and the frequencies of red blood cells, platelet concentrate, and plasma per bag were 0.5%, 4.2%, and 0.4%, respectively. Propionibacterium acnes was detected in 1 of 29 cases (4%) in which blood culture was performed.
Conclusion: Construction of a reporting system in cooperation with a hospital blood transfusion department is indispensable for tracing transfusion reactions, and development of the Hemovigilance System will contribute to transfusion safety in Japan.
This study was supported by a grant from the Ministry of Health, Labor and Welfare in Japan.

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ANTI-MAJOR HISTOCOMPATIBILITY COMPLEX CLASS I-RELATED CHAIN A ANTIBODIES IN BLOOD COMPONENTS IMPLICATED IN TRALI AND VOLUNTEER BLOOD DONORS IN JAPAN

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Background: Transfusion-related acute lung injury (TRALI) is a life-threatening adverse effect of transfusion that has been the leading cause of transfusion-related death. Antibodies to human leukocyte antigens (HLA) in donated blood have been implicated as a cause of TRALI. Supplying fresh-frozen plasma predominantly from male donors resulted in a substantial decline in TRALI cases in the United Kingdom.
Aims: As with HLA antigens, non-HLA antigens, such as the polymorphic major histocompatibility complex class I-related chain A (MICA), expressed on endothelial cells have been implicated in the organ allograft rejection. As neutrophils and endothelial cells are pivotal in the pathogenesis of TRALI, the prevalence of MICA antibodies was determined.
Methods: Detection of anti-MICA antibodies was performed by Luminex technology. Blood components implicated in TRALI (n = 11) and sera of randomly selected volunteer blood donors (n = 256; age 16–69 years) were tested by LABScreen MICA Single Antigen Antibody Detection Test (One Lambda Inc). Due to higher than acceptable background fluorescence with the MICA*019 beads, positive samples only for MICA*019 were not counted.
Results: HLA antibodies were detected in 7 of 11 TRALI blood components (four class I and five class II) and human neutrophil antigen antibody was not detected. Antibody to MICA was confirmed for one of 11 TRALI blood components (MICA*001, 007, 018). Unfortunately, typing of the TRALI patient for MICA antigen could not be done, because DNA of the TRALI patient was not available. Antibody to MICA was confirmed for 27 (10.5%) of 256 volunteer blood donors (male 134 and female 122). Male and female donors and young and old donors showed similar levels of sensitization to MICA.
Summary and conclusion: We identified one of 11 TRALI blood components as positive for MICA antibody. The prevalence of MICA antibody among volunteer blood donors was estimated around 10% in Japan. Further analyses of the role of MICA antibody in the pathogenesis of TRALI and the mechanism of MICA alloimmunization in Japanese blood donors are warranted.

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THE TRENDS OF DAILY BLOOD USAGES AND INVENTORY LEVELS: KOREA BLOOD INVENTORY MONITORING SYSTEM

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Background: There had been no national surveillance program for the collection and monitoring of blood use and inventory at hospitals. Korea Centers for Disease Control and Prevention (KCDC) has developed and run a web-based program for the National Surveillance System (KBIMs; Korea Blood Inventory Monitoring system) based on sentinel hospitals since December, 2008.
Aims: The purpose of the current study was to evaluate the results of blood usage, inventory levels by Korea Blood Inventory Monitoring System, and survey indicators of monitoring blood shortage.
Method: Hospital's daily data could transfer BMS (Web-based program for KBIMs, Blood Monitoring System) through semi-automated submission, and then the program automatically computes blood inventory level, daily blood usage and other some indicators monitoring. Total of 29 sentinel hospitals were participated in KBIMs and we collected voluntary reported data of them to analyze the characteristics of blood supply and usages in Korea.
The indicators of monitoring blood shortage such as the blood inventory ratio, the minimal inventory ratio, the ideal inventory ratio and the daily blood usage index has been developed and used to analyze the result from Dec. 2008 to Jun. 2009. 'The blood inventory ratio' is defined as ratio between the daily blood stockpile and average daily usage of past year. 'The ideal blood inventory ratio' is defined as ratio between the blood stockpile of given day and 5 times of average daily usage of past year. 'The minimal blood inventory ratio' is defined as ratio between the blood stockpile of given day and two times of average daily usage of past year. 'The daily blood usage index' is defined as ratio between the daily usage on a given day and daily average usage of past year. The blood inventory ratio

by the magnitude of RBCs usages levels (5,000~9999 units, 10,000~49,999 units and 50,000 + units, respectively) of each hospitals was analyzed by ANOVA test.

Results: The blood inventory ratio defined inventory blood for the based condition, daily inventory level on a given day/ daily average usage, and it was 4.32~6.98. The blood inventory ratio by the ABO blood type was 5.51 ± 0.49 for A types, 5.96 ± 0.49 for B types, 6.35 ± 0.44 for AB types and 6.23 ± 0.54 for O types. The blood inventory ratio by the magnitude of RBCs usages levels of each hospitals was significantly differ among the three group ($P < 0.001$). The ideal blood inventory ratio and the minimal inventory ratio were 0.86~1.40 and 2.16~3.49, respectively. The daily blood usage index was showed 0.64 ± 0.06 for weekend day and 1.07 ± 0.09 for weekday.

Summary/Conclusions: The blood inventory ratio of RBC products has been seen to have 5~6 times than that of average daily usage of past year Dec. 2008 to June 2009. Daily average usage is almost same as the last year and the lack of blood can not been seen. The blood inventory ratio was lowest in the group that the magnitude of RBCs usages level was the highest.

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BLOOD COMPONENTS CONTAINING HIGH-MOLECULAR-WEIGHT IGE INDUCE MAST CELL DEGRANULATION

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Background: Anaphylactic transfusion reaction (TR) is one of the major TRs in Japan. In most cases of anaphylactic TR, however, the mechanisms are unclear. During the haemovigilance survey among the cases of anaphylactic TR, we found a blood component that could activate cord-blood-derived mast cells in vitro. We also found that another blood component manufactured from the same donor was associated with anaphylactic TR.

Aims: We attempted to identify the mast cell activating factor contained in the blood component that induced mast cell degranulation.

Methods: Plasma samples were collected from the donor of the blood components. The mast cell activating factor was isolated in one of the plasma samples by three-step chromatography, and the mast cell degranulation activity of chromatographic fractions was examined. The proteins in selected fractions were separated by nonreducing SDS-PAGE, and the separated proteins corresponding to the detected bands were analyzed by tandem mass spectrometry. It was also examined whether the identified protein possessed mast cell degranulation activity.

Results: Two protein bands with high molecular weight (about 500 kDa and over) were detected by electrophoresis followed by silver staining of the chromatographically isolated fractions that activate mast cells. The proteins of the two bands with high molecular weight were identified as IgE by tandem mass spectrometry, which was confirmed by western blot analysis using an HRP-conjugated anti-IgE antibody. The mast cell degranulation activity of the fractions was decreased by incubation with an anti-IgE antibody or with standard IgE. The activity in the original plasma sample was also decreased by coincubation of the sample and standard IgE. The high-molecular-weight IgE (HMW IgE) and mast cell degranulation activity were also observed in the plasma samples isolated from the transfused blood component and collected from the same donor on other dates. Between 1997 and 2008, the mast cell degranulation activity, the IgE concentration, and the HMW IgE concentration in the donor plasma increased gradually and proportionally. Several high IgE sera from atopic patients without detectable HMW IgE did not show mast cell degranulation activity.

Conclusions: The mast cell activating factor contained in the blood components derived from the index donor is the HMW IgE. These blood components might have induced mast cell degranulation and anaphylactic TR in transfusion recipients, even though the recipients did not have antibodies to the components of the blood product or antigens recognized by the antibodies in the blood product.

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HAEMOVIGILANCE DATA FOR FIVE YEARS BY JAPANESE RED CROSS BLOOD SERVICE: TRANSFUSION-RELATED ADVERSE REACTIONS AND INFECTIONS FROM 2004 TO 2008

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Background: Japanese Red Cross Blood Service Headquarters has been collecting information about suspected cases of transfusion-related adverse reactions and infections from medical institutions via medical representatives since 1993. In case of severe or unknown reactions and infections, they are reported to the Ministry of Health, Labor and Welfare, in accordance with the Pharmaceutical Affairs Law. Analysis of the recipient's pre- and post-transfusion samples and repository samples of the implicated donations is performed to evaluate causal relationship between the events and transfusions. Repository samples of all donations are stored for 11 years for the purpose of look-back study and analysis of the suspected cases of transfusion transmitted infections (TTI).

Methods and results: A total of 9 194 suspected cases were reported for these five years (1 943 cases in 2004, 1 882 in 2005, 1 828 in 2006, 1 814 in 2007, and 1 727 in 2008) which included 7 949(86.5%) cases of non-hemolytic reactions, 1 051(11.4%) of suspected TTI, 142(1.5%) of hemolytic reactions, 30(0.3%) of suspected TA-GvHD, and 22(0.2%) related to plasma derivatives. Of the 7 949 non-hemolytic reactions, there were 2,771(34.9%) urticaria, 1 024(12.9%) febrile reactions, 887(11.2%) anaphylactic reactions, 1 289(16.3%) anaphylactic shock, 307(3.9%) hypotension, 861(10.9%) dyspnea, 212(2.7%) transfusion-related acute lung injury and 579(7.3%) others. 19 cases were excluded by physicians concluded there were no causal relationship to transfusions after reporting. The suspected TTI cases were evaluated using individual NAT of repository samples from the implicated donors or using blood culture of implicated components. If the repository sample is positive for the related virus, the viral genome sequence is compared to that from the recipient's sample. There were 54 HBV cases, 3 HCV, 6 HEV and 4 human parvovirus B19 which were likely to be associated with transfusion. Five cases of bacterial infection were possibly associated with the contaminated blood components proven by blood culture. A total of 54 cases of HBV infection were reported, which included 29 cases reported voluntarily from medical institutions, 19 revealed by look-back study, and 6 by the follow-up research. Of the 48 cases found by voluntary reports and look-back study, 31 implicated donations were in the window period, especially six were in the window period of individual NAT. The remaining 17 cases seem to be associated with donors with low viral load in chronic phase of infection. There were five bacterial infection cases.Three cases were related to platelet concentrate contaminated with Staphylococcus aureus (2) and *Streptococcus dysgalactiae* ssp. equisimilis (1), two were related to red blood cells contaminated with *Yersinia enterocolitica*. Fatal was one case with *Staphylococcus aureus*. No TA-GvHD cases were confirmed except one suspected case which was caused by blood from patient's family collected in the hospital and used without irradiation.

Conclusions: In order to improve transfusion safety, it is necessary to monitor the safety of transfusion by implementing national haemovigilance system together with the government and carrying out appropriately adverse reaction and infectious disease reporting system and look-back study. It will contribute to establishment of safety measures of blood components and products in the future.

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ELEVATION OF SERUM OR PLASMA TRYPTASE CONCENTRATIONS IN PATIENTS WITH ANAPHYLACTIC/OID SHOCK

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Background and objective: Little is known about the mechanisms that generate anaphylactic/oid transfusion reactions in many cases. We investigated whether the measurement of the change in the serum or plasma tryptase concentration in the patients who suffered from the transfusion reactions is useful to understand the mechanisms.
Methods: A total of 1382 cases of voluntarily reported nonhemolytic transfusion reactions were examined for patients' serum or plasma tryptase concentrations. Patients' serum or plasma samples collected in the hospitals before the start of the transfusion and after the occurrence of nonhemolytic transfusion reactions on the same day were examined. Tryptase concentrations of the samples were measured using the ImmunoCAP Tryptase kit (Phadia AB, Uppsala, Sweden) and the differences between those before and after the transfusion reactions were evaluated according to the categories of transfusion reactions such as anaphyalactic/oid shock, anaphylactic/oid reactions, urticaria, TRALI, hypotension, respiratory distress, febrile reaction, and others.
Results: There were statistically significant increases of serum or plasma tryptase concentrations of the samples collected after transfusion reactions in the categories including the cases of allergic transfusion reactions, such as anaphylactic/oid shock, anaphylactic/oid reaction, and urticaria. Especially in the category of anaphylactic/oid shock, the degrees of tryptase concentration elevations were highest. Apparent increase in tryptase concentration, ≥ 10 μg/L, was observed after transfusion reaction in 87/245 (35.5 %) cases with anaphylactic/oid shock.
Conclusion: In many cases of anaphylactic/oid shock, it is suggested that mast cell degranulation and tryptase release are suggested to be related to the development of the symptoms. The measurement of the serum or plasma tryptase concentration in the patients with anaphylactic/oid shock is thought to be valuable to analyze the mechanisms of the transfusion reactions.
Table to follow
Picture 1: http://www.eventure-online.com/parthen-uploads/img1_109941.jpg
Table 1: Increase in serum or plasma tryptase concentration

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RAPID DETECTION OF ANTI-PLASMA PROTEIN ANTIBODIES USING SURFACE PLASMON RESONANCE

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Background: Anti-plasma protein antibodies, produced in patients' blood, are considered a possible cause for anaphylactic transfusion reactions. For example, anti-IgA antibody, which is produced in IgA deficiency that is prevalent in Caucasians, and anti-haptoglobin (Hp) antibody, which is produced in Hp deficiency that is reported in Asians, are considered risk factors for severe anaphylactic transfusion reactions in these patients. Therefore, rapid diagnosis of individuals with anti-plasma protein antibodies is important to prevent the development of anaphylactic transfusion reactions and/or to support further transfusional requirements of these patients.
Aims: The aim of this study is to evaluate a new, rapid detection method for anti-plasma protein antibodies in patients' sera using surface plasmon resonance (SPR).
Material and methods: (1) Instrument: SPR signals caused by a change in mass generated by antigen-antibody interactions were monitored using a BIACORE2000 and analyzed using the BIAevaluation software (GE Healthcare co., Uppsala, Sweden) (2) Preparation of sensor chips: Purified plasma proteins, such as IgA, Hp and the ninth component of complement (C9) were immobilized on each flow path of a CM5 sensor chip using N-hydroxy-succimide and N-ethyl-N'- (3-dimethylaminopropyl) carbodiimide hydrochloride yielding three kinds of plasma preotein immobilized surfaces of ca.18000 resonance units (RU). 3) Detection of anti-plasma protein antibody: Serum samples collected from patients experienced nonhemolytic transfusion reactions and had previously been judged positive for anti-plasma protein antibodies using ELISA were assayed. Normal healthy donor sera were also assayed as negative controls. Sera were diluted in the running buffer, 10 mM HEPES, 3.4 mM EDTA, 150mM NaCl, 0.05%(V/V) Tween 20 (pH 7.4) and injected into the plasma protein immobilized-sensor chips followed by further injection of anti-human IgG mouse monoclonal antibody solution. Sensitivity, specificity and reproducibility of the SPR signals were analyzed.
Results: In addition to direct binding of the antibodies to the antigenic plasma proteins, the existence of IgG class antibody was confirmed by increasing signals from the anti-human IgG mouse monoclonal antibody bound to the patients' IgG antibody. Anti-plasma protein IgG antibodies could be detected when the extent of the increment of the signals from the anti-human IgG mouse monoclonal antibody exceeded 50 RU (approximately 50 pg/mm2). One cycle of the test, including regeneration of the sensor chip, was completed within 15 minutes. All 12 samples with anti-plasma protein antibodies, including 3 with anti-IgA, 8 with anti-Hp and 1 with anti-C9 were judged to be positive for antibodies against the respective plasma proteins. The observed levels of the SPR signals were reproducible.
Conclusions: This method using SPR was sensitive and easy to perform. The results are obtained within several minutes. This technique can be recommended to determine anti-plasma protein antibodies in patients with anaphylactic transfusion reactions.

7.2. Cellular Therapies Processing, storage and release

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DEVELOPMENT OF A NEW CLOSURE DEVICE CONCEPT TO OPTIMIZE WORKFLOW, WORKLOAD AND PRODUCT QUALITY IN BLOOD COMPONENT

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Background: Since the introduction of flexible blood bag systems the closure devices (bag breakers) remained nearly unchanged. Staff members often complain about mechanical damage of fingers, joints and tendons as well skin deteriorations that represent symptoms of Repetitive Strain Injuries (RSI). This, finally, may lead to haemolysed RCCs caused by insufficiently broken closure devices and additional sick leave of staff members which causes an enormous money drain from blood services.
Aim: Development of a technical concept to avoid RSI and consecutive haemolysis in blood products.
Methods: The redesign of the closure device alone would not have solved all above mentioned problems. A complete concept including an automated opening of the closures was developed consisting of blood bag systems containing a CompoFlow® closure and two different opening devices (blood component separator CompoMat G5® and battery driven handheld opener CompoSure®).
Results: During feasibility studies at Blood Research Center of CBS in Canada with CompoFlow blood bag systems the device showed an excellent performance with no impact on haemolysis or activation of platelets compared to standard breakers. The flow characteristics of CompoFlow were much better (free flow space nearly double). Preliminary data of more than 5 000 processed CompoFlow bag systems in combination with CompoMat G5 at German Red Cross BDS Baden-Baden and smaller studies in Modena/Italy and Mainz in combination with CompoSure clearly demonstrated the superiority of the concept. Processing time was significantly reduced even with the use of CompoFlow bag systems alone. During the field tests a decrease of 30–50% could be shown in combination with CompoMat G5.
Conclusions: Haemolysis is the most common form of noxa that beside leakages lead to an unnecessary discard of blood products. It is possible to reduce the occurrence of haemolysis by technical measures like the implementation of the Com-poFlow Concept. Further calculations will show not only the financial benefit but also a better supply situation for urgently needed blood products. On the other hand the CompoFlow system being an integrated solution to avoid RSI at the most critical production step of opening bag breakers adds additional benefit in respect of haemolysis avoidance. The concept is designed to keep the process of component preparation very simple with the highest staff satisfaction by avoiding painful pinch grip opening movements. In combination with the CompoMat G5 even staff in contact with blood bag systems will get fewer skin lesions, tendinitis and carpal tunnel syndrome. All this leads to lower staff replacement cost and a higher job satisfaction.

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ASSESSMENT OF T-CELL PROLIFERATIVE RESPONSES IN BLOOD UNITS GAMMA IRRADIATED WITH VARIOUS DOSES AND STORED SUBSEQUENTLY

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Background: Transfusion associated graft versus host disease can be prevented by treating cellular blood products with gamma irradiation (GI). Variations exist in the dose and routine protocol for GI.
Aim: To assess the influence of various doses of GI and subsequent storage on T cell proliferative responses in blood units.
Material and Methods: Whole blood units (N = 5 each) were GI using a Cobalt - 60 source on day-1 with 15Gy, 25Gy, 50Gy dose. Samples were collected at 1 hour, 24 hours, 72 hours, 5 days and 7 days intervals after GI and analyzed for PHA induced T- cell proliferation in the presence of IL2. Non-GI whole blood units (N = 5) served as controls.
Statistical Methods: Correlation analysis, Regression analysis.
Results: Compared to responses prior to GI, the proliferative response of lymphocytes was 34.1 % with 15Gy, 18.8% with 25Gy and 1.5% with 50Gy at one hour post- GI. Activated T- lymphocytes and NK cells (lymphocytes responding to IL2 alone) were more sensitive to GI. By day-3, a significant (50%) decline in the proliferative response of the T- lymphocytes was observed in the whole blood units stored without GI. This response further declined to a negligible level (<10%) by day-7. A linear co-relation between % T-cell proliferative response and dose of GI ($P < 0.001$) was seen at 1 hr post-GI. Thus the effect of GI on inactivation of T-lymphocytes was dose dependent. Regression analysis ($R2 = 0.82$) suggested proliferative response of 13% at 30Gy and 8% at 35Gy.
Conclusion: For GI of blood units more than 3 days old, a dose lower than 25Gy may suffice. However, a higher dose (35Gy) may be required for GI of fresh blood units for immediate release.

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DOES LEUCODEPLETION FOLLOWED BY GAMMA IRRADIATION PERFORMED AT VARIOUS TIME PERIODS DURING STORAGE AFFECT THE QUALITY OF RED BLOOD CELLS?

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Background: Leucodepletion (LD) and Gamma irradiation (GI) of red cell units affects their quality adversely. If not performed early during their storage period these interventions may lose their relevance. Currently at our blood centre LD within day seven and GI just prior to issue of Red cells is practiced.
Aim: To identify the safest and logistically feasibly protocol for LD and subsequent GI of red cell units.
Material and Methods: Six protocols were designed depending upon whether LD and / or GI were done on day1, day7, and day 14 of storage. Laboratory LD filters and blood irradiator with Cobalt (60) source were used for LD and GI respectively. Packed red cells (pRBC) (N = 30) with SAGM or Adsol were studied. pRBC units (N = 5) which were not LD or GI served as controls. All units were stored up to day 28, sampled every week and analyzed for hemolysis, K+, LDH, pH and hematological parameters.
Statistical Methods: Paired 't' test, Linear regression analysis.
Results: Hemolysis, K+ and LDH increased with storage. LD and GI, both caused a significant increase ($P < 0.01$) in hemolysis of red cells. The protocol of LD on day1 and GI on day14 with further storage upto day 28 was found to be the safest protocol ($R2 = 0.859$). LD performed beyond day 7 and GI beyond day 14 was associated with unacceptable levels of hemolysis on day 28 of storage. Leucodepletion showed significant ($P < 0.05$) reduction in GI induced hemolysis but had no effect on K+ leakage.
Conclusion: Due consideration to age of the unit and storage period after GI should be given. LD preferably on day 1 and certainly before day 7 and GI up to day 14 followed by subsequent storage for 14 days is suggested as safest and logistically feasible protocol.

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REPORTING OF ACTUAL AND NEAR-MISS EVENTS FOR TRANSFUSION MEDICINE: IMPROVING TRANSFUSION SAFETY IN ISFAHAN BLOOD TRANSFUSION ORGANIZATIN IN 2006-2007

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Background: Errors are common in blood banks but fortunately, Mortality and Morbidity are rare. If we focus in on them, it presents the system strengths and weaknesses before they occur then we may improve blood safety. This study was conducted to assess no-fault error reports and the corrective procedures.
Aims: This study was conducted to assess no-fault error reports and the corrective procedures.
Methods: In this prospective study, 201 reports from EBTO and four Hospital (Community) being sent over 12 months in 2008 were assessed. When the errors Occur, Employees were filled in the questionnaires then analyzed.
Results: The reports were 201(184 cases in EBTO and 17 cases in the hospitals). 99% (198 cases) was near-miss Events and actual events were in 2 cases. 75.5% were human errors that 55% (101 cases) related to computer registries in EBTO and 41% (7 cases) to sampling.
Summary and conclusions: Some near-miss events were unplanned and another planned such as using the algorhythm (1 to 5). During present study, IBTO software was upgraded but it is necessary to create a unique classified event reporting system with potential standard causes

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ENGRAFTMENT AND ADVERSE EFFECTS OF DIMETHYL SULFOXIDE-DEPLETED PERIPHERAL BLOOD PROGENITOR CELLS

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Background: Peripheral blood stem cell transplantation (PBSCT) with cryopreserved PBSCs is widely used for the treatment of hematological disorders. The most commonly used cryoprotectant is dimethyl sulfoxide (DMSO), which is associated with adverse effects when thawed PBSCs are infused without its depletion.
Aims: The aim of this study was to assess whether the depletion of DMSO from thawed PBSCs is safe for hemopoietic cell engraftment and reduces adverse effects associated with DMSO.
Methods: From December 1992 to September 2008, 48 and 21 patients received autologous and allogeneic PBSC transplants, respectively. In the autologous PBSCT group, PBSCs were mobilized with high-dose chemotherapy following granulocyte colony-stimulating factor (G-CSF) administration. Conditioning regimens were high-dose chemotherapy regimens. In the allogeneic PBSCT group, donors were administrated G-CSF to mobilize PBSCs. Conditioning regimens were myeloablative or reduced-intensity regimens. PBSCs were collected using an automated continuous-flow blood cell separator (Spectra; Cobe, Englewood, CO). Sixty ml of the collected PBSC solution was mixed with 40 ml of RPMI-1640 medium supplemented with 2 000 units of heparin. Then, the cryoprotectant solution containing 50 ml of 24% hydroxyethylstarch, 10 ml of 99% DMSO (CP-1; Kyokuto Pharmaceutical Industrial Co., Ltd., Tokyo), 32 ml of 25% human albumin, and 8 ml of saline was added. The final concentration of DMSO was 5%. The bags containing the cell mixture were stored at -80 or -120?C for one year. On the day of transplantation, cryopreserved PBSCs were thawed in a 37°C water bath and centrifuged to remove the supernatant. The solution of 200 ml of RPMI-1640 medium supplemented with 30 ml of acid citrate dextrose (ACD-A) solution (RPMI/ACD) was replaced and mixed. Before the infusion of PBSCs, patients received premedication with 100 mg of hydrocortisone. G-CSF was administered to 68 of all 69 patients after PBSCT.
Results: In the autologous PBSCT group, the median number of colony-forming unit granulocyte-macrophages (CFU-GM) before cryopreservation and infusion was 9.1–10e5 (29 patients) and 5.3–10e5 cells per Kg body weight (48 patients), respectively. Similarly, in the allogeneic PBSCT group, the median number of CFU-GM before cryopreservation and infusion was 6.3–10e5 (9 patients) and 7.2–10e5 cells per Kg body weight (21 patients), respectively. The median neutrophil recovery (≥ 0.5–10e9/L) in the autologous and allogeneic PBSCT groups was 11.5 and 13.4 days, respectively. The median platelet recovery (≥20–10e9/L) without platelet transfusion in the autologous and allogeneic PBSCT groups was 12.9 and 23.2 days, respectively. Late engraftment failure was observed in one patient. Nine patients showed mild adverse effects related to the infusion of PBSCs as follows: grade 1 gastrointestinal symptoms, six patients; grade 1 headache, one; and grade 2 headache, two.
Conclusions: Our results suggest that the depletion of cryoprotective agents from thawed PBSCs and then replacement by RPMI/ACD may be safe for hemopoietic progenitor cell engraftment and reduce the toxicities of cryoprotective agents.

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TEST NAT APPLICATION TO BLOOD CORD DONATION

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Background: The use of the staminal cells from blood is a complementary therapy to the transplation of bony marrow for both adult patients and pediatic age with or without hematologic neoplastic disease. To this end have been prepared units of cord blood cells collected and stored in delivery room. Some controls have been made in order to check the stability during the storage and to establish the immunologic features of compatibility between donor and recipient. The Campania region, following ordinance number 219, October 21, 2005, has promoted the opening of such 'cord banks'. As a reference centre of NAT testing (A.O.R.N.A. Cardarelli, Naple,Italy) we are receinving, since June 2003, from the cord bank Center Basco A.O. Pausilipon, blood samples from cord donors.
Methods: Till now our center has tested 1 924 samples from the cord bank Basco using the NAT Procleix Ultrio assay, by Chiron, Novartis company, in single unit. This assay allows to detect three viruses (HCV and HIV RNA and HBV DNA) in a short time (100 samples in four hours and half).
Results: Two samples were reactive to the NAT testing. The following esecution of the Discriminatory test has allowed to define an HCV RNA and HBV DNA reactivity resplectlively. The samples are reactive also to the serological research of anti-HCV and HBsAg.
Conclusions: Frequentely a donor of biological material is not aware of his reactivity to an viral agent and he learns it only after an occasional event as a blood donation. The donation of cord cells should follow the same procedure used for the blood donation, specially because of his storage that could be a risk for both a potential recipient and the other units stored in the same 'cord bank'. It's therefore needed the screening for viral agents using NAT testing, as for blood units, immediately after the cord donation and before proceeding to cryoconservation.

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PLATELET GROWTH FACTORS ENHANCE OSTEOGENIC DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELLS ON CALCIUM PHOSPHATE SCAFFOLDS

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Introduction: Mesenchymal stem cells (MSCs) are nonhematopoietic stem cells that are typically obtained from bone marrow. They were first

described by Fridenstein et al. in 1976, which identified an adherent, fibroblast-like population in adult bone marrow. MSCs reside in diverse tissue such as adult and fetal bone marrow, umbilical cord blood and adipose tissue. MSCs are multipotential cells capable of differentiating into osteoblast, chondrocytes and adipocytes. Mesenchymal stem cells applied to bone substitution materials can improve bone haling. Bone healing is mediated by different growth factors. The major source of autologus growth factors (PDGF, TGF, EGF, IGF, VEGF) are localized in platelets. Growth factors are released upon activation of thrombin and calcium at fracture site. The aim of our study was to investigate the effect of platelet growth factors on the differentiation of human mesenchymal stem cells.

Material and methods: Human MSCs were obtained by aspiration of 10 ml of bone marrow from normal volunteer donor. MSCs were seeded in DMEM-LG medium with 10% FBS and incubated at 37°C with 5% humidified CO2. When confluence was reached at 80–90%, the cells were applied for osteogenic differentiation. The platelet gel is formed by adding calcium and thrombin to platelet rich plasma (PRP). Treated PRP was incubated and then Sample was centrifuged and platelet gel supernatants harvested. Expanded cells were seeded on calcium phosphate scaffold and then supplement with 10% FBS (control) and 10% platelet growth factors (test). After 15 days, Cells growth and morphology on scaffold were analyzed by a scanning electron microscope (SEM). Total alkaline phosphatase activity measured to confirm osteogenic differentiation.

Results: The results of SEM demonstrated that calcium phosphate scaffold has the mechanical stability to hold the cells. The entire scaffold was covered with differentiated osteoblast by day 15 of cultures. The alkaline phosphatase activity of differentiated cells was higher in the culture with platelet growth factors. We demonstrated that platelet growth factors provide a significantly higher effect on osteogenic differentiation MSCs than FBS.

Conclusion: platelet growth factors can be used in place of FBS to provide a safer and more effective culture condition to differentiated MSC for clinical purpose.

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STORAGE OF PLATELETS WASHED WITH M-SOL AND COMMERCIALLY AVAILABLE ADDITIVE SOLUTIONS

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Background: The platelets (PLTs) washed with additive solutions (plasma carryover, approx. 5% or less) have been used successfully in patients who cannot tolerate adverse reactions such as anaphylaxis or febrile non-hemolytic reactions after PLT concentrate (PC) transfusion. Previously, we developed a novel additive solution (M-sol) for PLT storage (TRANSFUSION 2007; 47: 960–965). The M-sol is prepared by mixing solutions approved for clinical use. Here we studied in vitro functions of PLTs washed with M-sol and other additive solutions such as PASIIIM and Composol.

Methods: The outdated PC (processed four or five days after collection) was divided into two equal aliquots (control group and test group). After centrifugation (2560 g, 10 minutes) of both aliquots and removal of supernatants as much as possible, pellet of control group was resuspended in M-sol and that of test group was resuspended in PASIIIM or Composol (day 0). These washed PLTs (173 ± 2 mL; 80.4-e4±8.9-e4 /uL) were stored at 20–24°C on flatbed shaker (50–60 cycles/min) in poly-olefin bag (KBP-1000FPN, Kawasumi Co., Ltd, Tokyo, Japan). The PASIIIM and Composol were prepared according to the previous report (TRANSFUSION 2006; 46: 236–243). The pH, mean PLT volume (MPV), percent hypotonic chock response (HSR) and PLT aggregation were measured by standard methods. P-selectin expression (%) on PLTs was measured by flow cytometry with fluorescence-coupled antibodies to the cell surface antigens CD62P and CD61. These parameters were measured on day 1, 3 and 7.

Results: The pH values of PLTs washed with M-sol (M-sol PLTs), PASIIIM (PASIIIM PLTs) or Composol (Composol PLTs) were well preserved up to day 7. The P-selectin values of M-sol PLTs were not significantly different from those of PASIIIM PLTs and Composol PLTs up to day 7. The MPV values of PASIIIM PLTs and Composol PLTs were significantly higher than those of M-sol PLTs on day 7. The percent HSR of PASIIIM PLTs and Composol PLTs were significantly lower than that of M-sol PLTs on day 3 and 7. The collagen and ADP induced aggregation of PASIIIM PLTs and Composol PLTs were weaker than that of M-sol PLTs on day 3 and 7.

Conclusions: The in vitro functions of M-sol PLTs were maintained better than those of PASIIIM PLTs and Composol PLTs on day 3 and 7. The results indicate that, in terms of the ability to preserve PLTs stably in low plasma concentrations (5% or less), M-sol is better than PASIIIM and Composol.

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THE CORRELATION OF RED CELL FRAGILITY WITH GLUCOSE LEVEL AND PH IN PACKED RED CELL AT STORAGE

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Background: The good Packed Red Cell (PRC) must meet the criterion that at least 70–80% of red cells viable or circulate in the recipient's circulation 24 hours post transfusion. According to Wolfe, viability correlates with the osmotic fragility of red cell membrane and loss of ATP. PRC with CPDA-1 as preservative can be stored up to 35 days, but UTDD PMI DKI Jakarta only permits a 14-day dating period. The shelf life of red cell was determined based on reports that patients who had received one month- old blood become icteric. Beside that, Priyatna et al showed that there was a significant difference of ATP level in fresh PRC compared to 2 weeks stored-PRC. ATP is one of the glycolysis product beside lactic acid, using glucose as substrate. If ATP correlates with fragility, do glucose and acidity also correlate with fragility?

Objective: To analyze the correlation of red cell membrane fragility with glucose and pH in PRC.

Methods: PRC were stored at 4 ± 2C for 0, 7, 14, 21, 28, and 35 days in cold room. Glucose, pH and osmotic fragility test were performed using samples from PRC. The data were analyzed statistically.

Results: There were significance decreased of glucose, pH and membrane fragility ($P < 0,05$). The decreasing of glucose at each observed time is significance if compared with glucose at 0 day because the glucose were consumed for glycolysis. pH was also decreasing from 7,56 ± 0,03 in day 0 to 6,81 ± 0,03 in day 35 because of lactate accumulation. The result of osmotic fragility test shows that on day 7, 23,45 ± 2,63% of red cell has hemolyzed and on day 14, 75,47 ± 2,18 % of red cell has hemolyzed. The correlation between glucose and pH, glucose and membrane fragility, pH and membrane fragility were strong and significant ($r > 0,8$ and $P < 0,05$). It showed that glycolisis is proceed in stored red cell but because no glucose were added and pH decreased, thus the glycolysis diminished. As the consequences,the red cell membrane fragility increased.

Conclusion: There is a correlation of red cell membrane fragility with glucose and pH in PRC.

Table 1: Correlation between glucose and OFT

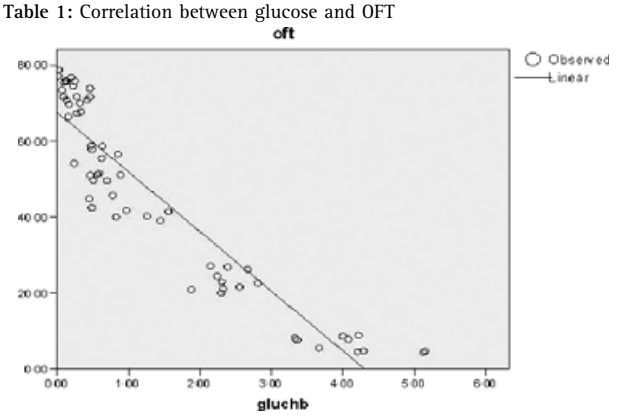
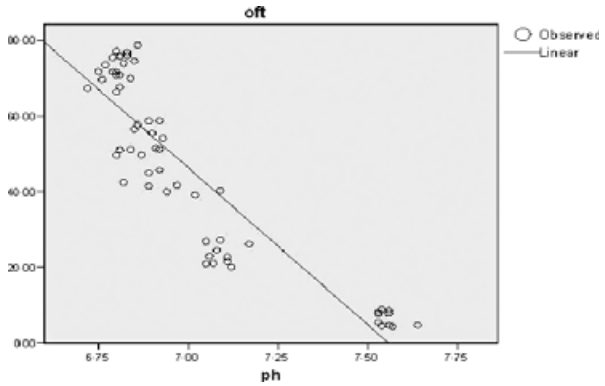


Table 2: Correlation between pH and OFT



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EFFECT OF PRE-STORAGE LEUCOREDUCED AUTOLOGOUS BLOOD ON EDEMA AND PAIN IN LEGS AFTER ORTHOPEDIC SURGERY

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Background: Leucofiltration of red blood cell products can reduce the number of platelets (PLTs) to approximate 5% of their pre-filtration level. This may be important because PLTs break down into microparticles during storage. We have already demonstrated that the increase of microparticles seen in unfiltered autologous blood is almost entirely suppressed by pre-storage leucofiltration. As platelet-derived microparticles have pro-coagulant activity, it may be that pre-storage leucofiltration of autologous blood can reduce unfavorable thrombotic events after orthopedic surgery.
Aims: To evaluate the clinical impact of filtering autologous blood collected for transfusion, we studied whether pre-storage leucoreduction could reduce clinical symptoms related to deep vein thrombosis (DVT), such as edema and pain in legs after orthopedic surgery.
Methods: This comparison trial took place at a single university medical center, and was approved by its institutional ethics committee. All patients signed written informed consent after the study objectives were explained to their satisfaction. Based on date of birth, patients were assigned to receive either leucoreduced (LR) or non-leucoreduced (N-LR) autologous blood, as processed and stored in approved autologous collection sets. As indices of DVT-related complications, edema and pain in legs after post-operative day 3 were investigated. Symptoms in the operated joint were excluded. Most patients were pretreated with heparin or factor Xa inhibitor for DVT prophylaxis.
Results: Five hundred sixty six patients were analyzed in this study (LR group: 287, N-LR group: 279). Only two cases in the LR group and one case in the N-LR group were diagnosed with DVT. The fraction of cases with edema and/or pain of legs after autologus blood transfusion in the LR group (95/279, 34%) versus the N-LR group (132/287, 46%) achieved statistical significance (Chi-square test, $P < 0.01$).
Conclusion: These results suggest that pre-storage leucoreduction of autologous blood reduces complications such as edema and pain in legs after orthopedic surgery. However, the difference in the occurrence of the symptoms between two groups is only 12%.

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A COMPARISON STUDY: TOTAL NUCLEATED CELL RECOVERY IN UMBILICAL CORD BLOOD COLLECTION BAGS

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Background: Stem cells derived from umbilical cord blood provides a life saving source of cells for transplantation to treat many diseases. Transplant success depends upon engraftment, and to optimize this clinical response an increased number of hematopoietic stem cells are required. Total nucleated cell count (TNC) is a screening parameter used as a global measure to predict the number of stem cells contained within each unit. Criteria has been established that allows the bank to screen units in order to proceed with processing, testing and banking of a unit likely to be selected for transplantation.
Aim: Technique and supplies used for collection of cord blood can impact the success of the volume retrieved. This report outlines our experience in the evaluation of two different collection bags for umbilical cord blood.
Methods: Cord Blood Units (CBU) were collected at hospitals following a baby's birth. The in-utero collection method was used for all units in the study. Products evaluated included the Baxter® and Pall® bags, both containing 35 mL of Citrate Dextrose Phosphate (CPD). Collection weights of units ranged from 80g to 110g. A comparison of products was conducted by capturing the following data points: 1) Weight of CBU; 2) Pre-processing TNC; and 3) Time from collection to testing (<24 hours or >24 hours).
The Sysmex® analyzer measured TNC for both products. Forty products were selected, twenty using the Baxter® bag and twenty using the Pall® bag. The storage/transport conditions for all units in the study were identical.
Results: The results are shown in Table 1 as follows.

Collection Bag Manufacturer	Time from Collection to Processing	Weight Range/ Unit	Pre-TNC (10e9) Value
Baxter® CPD	< 24 hours	80 – 110g	0.3925
Baxter® CPD	24-72 hours	80 – 110g	0.3515
Pall® CPD	< 24 hours	80 – 110g	1.2531
Pall® CPD	24-72 hours	80 – 110g	0.9681

Comparison showed a significant difference in TNC recovery between collection bags with the greater recovery in the Pall® bag. Time interval from collection to processing was measured and showed a slightly lower TNC recovery at greater than 24 hours for both products. In an earlier study, TNC recovery was found to significantly decline in units collected at greater than 72 hours prior to processing/testing. Ease of use for each product was evaluated and both were equally viable in operation. The comparison of the ''pre''versus ''post''processing TNC values was not found to be a variable in this study. The results of the study have supported the selection of the Pall® CPD sterile collection bag. As the Pall® bag is sterile, it eliminates the use of an additional sterile connecting device for caesarean section deliveries.
Summary and conclusions: This study resulted in the selection of the Pall® CPD collection bag due to the increased recovery of TNC and ease of use. In addition, this bag allows for modification and improvement to the existing kit packaging, thus offering a cost savings in transportation fees as well as elimination of the additional sterile connecting device.

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AN AUTOMATIC SYSTEM FOR BLOOD COMPONENTS IDENTIFYING,PRINTING AND LABELING,CLASSIFYING AND COLLECTING

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Aim: To design an automatic system for packed blood components identifying, printing and labeling, classifying and collecting to replace a manual operation.
Method: The system consisted of 8 function modules:
(1) Packed blood components feeding module;
(2) Barcode reading module;
(3) Color discrimination module(which can identify the kind of component, only between the red blood cell and plasma);

(4) Components weighting module(which can distinguish 1U or 2U of red blood cell,and content of plasma);
(5) Printing and labeling module(the final big label);
(6) Check point module(compare the barcode in final label with the initial one, in order to make sure it is correct);
(7) Classification and collection module(using a magic hand to classify the A,B,O,AB types components and pick out the test-positive components);8)information center (a computer including specialized software).
Results: 2500 packed blood components were processed using this system. The speed of process was nearly six pieces per minute for operation by one worker. 2491 of 2500 were processed without problems. The bar codes on nine bags could not be read and these were rejected at the checking stage. All the test-positive components were picked out.
Conclusion: This automatic system can simplify the process after blood components have been separated. It can increase the efficiency and decrease the time of components at room temperature.

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TRANSFUSION OF PLATELET CONCENTRATE WASHED TWICE WITH M-SOL PROVIDED ADEQUATE POST-TRANSFUSION PLATELET COUNT INCREMENTS IN AN ANHAPTOGLOBINEMIC PATIENT WITH ANTIHAPTOGLOBIN ANTIBODIES

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Background: Depletion of plasma from platelet concentrate (PC) is often useful for patients who have recurring adverse reactions to plasma-containing PC. In these cases, PC is usually washed only once with saline solution including ACD-A solution (A-SOL) or various medium. Here, we report a haptoglobin deficiency patient with antihaptoglobin antibodies for whom PCs washed twice were needed to prevent transfusion-related adverse reactions. There was quite a difference of clinical effects after transfusion of washed PC depending on the washing medium, namely A-SOL and a novel additive solution, M-SOL.
Methods and results: An 8-year old boy with acute lymphoblastic leukemia received intensive chemotherapy in our hospital. He had shown no significant transfusion-related adverse reactions despite transfusion of 14 units of leukocyte-reduced red cells concentrates (RCC-LR) and 150 units of leukocyte-reduced PC (PC-LR) (1 unit = 0.2x10E11cells). However, he developed hypotension, respiratory distress, skin rash, and cough after transfusion of 2 units of RCC-LR in December 2008. He again complained of wheezing, respiratory distress, cough, and itching after another transfusion of RCC-LR. Even when he received RCC-LR washed once with M-SOL, he developed itching and urticaria. Since analysis of his serum revealed that he had haptoglobin deficiency and antibodies against haptoglobin, we decided to wash both RCC-LR and PC-LR twice afterward. When PC-LR was washed once with A-SOL followed by M-SOL at the Mie Red Cross Blood Center in Japan, transfusion of the washed PC-LR significantly increased the platelet levels without adverse reactions. However, because the center stopped washing PC-LR in March 2009, we decided to wash PC-LR by ourselves. We washed PC-LR twice with only A-SOL and the washed PC-LR was transfused to the patient immediately after washing. Despite the transfusion of 10 units of washed PC-LR on March 30, platelet levels in the patient continued to decrease from 24 000/μl on March 30 to 17 000/μl on March 31 and to 9 000 /μl on April 1. Platelet levels were not changed again after transfusion of another 10 units of washed PC-LR prepared in the same way. We therefore changed the washing medium from A-SOL to M-SOL and found that, after transfusion of 10 units of PC-LR washed twice with M-SOL, platelet levels increased from 8 000 /μl on April 2 to 36,000 /μl on April 3. Platelets levels further increased to 54 000/μl on April 4 after another transfusion of PC-LR washed with M-SOL. Thereafter, in spite of frequent transfusion, PC-LR washed twice with M-SOL has provided adequate post-transfusion platelet count increments until now.
Summary: Our case suggests that it is important to use appropriate medium such as M-SOL at least at the second round of washing process when we wash PC-LR more than twice before transfusion.

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ESTABLISHMENT OF STANDARDS FOR PROCESSING CELLULAR THERAPY PRODUCT ROUTINELY USED FOR HEMATOPOIETIC STEM CELL TRANSPLANTATION IN JAPAN

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In Japan, about 4 000 hematopoietic stem cell transplantations (HSCT) are currently performed for various hematologic and non-hematologic disorders in about 200 hospitals per year. However, there have been no regulations or guidelines for processing cellular therapy product routinely used for HSCT. Therefore, the Japan Society of Transfusion Medicine and Cell Therapy (JSTMCT) in collaboration with the Japan Society for Hematopoietic Cell Transplantation (JSHCT) are planning to establish guidelines 'Japanese Standards for Processing Cellular Therapy Product Routinely Used for Hematopoietic Stem Cell Transplantation' for all hospitals and related personnel performing HSCT. The objects of this guideline are all the hospitals and related personnel where HSCT is performed. According to the nation-wide survey performed by JSTMCT, it is likely that the numbers of medical staffs and equipments are insufficient in many hospitals. Although this guideline is based on the world-wide standard, FACT-JACIE 3rd edition and are intended to be minimum standards, some modifications were made to reflect the present situation of most hospitals. The guideline includes; 1 Objective, 2 Application, 3 Product Collection, 4 Personnel, 5 Equipment and Facility, 6 Policies and Procedures, 7 Distribution, 8 Storage and Thawing, 9 Sample Storage, 10 Infusion, 11 Disposal, and 12 Review. Appendices include outlines of each procedures related to transplantation and examples of standard operation procedure (SOP) and record forms. The established standards are to be uploaded at the JSTMCT website so that each individual could access and download SOP and record forms, which can be revised for use at each hospital. Now the draft is ready for the public comments and is expected to be published by the end of this year. The accreditation system is also planned to be established after these Standards are published.
Recently, peripheral blood stem cell (PBSC) collection is under consideration to be included in the unrelated donor program by JSHCT, JSTMCT and Japan Marrow Donor Program (JMDP). All hospitals and physicians involved in the unrelated donor program also shall be required to implement the specific standards for safe execution of harvesting, processing, cryopreservation and shipping of bone marrow and PBSC products.
This project is supported by grant from the Ministry of Health, Welfare and Labor of Japan.

7.3. Cellular Therapies Clinical developments

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AGE OF TRANSFUSED BLOOD: MANAGEMENT IS MORE IMPORTANT THAN PHYSIOLOGY

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Background: There is controversial data on the influence of the age of transfused blood on short-term mortality. The goal was to examine if this is observed in our multisectoral clinic.
Methods: We compared the age of red blood cells transfused to patients with the different outcomes in a multisectoral hospital in 2008. A total of 818 patients received 2085 red blood cell (RBC) units. A total of 63 recipients who died in hospital were transfused with 335 RBC units and 755 survived recipients received 1750 RBC units.
Results: The duration of storage for RBCs transfused to patients with lethal outcomes was 11.1 % less than in the group of survived patients ($t = 3{,}43$; $P < 0{,}001$). The quantity of blood stored more than 14 days prior to transfusion also was 19.6 % more for the survived patient group ($\chi 2 = 7{,}66$; $P = 0{,}0056$). Patients with lethal outcomes received 36.1 % more transfusions during night hours and public holidays in comparison with survived patients ($\chi 2 = 18{,}22$; $P < 0{,}001$). Only 38.3 % (798 units) of red cells were prepared in plastic bag systems with additive solution (CPD/SAGM). 7 units of washed RBCs were transfused to survived patients. Other cells were prepared from whole blood with CPDA-1 and contained a volume of plasma. The number of RBC in additive solution was 17.3 % more for the survived patient group ($\chi 2 = 3{,}96$, $P < 0{,}05$).
Conclusions: Transfusions during public leisure-hours and transfusions of RBCs in CPDA-1 were associated with a reduced short-term survival. Our retrospective study did not demonstrate a link between mortality and older blood transfusion units.
Acknowledgment We thank Anton Shchepetov and Iwona Walicka for help with translation

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AUTOLOGOUS RICH-PLATELET GEL USED IN ORTHOPAEDICS

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Various growth factors are involved in the fracture healing process. The most important of them are: transforming growth factor b (TGF-b1, TGF-b2), insulin like growth factor (IGF), platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF). Platelet cells contain a big concentration of these factors. Lastly these factors are used often in orthopaedics and trauma clinics as concentrated platelet gel.
In this study we bring our experience of using this rich-platelet gel in orthopaedic surgery aiming to evaluate the efficacy of a platelet gel rich in growth factors in the treatment of pseudoarthrosis. During a period of one year (June 2008-June 2009) we used the rich-platelet gel in 29 patients in orthopaedic clinic. All these patients had a pseudoarthrosis (7 in upper extremity and 22 in lower extremity) non responding by previous interventions. Regarding to their age there was a variation from 29 – 83 years old. All the treated patient were not infected. The reason of pseudoarthrosis was the internal fixation failure or broken synthesis plate or atrophic pseudoarthrosis. Before the intervention from all the patients were taken 36 ml of their own blood in tubes with anticoagulant substance (sodium citrate). This blood were centrifuged for 6 minutes in 1100 r/min and the separated plasma with platelet was dropped in another tube containing calcium chloride helping the gel forming and centrifuged for 15 minutes in 1500 r/min. The rich-platelets gel for 30 minutes was ready to use in operating theatre. All the procedure was done in strong sterility conditions. After the fracture synthesis and before closing the wound was induced in the decorticated fracture site the rich-platelets gel.
The patients were followed up in time and we took X-ray data about bone consolidation. After four weeks 18 of patients had initialized bone healing, referring to the X-ray result where the callus was present. At the 8-th week 30 patients had good result of callus formation. 1 patient had a second trauma during the treatment on the 7-th week. At the 16-th week 19 patients had good bone consolidation and we consulted the patients to have controlled full weight bearing motions. After 20 weeks all the patients had good bone consolidation and free motions.
The application of rich-platelet gel in treatment of bone tissue regeneration, combined with bone synthesis material (plate and screws or external fixation) appropriately into the overall management and multidisciplinary care of the patient, is being shown to be effective. We found a clearly faster response as well a much higher percentage of healing than in the cases previously treated without this therapy.

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INVESTIGATION INTO THE BLOOD TRANSFUSION STATE OF 73 JAPANESE WITH URINARY BLADDER CARCINOMA (CA)

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Seventy-three Japanese diagnosed with bladder carcinoma (Ca) from April, 2007 to June, 2009 were studied here to investigate into their blood transfusion state. Their bladder Ca belonged to urothelial Ca of G2 to G3 combined with pT1 to pT4Tis invasion. Among them, 18 (25%) cases received an operation of total bladder and prostate resection. The 18 showed the lowest serum calcium of 7.1 mg/dl with mean hemoglobin of 10.2 g/dl. Fibrinolysis was a characteristic feature, in which they showed low levels of plasminogen and antithrombin III and prolonged times of PT and APTT. Ten (55%) of the 18 were transfused autologous blood of 2–6 (4.4) units (U) and 7 (70%) of the 10 were injected with erythropoietin (Epo) before their blood collection. Among the 7 with Epo, 5 cases had a bad prognosis based on advanced Ca invasion of pT3a to pT4Tis. During the operation, each case of the 18 was transfused mean U of allogenic blood as follows: red blood cells (RCC-LR) of 6.7 U and plasma (FFP) of 6.9 U. Five of the 18 were transfused only autologous blood. The case bled massively had RCC-LR of 18 U, FFP of 14 U and platelet (PC) of 10 U. Ten of the 18 and 15 of non-operative 55 had unexpected blood transfusion. Many of them had pre- and post-operative bleeding episodes and bleeding episodes from bladder Ca spontaneously or after biopsy. Three with severe anemia of chronic renal failure were treated with Epo. Three (4%) of the 73 died of advanced Ca metastases, whose abnormal coagulation became a dangerous factor of thrombosis with fibrinolysis

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PRE-TRANSFUSION TESTS IN PATIENTS PREPARING FOR ELECTIVE SURGICAL, ORTHOPAEDIC AND GYNAECOLOGICAL INTERVENTIONS

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Introduction: Pre-transfusion testing includes several immuno-haematological tests of the blood samples from the patient and the donor (determination of ABO and RhD blood group, screening tests for the detection of presence of clinically important anti-erythrocytes antibodies and their identification, compatibility test), in order to obtain safe blood for transfusion and to prevent hemolytic post-transfusion reaction.
Aim: Routine detection of the presence of anti-erythrocytes antibodies in the serum in patients preparing for operation has a great advantage over detection of antibodies by performing cross-reaction, because the tests are done before the transfusion so that there is enough time for identification of the antibodies, determination of their clinical importance and obtaining

compatible blood for transfusion. Moreover, compatibility test is not necessary for operations for which big blood loss is not expected.
Method and materials: At the Department of Transfusiology,,General Hospital,, Strumica, in 2008 were taken 295 blood samples from patients preparing for surgical, orthopaedic and gynaecological interventions for which a blood loss of less than 500 ml. is expected and for which there is usually no demand for blood for transfusion. The following tests were performed for all of them:
1. Determination of ABO and RhD blood group using two methods:
- With anti A, anti B, anti AB and anti D, monoclonal tests serums on a tile.
- With microgel agglutination technique with microcards (DaiClon ABO/RhD for patients). For reverse grouping test erythrocytes O.A.B. are used.
2. Detection of anti-erythrocytes antibodies:
- By using IAT in microcard with added anti IgG, anti C3 and Liss, as well as double cell panel (microcard ID Liss/Coombs and ID DiaCell I + II).
3. Laboratory tests
4. Screening haemostatic tests.
Results: Out of 296 patients, 131 were diagnosed with,Cholecistitis chronica calculosa'', 95 with, Myoma uteri'', 25 with fracture of the forearm or the lower leg, 19 with, Prolapsus uteri'', 13 with, Cystis ovarii'', and 12 with, Gangrena pedis''. ABO and RhD blood group were determined for all patients, and screening for anti-erythrocytes antibodies was performed. There was positive IAT in 5 patients (between 1+ and 3+). The laboratory tests and screening homeostasis were normal. Compatibility test was done only for those who had positive IAT, in order to ensure compatible blood for transfusion. For all the patients blood/blood components requests and blood samples were submitted. During the operation only 24 patients needed blood and blood components. The requests for blood for these patients were made by phone, as the forms for blood/blood components request had been filled in and submitted before the operation.
Conclusion: For elective operations for which small loss of blood is expected, when the tests are done completely before the operation, only the ''type and screen''test is enough from the immuno-heamotological tests, without conducting compatibility tests. In this way, there is more rational use of blood and there is no need of keeping reserved blood. On the other hand, the previously conducted tests guarantee that there will not be any difficulties in supplying compatible blood for transfusion, if the need arises.

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DEEP VEIN THROMBOSIS SCREENING OF HIP ARTHROPLASTY PATIENTS IN LEUKOREDUCED AND NON-LEUKOREDUCED AUTOLOGOUS WHOLE BLOOD COHORTS

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Background: Leukodepletion of allogeneic blood components is beneficial to certain patient populations, but possible benefits of pre-storage leukodepletion of autologous whole blood are unclear. We recently reported that prestorage leucofiltration prevented the accumulation of platelet-derived microparticles in whole blood derived from healthy volunteer donors. Because platelet-derived microparticles might participate in coagulation, inflammation and immunity, we expected that prestorage leukodepletion might reduce the risk of postoperative embolic events such as deep vein thrombosis in orthopedic surgery patients.
Aims: To compare surgical patients for evidence of deep venous thrombosis, from matched cohorts of those whose autologous blood was, or was not, leukodepleted.
Methods: This randomized control, cohort study trial took place at a single university medical center, and was approved by its institutional ethics committee. All patients signed written informed consent after the study objectives were explained to their satisfaction. From January 2008 to June 2009, 215 patients undergoing elective total hip arthroplasty were enrolled. Patients were randomly assigned to either prestorage leukoreduced autologous whole blood transfusion (LR group) or conventional unfiltered autologous whole blood (non-LR group). Leukodepletion for the LR group was done with the Sepacell Integra system (Asahi Kasei Medical, Tokyo, Japan), while blood from non-LR group patients was stored without filtration. Perioperative prophylaxis for deep vein thrombosis included mechanical thromboprophylaxis with IPC (intermittent pneumatic compression) and GCS (graduated compression stocking) as long as possible. Pharmaceutical prophylaxis, including low-molecular-weight heparin, followed ACCP (American College of Chest Physicians) recommendations without hemorrhagic complications. Duplex sonography was performed within14 days of surgery in all patients. A single vascular surgeon, blinded to the study arm of each patient, performed ultrasonography in all cases. Compression ultrasonography and duplex Doppler ultrasonography were used from calf to groin in bilaterally. Patients suspected of DVT were further evaluated by enhanced spiral computed tomography. Data were collected on postoperative complications such as deep vein thrombosis, pulmonary embolism, surgical site infection, and length of hospital stay.
Results: A total of 167 surgical patients (83 LR, 84 non-LR) were analyzed by postoperative ultrasonography5 to 14 days (median 8) after total hip arthroplasty. In the LR group, no patient was diagnosed with DVT. In non-LR group, one patient was suspected but was ruled out by helical computed tomography. No difference was observed in other postoperative parameters including surgical site infection, incidence of fever or length of hospital stay between LR and non- LR groups.
Conclusion: Pre-storage leukofiltration of autologous whole blood reduces the accumulation of platelet-derived micoparticles, but our duplex sonography investigation could not demonstrate that leukofiltration of autologous whole blood improved postoperative embolic complications in hip joint surgery patients.

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SUPPRESSION EFFECT OF CXCR1 AND CXCR2 RECEPTORS ON MEGAKARYOCYTE PROGENITOR CELLS DERIVED FROM UMBILICAL CORD BLOOD CD133+ CELLS

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Objective: Most studies have reported that some CXC chemokines including NAP-2, IL-8, and PF4, which expressed by megakaryocytes in megakaryocytopoiesis and affect cell expansion and differentiation of hematopoietic stem cells. Previous studies have shown that these chemokines inhibit megakaryocytopoiesis. The receptors for NAP-2 and IL8 and likely PF4 were reported to be CXCR1 and CXCR2. The aim of this study was to investigate the effect of inhibition of CXCR1 and CXCR2 on differentiation of umbilical cord blood (UCB) CD133+ cells into megakaryocyte progenitor cells.
Method: Umbilical cord blood CD133+ cells were separated by magnetic cell sorting method. CD 133+ cells were placed immediately after selection, in a serum free medium supplemented with IL3, IL 6, TPO, and stem cell factor (SCF) as well as 5% CO2 for 12 days as control cells. To investigate the effect of receptor inhibition, the CD133+ cells were cultured under a same condition with cocktail cytokine and divided into three groups : the first and second group, CXCR1 and CXCR2 were separately blocked by neutralizing monoclonal antibody. The third group, both receptors were blocked by Anti CXCR1/CXCR2 neutralizing monoclonal antibodies. After 7, 12 days of culture, expression of the CD41 and CD61 antigen as megakaryocyte progenitor cell markers was evaluated by flow cytometry.
Results: The results showed that inhibition of CXCR1 and CXCR2 receptors together caused an increase in CD61 expression on days 7 and 12 in comparison to cells treated with IL3, IL 6, TPO and SCF ($P < 0.05$). Inhibition of both receptors showed an increase expression of CD41 on days 7

and 12, but this increase was significant just on day 12 ($P < 0.05$). Although inhibition of CXCR1 and CXCR2 alone augmented CD41 and CD61 expression on days 7 and 12, this increase was not significant ($P > 0.05$).
Conclusion: CXCR1 and CXCR2 receptors play a potent role in the suppression of megakaryocytopoiesis through their ligands. We demonstrated that the inhibition of this suppressive effect could increase differentiation of UCB CD133+ cells into megakayocyte progenitor cells.

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DETERMINATION OF REFERENCE RANGE FOR PARAMETER PRODUCED BY A MULTIPLATE FOR PLATELET FUNCTION ANALYZER

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Background: A point-of-care monitoring of platelet function can be used for identifying groups of peri-operative patients at a high risk of transfusion of platelets, especially with intake of anti-platelet agents, or screening of donors for plateletpheresis. Multiplate (Dynabyte, Germany) is a new point-of-care equipment and has been developed to measure platelet aggregation whole blood.
Aims: The objective of this study was to determine the reference range of parameter produced by a Multiplate in healthy volunteers in Korea to be helpful to interpret the results from Multiplate.
Methods: Blood was collected using tubes with hirudin (Multiplate Service GmbH, Germany) and 3.2% sodium citrate (Becton Dickinson, USA) from 35 healthy volunteers (female 18, male 17) without history for any medication. Whole blood platelet aggreagations triggered by the Multiplate ADP, ADP-high sensitive (only in hirudin blood), collagen, TRAP or arachidonic acid (ASP test) were investigated using Multiplate platelet function analyzer in both hirudin- and citrate blood, and the area under the curve (U) was quantified. Reference ranges were determined from central 95 percentile of results with outlier test.
Results: The reference ranges were 38–107 U for ADP, 13–91U for ADP-high sensitive, 53–112 U for collagen, 81–163 U for TRAP, and 64–156 U for arachidonic acid in hirudin blood, and 18–119 U for ADP, 26–108 U for collagen, 49–149 U for TRAP, and 32–117 U for arachidonic acid in citrate blood. There were significant correlations of the results between hirudin blood and citrate blood for ADP, arachidonic acid and collagen, but not for TRAP. The results for collagen, arachidonic acid and TRAP from hirudin-treated blood were significantly higher than those of citrate-treated blood, but not that for ADP.
Conclusions: The reference ranges according to the type of anticoagulant are expected to be helpful to interpret the results from Multiplate for identifying patients not only with dug resistance to anti-platelet agents but also at a high risk of platelets transfusion or for screening of donors with decreased platelet function.

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IMMATURE PLATELET FRACTION IS A USEFUL MARKER TO PREDICT PLATELET RECOVERY AND PLATELET TRANSFUSION REQUIREMENT IN CANCER PATIENTS AFTER CHEMOTHERAPY AND HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: The ability to predict platelet recovery after chemotherapy and hematopoietic stem cell transplantation (HSCT) allows a more reasoned approach to platelet transfusion. Reticulated platelets analyzed by flow cytometry using thiazole orange have been used to predict platelet recovery after chemotherapy and HSCT. However, it is difficult to apply this method to clinical determination of reticulated platelets because standardized methods of flow cytometric techniques to measure reticulated platelets have not yet been established. Recently, the immature platelet fraction (IPF) monitored by the automated hematology analyzer, XE-2100, was found to be a useful parameter of a thrombopoietic activity in bone marrow.
Aims: In the present study, we assessed the clinical utility of the percentage of the IPF (IPF%, a percentage value of IPF to the total optical platelet count) to predict platelet recovery after chemotherapy and HSCT.
Methods: Blood samples obtained from 114 healthy individuals (41 males and 73 females; age, 51 ± 21) were used to establish a reference range in health for the IPF%. To examine relationship of the IPF% to platelet count, the IPF% was measured in 100 patients without dysfunction of bone marrow (50 males and 50 females; age, 53 ± 25). IPF% was serially monitored in 22 patients with hematologic malignancies (15 with acute myeloid leukemia, 2 with acute lymphoblastic leukemia, 4 with non-Hodgkin lymphoma and 1 with multiple myeloma) and one with rhabdomyosarcoma who received 55 courses of chemotherapy. In addition, IPF% was serially monitored in 8 patients with hematologic malignancies undergoing 11 courses of autologous or allogeneic HSCT. Blood samples collected with EDTA-2K were subjected to measurement of platelet count, IPF absolute count and IPF% using the XE-2100 within 3 h after collection.
Results: The IPF% in 114 healthy individuals was 2.8 ± 1.4%. There was an inverse correlation between platelet count and IPF% ($r = 0.319$, $P = 0.0012$). In cancer patients undergoing chemotherapy and HSCT, a transient increase of IPF% (IPF% peak) preceded the recovery of the platelet count to more than 30000/μL by 1 to 11 days. In cases undergoing chemotherapy with more than 10% of the IPF% peak value, platelet recovery (> 30000/μL) occurred significantly earlier than in cases with 6–10% of and less than 6% of the IPF% peak value (2 days versus 4 days and 5 days, $P < 0.05$, respectively). In cases undergoing autologous or allogeneic HSCT with more than 10% of the IPF% peak value, platelet recovery occurred earlier than in cases undergoing allogeneic HSCT with less than 10% of the IPF% peak value (2 days versus 3 – 11 days). In contrast, because the IPF absolute count paralleled the platelet count after chemotherapy and HSCT, the IPF absolute count was not useful to predict platelet recovery.
Conclusion: The IPF% peak value is a useful parameter for predicting platelet recovery after chemotherapy and HSCT. Therefore, the measurement of the IPF% has the potential to allow optimal platelet transfusion.

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ROLE OF SOME BIOCHEMICAL MARKERS IN PATIENTS WITH THROMBOSIS

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Introduction: Thromboembolic disease a major cause of morbidity and mortality in Iranian population. Biochemical markers may be have important role for thrombosis.homocysteine is one of the biochemical marker that play a important role for formation of thrombosis. Vitamin b12 and folate are major vitamins in homocysteine metabolic pathway, so deficiency of them affects plasma total homocysteine. This can cause homocysteine accumulate in the blood. So we aimed to evaluate the relationship between thrombosis and these biochemical markers.
Material and methods: We measured fasting plasma homocysteine in patients and control groups by elisa method, meanwhile the second blood sample is collected for measurment of serum Vitamin B12 and Folate by ria in patients group. The results enterde in SPSS statistical program and analysed statistically using t-test, Chi-square and also estimated risk of thrombosis.
Results: Total of 100 patients with arterial thrombosis and 68 healthy control subject were included in this study. A statistically remarkable difference was observed between the mean of fasting plasma total homocysteine in patients and the mean of plasma total homocysteine in control ($P < 0.001$). Serum vit b12 and folate concentration were significantly lower in patients With hyperhomocysteinemia when we compared with control group ($P < 0.001$).
Disccusion: In our study according to odds ratio (or = 2.72) shows that hyperhomocysteinemia is an independed risk factor for thrombosis in

iranian population. There was significant correlation between increase plasma homocysteine and decrease serum vit b12 in thrombotic patients. We conclude that low vit b12 and folate concentrations are associated with an increased risk of thrombosis. For undestanding the effects of vit b12 and folate on thrombotic patients, more detailed follow-up studies with long priod are needed.

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REGULATORY T CELL RECOVERY AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Appropriate immune reconstitution after allogeneic hematopoietic stem cell transplantation (allo-HSCT) is essential to avoid life-threatening graft-versus-host disease (GVHD) and graft failure, and to induce enough graft-versus-leukemia(GVL) effect. It has been suggested that regulatory T cells (Tregs) prevent GVHD by inhibiting the proliferation and function of conventional T cells. However, the exact function of Tregs in immune reconstitution after allo-HSCT is unclear.

To assess regulatory T cell recovery after allo-HSCT, we are investigating the diversity and distribution of regulatory T cell in patients who received allo-HSCT.

Blood samples were sequentially taken from 21 patients. Samples from nine healthy volunteers were used as controls. The derived mononuclear cells were stained and analyzed by flow cytometry. Comparing CD4+CD25highFoxp3 cells in pre-transplant patients (n = 21) with normal controls (n = 9), these cells were less numerous in pre-transplant patients (34.2 ± 38.3/μL vs 119.40 ± 45.03/μL, p''0.01) and comprised a smaller fraction of the white cell count (1.17 ± 0.46% vs 2.01 ±0.68%, p''0.05). Even on day 200 after allo-HSCT the number and fraction of CD4+CD25highFoxp3 remained low (28.7 ± 19.4/μL and 0.53 ± 0.23%, $P < 0.05$), whereas white blood cells in general and lymphocytes in particular recovered faster. One patient who developed acute GVHD had a much lower number and fraction of Tregs (11.2/μL and 0.24%) on day 30. In conclusion, we found that pre-transplant patients have fewer Tregs than healthy subjects and there is a delay in recovery of Tregs up to 200 days after allo-HSCT even in the uneventful patients. The very low number of Tregs may play a role in the occurrence of GVHD.

8.1. Novel Developments Alternatives to blood transfusion

P-280

TRANSPORT OF AUTOLOGUS BLOOD TO EXTRA-TERRITORIAL STRUCTURES IN OUR COUNTRY

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Background: In our country there was numerous 'trips of the hope', many patients bring from the regions of the south Italy toward sanitary structures of the center-north of the our country, for the most part to be submitted to surgical intervents or therapies that he holds, rightly or wrongly, not to be able to be guaranteed from the sanitary structures of his own place of origin in suitable way or in acceptable times.
A great part of surgical intervents, that contribute to the migratory flow, concern orthopedics, this kind of surgery also need a great transfusional support. All the candidates to orthopedic interventions of any nature, must preventively be valued to eventually enlisted in a program of autotransfusion.
In the routine the selection is effectuated in the structure where take place the surgical intervention, or in the transfusional Service Trasfusionale near the place of residence of the patients Subsequently the patients are sent to the Transfusional Service near their address. This kind of organization can result in a series of problems that may have a difficult solution. We have make the question as to organize the best kind of transport for the autologus blood to extraterritorial structures.
Methods: Scientific literature doesn't signal cases of attributable infectious illnesses with happened during the transport of blood and his components. The italian normative imposes particular precautions for the transport of the biological materials. The Transfusion Services of reference not send the blood but conferring responsibility to the patients. Our Transfusion Center completed once the autransfusion program but it is not able to directly handle the consignment.
Results: We submit to the patient detailed and explicit written and oral information and on the formalities of maintenance and transport of the blood. Also in relationship to the vector from them select for the trip (car, train, airplane, etc.) and of the necessary time to reach destination. At the same time we provide to point out the thermal containers to purchase and to view them before the use. In the case in which the separation of the blood have producted fresh frozen, we furnish the address of manufacturing firms of dry ice and the relative containers. Finally, during the departure, our personnel handles the wrapping to the packing and the issue of the necessary documentation of trip and the beads of accompaniment.
Conclusions: The assistance furnished results pleasant, clear and comprehensible. The emocomponents so submitted to the patients they have always reached destinations in excellent state of maintenance. Nevertheless some insurgent problem list recently. Often we need to sent blood with antibodies against the virus C of the hepatitis, this has made us to reflect if that are the best solutions can be looked for transport of the biological materials. Other problems are manifested on the airplane for the scurity.

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AUTOLOGOUS BLOOD TRANSFUSION - IS IT REALLY SAFE?

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Autologous blood is nowadays considered the safest blood for transfusion, since the risk of immunological reactions and blood-borne infections caused by transfusion of allogeneic blood can be avoided. However, in Japan, the safety of allogeneic blood, which are provided by the Japanese Red Cross Blood Center, has increased tremendously in recent years, since they are tested for viral infectious markers by the nucleic acid amplification test, subjected to prestorage leukocyte reduction (preLR) as well as removal of the initial blood flow at collection, and also irradiated blood can be provided, as necessary, to prevent PT-GVHD. Although rare, infected blood can slip through the NAT test and be transfused. To detect those infected by blood transfusion, and adequately manage them, the retrospective analysis was started, and the patients scientifically proven of blood-borne infection can apply for the Relief System for Injury to Health with Biological Product Usage of the Ministry of Health, Labour and Welfare. Therefore, not only the blood product manufacturing techniques have improved but the governmental measures to protect the victims of health hazards of blood transfusion have been established.
On the other hand, autologous blood is collected in-hospital and manipulated in blood transfusion services. One problem most hospitals in Japan face is the staff shortage in blood transfusion services, where the full-time transfusionist is frequently not appointed. Additionally, most hospitals lack the appropriate systems for collection, manipulation, control and provision of autologous blood. Therefore, there may be increased risk of adverse reactions during autologous blood collection, as well as of inappropriate manipulation of autologous blood, which may result in transfusion reactions or eventually wastage of autologous blood.
Considering these facts, there is need to reassess the real safety of autologous blood.
To improve and guarantee the safety of autologous blood transfusion, the following topics must be considered: (1) establishment of the appropriate system of autologous blood, from collection to provision, (2) trained and specialized staff to deal with autologous blood, (3) testing for bacterial contamination, (4) checking the adverse reactions and the causes of autologous blood wastage.
To solve these problems, the autologous blood outpatient clinic was established in our university hospital, where the full-time transfusionists consult the patient to decide on the best collection schedule, blood is collected under strict monitoring and observation of the patient by the specialist nurses and transfusionists, and the collected blood is manipulated by specialized laboratory technologists. All the system is strictly controlled by the computer system to prevent blood-patient exchange. And the adverse effects of transfusion are reported to the blood transfusion service, and by analyzing the data, the preventive measures are established. The enhanced bacterial detection system (eBDS) was used to test bacterial contamination of autologous blood, confirming the safety of the products, and a study was conducted to confirm the benefits of preLR in autologous blood.
To guarantee safety of autologous blood transfusion, the establishment of an autologous blood transfusion outpatient clinic is effective, avoiding the adverse effects of and wastage of autologous blood, and preLR should be implemented.

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ANTIOXIDATIVE ENZYMES CONJUGATED POLYHEMOGLOBIN CAN RELIEVE OXIDATION TOXICITY INDUCED BY H2O2

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Background: Reactive oxygen species (ROS) induced during ischemia reperfusion, especially H2O2, may be a potential threat to the application of hemoglobin-based-oxygen-carriers, but less is focused on the oxidation toxicity of vascular endothelial cells induced by H2O2 during the application of hemoglobin-based-oxygen-carriers.
Aims: We used hydrogen peroxide(H2O2)co-incubated HUVEC (Human Umbilical Vein Endothelial Cells) as a cell model to explore the oxidation toxicity to the endothelial cells and the potential signaling pathway of oxidation along with the protective effect of two candidates of hemoglobin-based-oxygen-carriers, Polyhemoglobin and Polyhemoglobin-SOD-CAT.

Methods: Intracellular variation of GSH was measured using the spectrometry. And NF-κB translocation was detected through immunofluorescence method. We also applied RT-PCR to assess the mRNA expression of endothelial nitric oxide synthase (eNOS), heme oxygenase-1(HO-1), c-jun and endothelin-1 (ET-1). Cell apoptosis, intracellular H2O2 content and extra cellular signal-regulated kinases1/2 (ERK1/2) phosphorylation were evaluated by flow cytometry. And Western Blotting was used to detect c-jun phosphorylation.

Result: Co-incubation with H2O2 caused HUVEC a significant decrease of GSH; evidently induced NF-κB translocation which consequently upregulated the expression of eNOS, HO-1 and leaded to the significant cell apoptosis, while ERK1/2 and c-jun, two signal transmitters of MAPK pathway, were greatly phosphorylated, which as a result caused a remarkable increase of ET-1 expression. Compared with the Polyhemoglobin, another candidate of HBOCs, Polyhemoglobin-SOD-CAT effectively eliminated the translocation of NF-κB and therefore abolished the upregulation of eNOS, HO-1 along with the relieved cell apoptosis. Moreover, Polyhemoglobin-SOD-CAT may also dispel the induction of MAPK pathway and hence removed the upregulation of ET-1, which may cause the vessel constriction during the application of hemoglobin-based-oxygen-carriers.

Conclusion: Reactive oxygen species (ROS) induced during ischemia reperfusion, especially H2O2, could cause a great damage to the vascular endothelial cells and induce endothelial cells to express the effect factor ET-1 that may lead to the vessel constriction. And PolyHemoglobin-SOD-CAT, which inhibited the H2O2-induced oxidative damage to vascular endothelial cells, could be a potential way of solution.

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PREOPERATIVE AUTOLOGOUS BLOOD TRANSFUSION IN JAPAN

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Background and aim: Preoperative autologous blood donation (PAD) is performed in order to avoid the complications of allogeneic blood transfusion. In Japan, Preoperative autologous blood transfusion (PAT) is actively performed in many institutions dealing with blood transfusion. It's clear that the good collaboration between surgeons and transfusionists is necessary for the safe and adequate preoperative autologous blood transfusion to be performed. In an attempt to realize on the present status and the problems of PAT in Japan, a survey was conducted by the Japanese Society of Transfusion and Cell Therapy (JSTC).

Methods: A questionnaire regarding transfusion practice in the hospital was sent to 7 857 hospitals in 2008. The similar questionnaire survey was performed for 4 consecutive years from year 2004 to 2007.

Results: A total of 3 208 institutions answered, and among them, 49.2% performed PAT. The mean yearly number of patients was 68 (range: 1–1,052), with 125 collections (range:1–1,373) and 220 units (range: 1–2,252) collected. Blood collection was performed by doctors in 53.9% of patients, by nurses in 30.0% and either in 16.0%. The collecting doctors were the surgeons in 86.0%, the transfusionists being involved in only 4.8%. The collected blood was mainly stored as whole blood (95.3%), without separation. Autologous fibrin sealant (FS) was prepared and used in 38 institutions (3.2%). During the 5-year period when the surveys were conducted, the number of PAT, calculated as the units of autologous blood/number of beds, was almost unchanged (0.52–0.56). The wastage rate of PAT was 12.0% (range:0–96.0). On the other hand, in Toranomon Hospital, more than 1 000 patients per year collect autologous blood at the preoperative period. About 80% of those patients receiving blood transfusion during operation are operated on only with autologous blood, and approximately 85% of them used fibrin sealant derived from the autologous plasma. One third of red blood products were frozen at -80 degree with glycerol. And the wastage rate of PAT was 33.3%.

Conclusion: In the present survey, almost half of the institutions performed PAT. In most institutions, surgeons, but not transfusionists, collected the autologous blood, which were mostly preserved as whole blood and stored at 4oC. No substantial change in the number of patients receiving PAT was observed during the last 5 years. On average, the wastage rate of PAT was 12.0%. At Toranomon hospital, an adequate system for PAT is established, with the full-time transfusionist performing the blood collections under monitoring by specialist nurses, and manipulation of the collected blood by specialist laboratory technicians. Furthermore, there is an active hospital transfusion committee, where the transfusion-related subjects, including autologous blood transfusion, are discussed, with consequent spreading of the knowledge on autologous transfusion among the surgeons. Thus, our results at Toranomon hospital are over the national average. PAT is popular and standard method in Japan, but efforts are needed to implement the safe and adequate system for its performance, improving the national average.

8.2. Novel Developments Biologicals

P-284

USE OF PLATELETS GEL IN MAXILLO-FACIAL SURGERY: OUR EXPERIENCE

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Background: The preparation and the use of the Platelets Gel (PG) in Maxillo-facial Surgery are used from many years. Particularly the PG represents an alternative to the glue of authologus fibrin finding application in different types of Maxillo-facial pathologies what: reconstruction of the mandibolar defects, oral pan-pipes and nasal breasts illness.
Methods: During the year 2008 we have treated in collaboration with the Maxillo-facial Surgery of our Hospital five clinical cases with PG autologo. Everybody. All the patients have reached the clinical observation with diagnosis of cystic lesion including tooth jaw. The first case, T.G., male of years 48, perform a series of pre-operating examinations of laboratory that show, platelets (PLT) 228×103/mm^3 and PT 10,4 sec; the second patient, S.T., female of years 29, show PLT 236×103/mm^3 and PT 9,8 sec.
The patients have been all submitted to surgical intervention of total enucleation of the cystic lesion, revision of the residual hollow, collecting of bony fabric that has been mixed to Fisiograft and finally, filling of the hollow. The PG has been prepared to level intraoperatorio in the following way: collecting of around 60 mls of whole blood tried with the automatic system ANGEL (DIDECO). Such procedure allows to get Plasma Rich in Plaques (PRP) that is mixed with the bon and Poor Plasma of Plaques (PPP) used for producing the trombina auotologa.
Results: The clinical control performed in the time on all the patients has showed that the hemostatic and anti-inflammatory effect results notably improved in comparison to the patients that are not treated of routine with PG, together to a clinical recovery, radiologic and histological more precocious.
Conclusions: The data suggest, that the use of the PG accelerates the recovery of the bony pathologies in the Maxillo-facial Surgery. Its characteristics of manageability and consistence make it a particular utility in the filling of hollow as those consequent to enucleation of this kind of cysts. The use, of the system Angel, results easy, allowing to get a final product standardized in reduced times and with a limited number of passages.

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USE OF PLATELETS GEL IN INTERVENTIONS OF RISE OF THE MAXILLARY BREAST: PRELIMINARY DATA

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Background: The lack of enough height of alveolar bone has been constituting for long time a limit to the insertion of fittings in the back part of maxillary bone. Such lack of height is the result of the regressive consequent alveolar bony reabsorption produced by the teeth and by the concomitant pneumatizzazione of the maxillary breast.
The law of Wolf establishes in fact that the bone is remodeled in operation of the strengths that practice on it; the bone needs stimulation to maintain its form and density and the teeth practice a strengths compression and traction on the alveolar bone. To return thickness to the bone and to allow the rehabilitation protesica of the superior arcade with fittings the serious atrophies, the most diffused technique consists in the rise of the maxillary breast (Sinus Lift).
The use of the Platelets Gel (PG) accelerates the processes of recovery (above all of the soft fabrics); it constitutes a valid contribution of platelets and fibrina. These components represent the necessary factors to guarantee a natural prevention against the infections, a meeting place hematoma and a natural contribution of catalysts of the processes of tissutal reparation In the present job we intend to appraise possible advantages in the use of the PG in interventions of rise of the maxillary breast.
Methods: During 2008 we have treated in collaboration with the Maxillo-facial Surgery of our Hospital five clinical cases of rise of the maxillary breast. In all has been effected in the immediate pre-operating collecting 60 mls of whole blood that has been tried with the automatic system 'Angel' (DIDECO). Such procedure allows to get Plasma Rich in Plaques (PRP) that is mixed with the autologus bone and PPP used for producing the autologus trombina.
Results: All the patients have reported an evident diminution of the painful, both type acute in the post-intervention that chronic in the following days, You swelling that he usually finds in this typology of intervention clearly results inferior. The recovery of the wound has happened without complications (deiscenze) and in rapid times. This is also verified for patient smokers.
Conclusions: In accord with the international literature, have recorded a meaningful improvement of the elapsed post-operating in the patients essays with PG, to demonstration of the by now undisputed effectiveness of the PG in the recovery of the soft fabrics. As it regards the bony neoformation, the data currently in our possession, with controls radiograficis to three months, don't allow us to draw certain conclusions, even if it would seem there is also in this case a tied up benefit to a more rapid benefit. The following controls and the amplification of the number of patients will allow us to furnish a great completeness on the aspect, still controversial, of the real advantage of the PG in the formation again plotted bony.

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TRANSIENT DOWN-REGULATION OF OSTEOPONTIN BY RNA INTERFERENCE IN HUMAN MESENCHYMAL STEM CELLS DELAYS IN VITRO OSTEOGENESIS

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Introduction: Mesenchymal stem cells or multipotent stromal cells (MSCs) isolated from the bone marrow have the capacity to generate heterotopic osseous cells such as osteoblast (OB). Osteopontin gene expresses during MSCs differentiation to OB cells. To determine whether down-regulation of Opn would affect on OB differentiation from MSCs, this study was conducted.
Methods: MSCs were isolated and characterized. OB differentiation media was added to the MSCs. After 5 day's, Opn-small interfering RNA (siRNA), formulated by lipofectamine RNAiMAX was transfected to the cells. Real-time PCR and Western blotting were used to quantify the mRNA and Opn protein levels. The OB differentiation character of transfected and non-transfected cells including mineralization, OB-specific gene expression (Osteocalcin) and alkaline phosphatase activity were analyzed.
Results: Sequence-specific siRNAs targeting Opn suppressed Opn RNA expression by 75% and also decreased Opn protein level by 65% in OB cells. The rate of mineralization, OB-specific gene expression and alkaline phosphatase activity in vitro were decreased in OB cells transfected using Opn-siRNA compared to controls ($P < 0.05$).
Conclusion: Our results suggested that down-regulated of Opn significantly decreased the osteocyte formation from MSCs during the in vitro osteogenesis process.

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HIGH EXPANSION AND GENERATION OF MEGAKARYOCYTES FROM UMBILICAL CORD BLOOD CD133+ CELLS

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Background: Umbilical cord blood transplantation due to low megakaryocyte progenitor cells has a delay in platelets engraftment. Ex vivo generation of megakaryocytes is crucial to rapid platelet reconstruction.

Aims: In this study, we optimized the best concentration of effective cytokines to expand hematopoietic stem cells and differentiation to megakaryocyte progenitors.
Methods: CD133+ hematopoietic stem cells from umbilical cord blood were isolated from cord blood and expanded in (SCF: 100 ng/ml, TPO: 100 ng/ml and IL-3:10 ng/ml in serum free stem span media) for 7 days and differentiated in (TPO: 100 ng/ml) for additional seven days. The developmental differentiation was analyzed by flow cytometry.
Results: TPO and SCF together with low concentration of IL3 were found to be optimum for expansion of CD133+ Hsc(46 fold of initial number expansion $P < 0.001$) at 7 days of culture. Interestingly, more than 90 % megakaryocytic differentiation at 14 days of culture, containing TPO in second week was seen. The megakaryocytic cells showed bright expression of CD41, CD42 and CD61.
Summery and conclusion: Ex vivo expansion of cord blood cells and differentiation to megakaryocyte cells can be obtained by selective cytokines in media.

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EFFECT OF DEXAMETHAZON ON HUMAN MESENCHYMAL STEM CELLS

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Background: Human Mesenchymal Stem Cells (HMSCs) have been characterized widely for a variety of clinical application as they easily isolated and expanded in a culture to produce large numbers of the cells for therapeutic approaches. Although it seems that there are not any chromosomal abnormalities in early passages of cultured HMSCs, in this study, we investigated that differentiation of primary passages of hMSCs to steoblast can generate immortal cells.
Material and methods: Mononuclear cells from bone marrow were isolated by density gradient centrifugation. The cell pellet was cultured and non-adherent cells were removed after 24 hours. To promote osteogenic differentiation, the cells were treated with beta-glycerol-phosphate ascorbic acid 2-phosphate and dexamethasone for 3 weeks. Osteogenic differentiation was assessed with alizarin red and alkaline phosphates staining. In order to determine the number and structure of chromosomes, each sample was investigated using standard GTG bandin technique.
Result: Regarding to our findings, under special circumstances and duo to genetic abnormality of donor B.M, dexamethazone can immortalize HMSCs.
Conclusion: Dexamethazoneon might play a role in induction of HMSCs immortalization and this point questioned the safety of dexamethazone for differentiation of HMSCs before their clinical use.