

Sunday 18 October
HSANZ Symposium: MPD incl CML

0900-1030
Hall C
0900

The Pathogenesis of Myeloproliferative Syndromes

Radek Skoda

University Hospital Basel, Experimental Hematology, Department of Biomedicine, Basel, Switzerland

An acquired somatic mutation in the JAK2 gene resulting in a valine to phenylalanine substitution at position 617 (JAK2-V617F) is present in the majority of patients with myeloproliferative disorders (MPD). The JAK2-V617F mutation is located in the "pseudo-kinase" domain of JAK2, which physiologically exerts an inhibitory effect on the kinase domain. The mutation is thought to de-repress the kinase activity by an allosteric mechanism. Expression of the mutated JAK2 cDNA in mouse models resulted in increased white blood cell numbers and red cell mass, recapitulating the MPD polycythemia vera phenotype. Mutations in exon 12 of the JAK2 gene have been identified in a subset of MPD patients negative for JAK2-V617F and mutations in exon 16 were found in 18% of patients with B cell ALL and Down syndrome. A number of observations suggest that JAK2-V617F in patients with MPD may be acting in concert with mutations in as yet unknown gene(s). Recently, mutations in the TET2 gene have been found in about 15% of patients with MPD and up to 30% of patients with myelodysplastic syndromes (MDS). The presentation will focus on the biology of the disease and the relation between the known mutations and the phenotype. In particular, the following questions will be addressed:

1. Why does JAK2-V617F cause 3 different clinical phenotypes?
2. Is JAK2-V617F the primary cause of MPD?
3. What is the role of JAK2 mutations in leukemic transformation?
4. How do mutations TET2 relate to the mutations in JAK2?

The currently available data suggest that JAK2 mutations may be sufficient to cause MPD. However, evidence has accumulated indicating that a considerable proportion of MPD patients carry additional mutations. Predisposition to acquiring JAK2 mutations can be increased in cases of familial MPD and the hereditary component is more frequent than is generally assumed.

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0945

Optimising Response in CML

Tim Hughes

Department of Haematology at SA Pathology, RAH, Adelaide, South Australia

(1) Is there really any important difference between the patient who achieves CCR but not MMR by 18 months and the patient who achieve MMR? We know that patients who achieve CCR by 18 months have a good prognosis and that patients who achieve MMR by 18 months do even better. The risk of disease transformation over the next 5 years is 4% compared to 1% in patients achieving MMR. Perhaps a more significant consideration is the risk of loss of CCR which is 24% in patients with CCR but not MMR by 18 months compared to 4% in patients with MMR ($p=0.001$). This data is based on the IRIS trial. A single centre study from the Hammersmith supports these findings.

(2) Does this failure to achieve MMR in a patient who has achieved CCR justify any change in management? We don't have enough data to answer this question but the following considerations may help.

(3) With a further 6-12 months of standard dose imatinib, what are the chances these patients will achieve MMR. Patients in the IRIS study who maintained the 400 mg dose of imatinib had a probability of MMR of 40% at 12 months and 56% at 24 months. An estimated 55% of patients who had achieved CCR but not MMR at 12 months went on to achieve MMR by 24 months. In the TIDEL trial where higher doses of imatinib were used 62% of patients in CCR without MMR at 12 months achieved MMR by 24 months. Patients in the TIDEL study had a dose increase from 600 to 800 mg /day (or maximally tolerated dose if they couldn't tolerate 800 mg) at 12 months.

(4) Is achieving MMR between 12 and 24 months just as favourable as achieving this response by 12 months?

The MD Anderson studies would support the notion that earlier molecular response is more favourable (ASH 2006) in terms of progression free survival. Our recent studies from the IRIS trial suggest that MMR achieved early or late are equally reassuring in terms of the risk of progression.

(5) If a higher dose of imatinib is used and is actually successful at reducing BCR-ABL to a lower level over the next 12 months has this really improved the prognosis? There is now emerging evidence from the MD Anderson studies suggesting that inducing an earlier and deeper molecular response using higher doses of imatinib leads to better progression free survival.

(6) Can we identify patients who are more likely to benefit from higher dose imatinib. We have recently demonstrated that an in-vitro assay to measure OCT-1 mediated influx of imatinib into blood cells taken from CML patients pre-therapy is predictive of molecular response to imatinib therapy. Patients who have low OCT-1 activity are more likely to have suboptimal response, especially if they receive dose modifications in the first 12 months of therapy. Patients with high OCT-1 activity generally do very well on imatinib, and dose intensity does not seem to be so important. Thus a patient who has not achieved MMR at 12 months and has low OCT-1 activity is quite likely to have a substantial improvement in molecular response if the dose is increased, whereas patients with high OCT-1 activity do equally well whether or not the dose is increased.

(6) Are plasma imatinib measurements helpful for optimising response? Studies from the French group and the IRIS study suggest that patients with higher trough plasma imatinib levels are more likely to achieve CCR and MMR. These studies suggest a role for measuring imatinib blood levels in patients who are not responding optimally, have compliance issues or are taking drugs that may affect imatinib metabolism.

In the future we may be able to use predictive assays like OCT-1 activity and ongoing monitoring with RQ-PCR for BCR-ABL as well as imatinib blood levels to determine where a dose increase will probably be beneficial and where it may not be worthwhile.

Sunday 18 October
ANZSBT Symposium: Transfusion Practice

0900-1030
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0900

Artificial Oxygen Carriers and Erythropoietin Use in Trauma Patients

Lena Napolitano

Abstract not received at time of going to print

Sunday 18 October
ANZSBT Symposium: Transfusion Practice

0900-1030
Hall B
0945

Intra-operative Blood Salvage

Dafydd Thomas

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The role of intra-operative blood salvage in blood conservation is now firmly established. Of all the autologous transfusion methods, intra-operative salvage has stood the test of time and is proving that an operative method of blood conservation can easily be incorporated into the vast array of techniques that are implemented to ensure an improved surgical outcome. Other methods of autologous transfusion have not been as successful for a variety of reasons, but salvaging autologous red cells at the time of surgical haemorrhage seems to be a very sensible option.

However there is a need to ensure that quality and safety issues are addressed and that standard operating procedures are followed to maintain the highest standard of care. Whilst decreasing exposure to allogeneic blood and preservation of existing blood supplies are both laudable aims, it is not good practice to replace a treatment that currently has such high quality assurance with an alternative, albeit an autologous option, that is not equally quality assured.

The UK has been obliged to look at alternatives to allogeneic transfusion mainly due to the vCJD problems, and although donor supplies remain healthy, demographic change and the impact of the precautionary approach to deal with prion disease may yet lead to a significant shortfall in allogeneic supplies. Anticipating this problem a significant amount of expertise and resource has been invested in developing educational and technical support for hospitals wishing to develop and promote intra-operative cell salvage programs. The UK Cell Salvage Action Group (UKCSAG) has developed educational material, technical factsheets and competency assessment literature to enable a standardized UK approach to teaching and training. The positive spin-off is that this work and supporting educational literature is available free of charge from the website – www.transfusionguidelines.org.uk.

Sunday 18 October
ASTH Symposium

0900-1030
Hall D
0900

Tissue Factor – In Health and Disease

Nigel S Key

University of North Carolina, Chapel Hill, NC, USA

Tissue factor (TF) is a 47kDa trans-membrane glycoprotein that when bound to FVII(a), initiates coagulation. The presence of extravascular TF has been recognized for well over a century, but a specific role in coagulation initiation was first proposed in 1905, when 'thrombokinase' was proposed as one of the essential 4 factors needed for coagulation by Morawitz. Until the 1990s, it was not appreciated that very low levels of intravascular TF can be detected in the circulation. Subsequently, in both human studies as well as in mouse models of thrombosis, much attention has focused on the measurement of TF procoagulant activity, particularly in circulating microparticles (MPs). MPs are sub-micron sized fragments of membrane and cytoplasm released by most cells that are undergoing activation or apoptosis. Detection of MPs in cell-free plasma by flow cytometry is a considerable technological challenge that has only recently been subjected to standardization efforts. However it is easier to measure TF procoagulant activity on MPs, and we, and others, have developed assays to address this issue. The data indicate that MP-TF activity is detectable in normal individuals, and is elevated in certain pathologic states. Perhaps the disorder in which the potential importance of circulating MP-TF is gaining increased acceptance is in the pathogenesis of cancer-associated thrombosis. Several groups have demonstrated elevated levels of MP-TF activity, particularly in patients with certain forms of malignancy such as pancreatic or non-small cell lung cancer. While these data are intriguing, there is as yet no good evidence from appropriately designed prospective studies that circulating MP-TF can be considered a true biomarker of thrombotic risk. Finally, although not as well studied in disease states, it should not be forgotten that cell-associated TF – particularly on monocytes, and probably also on activated platelets – may be equally or even more important than the pool of TF which is present on derived MPs.

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ASTH Symposium

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Stratifying Risk of Recurrent Venous Thrombosis

Paul Kyrle

Medical University of Vienna, Austria

Over the last years several clinical and laboratory risk factors of recurrent venous thrombosis have been identified. These include male sex, proximal deep-vein thrombosis or pulmonary embolism, recurrent venous thromboembolism, residual vein thrombosis, elevated levels of coagulation factors, natural coagulation inhibitor deficiencies, antiphospholipid antibodies or hyperhomocysteinemia. The impact of many of these risk factors on the recurrence risk is however moderate or is even regarded controversial. Determination of some laboratory markers of thrombophilia either lacks standardization or is too elaborate for routine purposes. Many patients with venous thromboembolism carry more than one risk factor and their combined risk of recurrence is unknown. Most importantly, clinical studies that would show a benefit of extended anticoagulation in patients with one of the aforementioned risk factors are lacking. Thus, routine thrombophilia screening is no longer warranted and predicting recurrence in an individual patient remains a major challenge.

Use of global coagulation markers that encompass the effects of clotting and/or fibrinolytic defects may improve risk assessment. There is now strong evidence that patients with a low d-dimer level after withdrawal of anticoagulation have a low risk of recurrence and may not benefit from long-term anticoagulation. Similarly, low in vitro thrombin generation is associated with a moderate recurrence risk. In a future step, global coagulation markers need to be integrated with clinical risk factors of recurrence. Validated and simple scoring systems may improve stratification of patients into low and high-risk categories regarding their recurrence risk and may optimize duration of anticoagulation.

Sunday 18 October
Nurses Symposium

0900-1030
Hall A
0900

Patient Blood Management

Sherri Ozawa

Englewood Hospital and Medical Center, Englewood, New Jersey, USA

The transfusion of allogeneic blood is an issue of vital importance to healthcare systems, consumers, and providers the world over. Constant and ever present concerns about existing and emerging pathogens, skyrocketing costs, and the struggle for adequate supply place increased focus on the pressing issues that surround this common procedure. Recent years have seen the publication of hundreds of studies and analysis that link the transfusion of blood products to poor patient outcomes, including longer length of hospitalization, increased morbidities, increased mortality, and many other serious negative complications. This information compels physicians, nurses, and other providers to dramatically change their use of transfusion from a default procedure to manage anemia to a relatively rare intervention when evidence of potential benefit outweighs risk. Statistics from throughout the world give evidence to the fact that clinicians use blood transfusions based on individual and institutional habit, instead of true clinical need. Such lack of an evidence based approach to this serious procedure repeatedly puts both patients and providers at risk.

Vital to this end is the concept of Patient Blood Management, an organized approach aimed at conserving a patient's own blood and minimizing or avoiding the need for the transfusion of allogeneic blood components. Patient Blood Management also proactively addresses the needs of patients for whom blood is not an option. This can be accomplished through the combined use of new and existing pharmaceuticals, devices, management strategies, and medical and surgical techniques which can obviate the need for transfusion, or at the very least ensure that benefit outweighs risk in a given transfusion related clinical situation.

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Nurses Symposium

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Patient Blood Management in Australia: Where are We Up to?

Barbara Parker

BloodSafe Program South Australia

Increased patients' exposure to allogeneic blood products has been associated with increased incidence of transfusion related complication and significant financial implications. Allogeneic blood remains a freely given but inherently limited resource.

Blood management is a philosophy to improve patient outcomes through the appropriate provision and use blood, its components and derivatives by integrating all available techniques to reduce or avoid the need for blood transfusion. It is a patient centred, multidisciplinary, multimodal, planned approach to patient care.

There is clear scope to optimise the management of transfusion practice using the Three Pillars of Patient Blood Management:

- Optimising pre-operative Hb through detection, diagnosis and treating reversible anaemia (eg iron deficiency)
- Minimising blood loss
- Optimising physiological tolerance of anaemia

Patient blood management programs are evolving in a number of states. This presentation explores some of the barriers and potential solutions, possible nursing models and outlines examples of blood management practices across Australia.

Sunday 18 October

BMTSAA Symposium: Regulation - Implementation and Impact

0900-1030

Meeting Rooms 1/ 2

0900

Regulation from the Auditor's Perspective

Wendy Harris

National Association of Testing Authorities (NATA), Silverwater, NSW

The presentation will discuss NATA requirements for accreditation of Apheresis units and where the accreditation of apheresis units is at present. It will also explain the process and what has been found in the first few assessments.

Sunday 18 October

BMTSAA Symposium: Regulation - Implementation and Impact

0900-1030

Meeting Rooms 1/ 2

0920

The Impact of Quality Management on a Collection Service

Bev Wake, Ann Canty, Rebekah Lamb and Peter Casey

Haematology Day Centre, Royal Adelaide Hospital, Adelaide, South Australia, Australia

The Haematology Day Centre (HDC) of the Royal Adelaide Hospital has a contract to collect haemopoietic progenitor cells and other blood components as required by apheresis for autologous or allogeneic transplantation with the Therapeutic Products Facility (TPF) of the Institute of Medical and Veterinary Science.

As part of the process of attainment of a Therapeutic Goods Administration (TGA) licence in 2003 by the TPF to manufacture therapeutic goods from human blood and blood components, the HDC implemented a quality system in accordance with the "Australian Code of Good Manufacturing Practice -Human Blood and Tissues 2000". The National Pathology Accreditation Advisory Council's (NPAAC) decision to include the collection process in apheresis units in a national standard as outlined in "Requirements for Procedures related to the Collection, Processing, Storage and Issue of Human Haemopoietic Progenitor Cells 2007" will impact on management of collection services and has resulted in review of documentation and practices in the apheresis setting.

No conflict of interest to disclose

Sunday 18 October

BMTSAA Symposium: Regulation - Implementation and Impact

0900-1030

**Meeting Rooms 1/ 2
0935**

NPAAC Guidelines and Requirements – Current Issues

Nancy Messino¹ & Annette Trickett²

¹Royal Children's Hospital, Melbourne, VIC, Australia; ²BMT Network NSW, Sydney, NSW, Australia

The National Pathology Accreditation Advisory Council (NPAAC) has recently released the third edition of the Requirements for Procedures Related to the Collection, Processing, Storage and Issue of Human Haemopoietic Progenitor Cells. In an effort to attain global harmonisation, this edition is based on the FACT-JACIE international standards, but formatted to NPAAC style.

The second edition NPAAC document was only a guideline and inspection / accreditation via NATA was voluntary. The third edition document contains standards, and compliance is mandatory.

The major differences between the second and third edition NPAAC documents are the inclusion of requirements for collection facilities and more stringent requirements for processing laboratories. This session will focus on detailing the new requirements and give suggestions on how the more challenging areas can be met.

Sunday 18 October
HSANZ Symposium: BM Failure

1100-1200
Hall C
1100

Pathogenesis and Treatment of Bone Marrow Failure

Neal S Young

Hematology Branch, National Heart, Lung, and Blood Institute and Center for Human Immunology, National Institutes of Health, Bethesda MD, USA

The human bone marrow failure syndromes include acquired aplastic anaemia, myelodysplasia (MDS), paroxysmal nocturnal haemoglobinuria (PNH), and constitutional Fanconi anaemia and dyskeratosis congenita (DKC). These diseases share pathophysiologic features and effective treatments, and clinical observations and basic laboratory studies have been mutually informative. In all the syndromes, deficient stem and progenitor cells characterize the haematopoietic failure and lead to pancytopenia and death from infection, bleeding, anaemia and the complications of chronic transfusion. In acquired aplastic anaemia, immunosuppressive therapy with antithymocyte globulin (ATG) and cyclosporine is effective in most cases, implicating an immune mechanism of marrow destruction. T cells producing type I cytokines induce apoptosis in hematopoietic target cells; this process is interrupted by ATG but the exact mechanism of action of effective therapies is not clear. Some MDS also responds to ATG, and target antigens resulting from chromosome aberrations have been identified. Mutations in genes of the telomere repair complex and related protective proteins are the aetiology of DKC, and these genes also are mutated in some adults with apparently acquired aplastic anaemia. In the laboratory, short telomeres lead directly to genomic instability and aneuploidy, and in the clinic they are the major risk factors for late clonal evolution to monosomy 7 MDS from aplastic anaemia; constitutional mutations in *TERT*, the telomerase gene, also occur in *de novo* acute myeloid leukaemia. Androgens, historic therapy for marrow failure, positively regulate *TERT*. Mutations in telomerase complex genes also affect regeneration and repair beyond the bone marrow, of lung (pulmonary fibrosis) and liver (cirrhosis). Aplastic anaemia thus is a model of genetic factors interacting with environment, resulting in organ failure and malignant transformation. Current and future treatments can target specific molecular pathways, in immune effector cells and hematopoietic target cells, and appropriate patient populations.

Sunday 18 October
ANZSBT Symposium: Transfusion Safety

1100-1200
Hall B
1100

Patient Safety and Blood Transfusion

Paul Ness

Transfusion Medicine, Johns Hopkins Medical Institutions, Baltimore, Maryland, USA

The only positive benefit of the AIDS epidemic and its associated publicity has been the increased utilization of alternatives to blood transfusion. Based upon the increased perception of the infectious risks, reduction of homologous blood transfusions has been recommended and the increased utilization of alternatives to transfusion such as autologous blood has taken place. Physicians are now encouraged to use drugs without biohazardous risks in favor of blood components. Patients with von Willebrand's disease are given DDAVP rather than cryoprecipitate to improve hemostasis, aprotinin and other fibrinolytic inhibitors have been used in high blood loss surgeries, many patients are receiving hematopoietic growth factors to prevent or treat perioperative anemia, and new agents such as recombinant VIIa have been used off-label for a growing list of hemorrhagic conditions. Older agents such as vitamin K and fibrin sealants are increasingly being used as adjunctive therapy for bleeding and hemorrhagic prophylaxis. This review will focus upon a number of pharmacologic agents that have the potential to reduce bleeding complications in patients undergoing surgery and thereby limit the quantities of allogeneic blood components they require.

In addition to autologous options including perioperative hemodilution and blood salvage the quest to provide transfusion safety may include the use of blood substitutes in the future or modifications of current blood components (leukocyte reduction, pathogen inactivation) that may reduce the risk of transfusion complications. A number of these approaches are under development and appear to be efficacious for some clinical indications. In other studies, drugs and growth factors that may reduce the need for transfusions may cause safety concerns such as tumor recurrence, renal dysfunction, and thrombotic complications. Although reducing the quantity of blood components that are transfused to a perioperative patient is an important goal, the ultimate goal of any transfusion alternative should be to permit the patient to undergo surgery with the lowest possible risk of morbidity or mortality. Reduction of blood transfusion should not be regarded as a primary endpoint or the ultimate goal; if reducing transfusion involves increased risks from adverse effects of drugs or other manipulations, the benefit to the patient may be minimized or in some cases eliminated.

Sunday 18 October
ANZSBT Symposium: Transfusion Safety

1100-1200
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1130

Pathogen Reduction by Inactivation Technologies – The New Frontier for Safeguarding Cellular Blood Products?

Ken Davis

Transfusion Medicine, SA Pathology – Royal Adelaide Hospital, Adelaide, Australia

Although the infectious risks of blood transfusion are remarkably small, the current multi-layered approach of donor screening, testing and deferral is unlikely to have as much impact in the future as it has in the past.

Pathogen inactivation [PI] technologies have all but eliminated the infectious risks of plasma-derived protein fractions and will potentially provide additional protection against both known and as-yet-unidentified agents.

The treatment of blood components using PI technologies continues to evolve and the impact of PI on product quality and recipient safety remains paramount and is yet to be fully determined.

The presentation will focus on the issues surrounding pathogen inactivation of blood components and how they contrast with those involved in plasma fraction manufacturing.

References

Prowse C: Properties of Pathogen-Inactivated Plasma Components. *Transfus Med Rev.* 2009 April 23 (2):124-133.

Proceedings of a Consensus Conference: pathogen inactivation – making decisions about new technologies. *Transfus Med Rev.* 2008 Jan;22 (1):1-34.

Barbara J. Bryant, MD; Harvey G. Klein, MD: Pathogen Inactivation The Definitive Safeguard for the Blood Supply. *Arch Pathol Lab Med.* 2007;131:719–733.

Sunday 18 October
ASTH Symposium: Current Controversies

1100-1200
Hall D
1100

Thrombophilia and Pregnancy Complications – To Test or Not to Test

Claire McLintock

National Women's Health, Auckland City Hospital, New Zealand

Until the mid 1990s, the connection between haematology, obstetrics and the thrombophilias was confined to the antiphospholipid syndrome - pregnancy complications such as recurrent miscarriage, fetal death and preeclampsia, in women with lupus anticoagulant, anticardiolipin and beta2 glycoprotein 1 antibodies. Thromboses and infarcts frequently found in the placenta of women with these pregnancy complications prompted studies to examine a potential and biologically plausible relationship between the inherited thrombophilias and placental mediated obstetric complications. As with inherited thrombophilias and venous thromboembolism, the promise of a simple blood test to identify women at risk from these serious pregnancy complications was just too alluring. The floodgates opened to release a deluge of case control and cohort studies investigating the association of various combinations of placental mediated pregnancy complications with inherited thrombophilias. Odds ratios and 95% confidence intervals emerged with widely ranging results. Extrapolation of odds ratios led many clinicians to believe that having one of the inherited thrombophilias increased the risk of women developing one a pregnancy complication de novo or having a recurrence of such a complication in a subsequent pregnancy. Moreover, in the absence any data from randomised clinical trials, many women were given thromboprophylaxis with low molecular weight heparins to prevent such pregnancy complications.

However, recently, the backlash has begun. Some authors¹ have challenged the importance of the inherited thrombophilias in the placental mediated pregnancy complications and called for an end to thrombophilia testing outside the setting of clinical trials. Two prospective studies have failed to confirm an increased risk of preeclampsia in women with thrombophilias. A nested case-control study from Canada⁴ showed no increase in the prevalence of FVL, PT20210 and the MTHFR polymorphism in women with preeclampsia (n=113) compared to women (n=443) with uncomplicated pregnancies, [OR 1.2 (95%CI 0.3-4.1); OR 1.1 (95%CI 0.1-8.8); OR 0.2 (95%CI 0.2 (0.1-1.0), respectively. A large prospective cohort study⁵ revealed similar rates of preeclampsia in women (n=134) who were heterozygous for FVL (n=5, 3.7%) compared to women (n=4751) without the mutation (n=141, 3%), [OR 1.3 (95% 0.4-2.8)]. Similarly, studies have failed to demonstrate that women with FVL or PT20210 are at increased risk of fetal loss.⁶ Entering the second decade of the new millennium what should clinicians do? To paraphrase the immortal bard, "To test, or not to test: that is the question."

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4. Coppens M, Folkeringa N, Teune MJ, et al. Outcome of the subsequent pregnancy after a first loss in women with the factor V Leiden or prothrombin 20210A mutations. *J Thromb Haemostat* 2007 Jul;5(7):1444-8

Sunday 18 October
ASTH Symposium: Current Controversies

1100-1200
Hall D
1130

To Bridge or Not To Bridge? – Managing Perioperative Anticoagulation

Christopher M Ward

*Northern Blood Research Centre, University of Sydney;
Department of Haematology and Transfusion Medicine, Royal North Shore Hospital,
St Leonards, NSW*

Longterm use of warfarin is increasing in the community, particularly for atrial fibrillation. Managing anticoagulation around major surgery or an invasive procedure can be challenging, with risks of both thromboembolism and bleeding. “Bridging” implies perioperative switching of patients from warfarin to (low molecular weight) heparin. There are little trial data to guide this practice, and a wide range of clinical opinion. It is important to consider the risks for each individual patient and their procedure. With most bridging protocols, there will be a short period of subtherapeutic anticoagulation during which the patient is at risk of thromboembolism (TE). We can calculate a daily risk of TE recurrence, but this may be significantly increased by surgery itself and postoperative immobilisation. Patients with low-risk atrial fibrillation (AF) may not require bridging anticoagulation and warfarin can simply be ceased 5 days prior to surgery. Similarly, patients with venous TE more than 3 months prior may only require prophylactic LMWH for bridging. The most problematic groups are patients with high-risk AF or mechanical heart valves; here, standard bridging protocols appear sufficient to prevent venous events but not stroke. More aggressive anticoagulation, such as a monitored unfractionated heparin infusion, may be needed to avoid arterial TE.

Bridging protocols can increase the rates of clinically relevant bleeding, particularly if heparins are restarted too soon after surgery. Bleeding events can trigger prolonged delays in anticoagulation and put the patient at risk of TE. For high-risk surgery, LMWH should be restarted at a prophylactic dose, or withheld for more than 24 hours. Successful bridging strategies require the cooperation of the surgical and anaesthetic teams. Prospective trials of bridging protocols are currently underway; these trials are clearly needed to guide clinical practice.

Sunday 18 October
1100-1200
Nurses Symposium: Extreme Haematology Nursing Out of the Metropolitan Area
Hall A
1100

Transfusion in the Top End

Julie Domanski
Royal Darwin Hospital, Darwin, Northern Territory, Australia

The role of the Northern Territory Transfusion Nurse is a unique and challenging one. Although the Northern Territory is one-sixth of the Australian landmass it has less than one percent of the population. It has the smallest population (220 000) and population density of all of the Australian states and territories.

The five Northern Territory public hospitals are located in Darwin, Alice Springs, Katherine, Nhulunbuy and Tennant Creek. These hospitals are not only isolated from the rest of Australia, they are also isolated by great distances from one another. The distance between Darwin and Alice Springs is 1500kms. Each hospital provides a transfusion service; however the three smaller hospitals hold only a small stock of blood products and provide a limited transfusion service.

The NT hospitals do not have a central managing body; each functions as an independent institution. At present there is limited or no co-ordination of nursing and medical education, pathology services and quality activities between the sites. This has proved to be quite a challenge for introducing standardised transfusion practice, performing transfusion quality activities, and co-ordinating transfusion education. The workforce in the NT, particularly in health, is very transient with many staff on short contracts from one to six months. Also many positions remain vacant for long periods of time as it is difficult to attract suitably qualified staff to work in remote areas. This can affect the level of transfusion service provided by each individual hospital.

The Transfusion Nurse currently reports to two senior staff members at the Royal Darwin Hospital. There is no direct reporting line that covers all five hospitals. The establishment of a territory-wide Transfusion Committee in March 2009 was a major milestone.

Sunday 18 October

Nurses Symposium: Extreme Haematology Nursing Out of the Metropolitan Area

1100-1200

Hall A

1130

Assisting the Development of Outpatient Day Centre in India

Lisa Elliot

Abstract not received at time of going to print

Sunday 18 October**1100-1200****BMTSAA Symposium: Post Transplantation Chimerism Analysis****Meeting Rooms 1/ 2****1100****Chimerism Analysis and Methodology – The RAH Experience**

Judy Stevens, Michael Vo, Monika Kutyna, Pamela Dyson, Ian Lewis
SA Pathology- RAH, Adelaide, South Australia, Australia

In transplantation medicine, chimerism refers to the co-existence of cells from two individuals in the one body. In patients who have undergone allogeneic transplantation, donor cells should be present in the blood or bone marrow. Chimerism testing quantitates the percentage of donor cells in the recipient and is used initially to monitor engraftment and later for possible relapse.

Quantitation of donor chimerism utilises differences in donor and recipient polymorphic genetic markers. We use a PCR-based method utilising polymorphisms known as short tandem repeat (STR) units -multiple copies of an identical DNA sequence, 2 to 5 base pairs long. A commercially available STR identity kit, called Identifiler is used, with probes for 16 different STR loci. STR units are highly polymorphic, and differences between the donor and recipient in at least some of the 16 loci are expected. Using this kit, all 16 loci may be tested with the aim of finding at least three informative markers.

Suitable informative markers are found by testing donor and recipient material collected prior to transplant and these markers are subsequently used for all testing. Generally, the first sample for chimerism testing is taken one month after the transplant and at regular intervals thereafter. Testing is done using either peripheral blood or bone marrow.

Chimerism analysis is a multi-step procedure comprising density gradient separation of mononuclear cells, sorting of cells into three subsets, subsequent extraction and amplification of the DNA followed by separation by genescan (capillary electrophoresis) technology.

Genescan results are analysed using Identifiler software. For pre-transplant material all sixteen markers are analysed, while post-transplant three markers are usually analysed.

We have utilised this procedure to follow engraftment in 19 patients with MM, 26 CML, 33 lymphoma and 77 AML. We have also performed testing on patients following double cord transplant.

No conflict of interest to disclose

Sunday 18 October

1100-1200

BMTSAA Symposium: Post Transplantation Chimerism Analysis

Meeting Rooms 1/ 2

1120

Chimerism and MRD post-SCT in children. A guide to immunotherapy in ALL?

Tamas Revesz, Heather Tapp, Colin Story, Judith Stevens & Pam Dyson
SA Pathology at Women's & Children's Hospital and IMVS, Adelaide

Stem cell transplantation is used to improve results in high-risk and in relapsed acute lymphoblastic leukaemia (ALL). Monitoring the level of minimal residual disease (MRD) and chimerism post transplant usually shows rapid reduction of MRD and establishment of full donor chimerism. If MRD remains detectable and/or chimerism shows increasing patient signal, it usually signals resistant disease and the prognosis is poor.

Recent advances in MRD and chimerism monitoring enable us to intervene in some cases of mixed chimerism. In the first instance this takes the form of rapid tapering of immunosuppression. If this approach doesn't work or the patient is already off immunosuppressive therapy, the next step is to introduce immunotherapy – preferably at a time when there is still relatively small leukaemia burden. This can be achieved through the use of donor lymphocyte infusions. The ultimate aim is to provoke grade 2 graft-versus host disease (GVHD) which in turn is treated promptly to prevent rapid deterioration due to progressive GVHD.

There is some evidence that early-stage GVHD is associated with increased leukaemia-free survival without long-term toxicity. Whilst this approach works in some of the patients, the majority who relapse post SCT seem to present with such rapidly expanding leukaemia that there simply isn't the time for immunotherapy to work.

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BMTSAA Symposium: Post Transplantation Chimerism Analysis

Meeting Rooms 1/ 2

1140

Chimerism Analysis and GvHD

Cheryl Hutchins

Abstract not received at time of going to print

Sunday 18 October
ASTH: Barry Firkin Oration

1200-1300
Hall C
1200

Guidelines for Venous Thromboembolism Prophylaxis –Complexity, Confusion, Controversy

John Fletcher

University of Sydney and Westmead Hospital, Sydney, New South Wales, Australia

Deep vein thrombosis (DVT) and pulmonary embolism (PE) are major health problems with deaths from venous thromboembolism (VTE) greater in number than deaths from bowel cancer, prostate cancer, breast cancer and road traffic accidents. VTE is responsible for 0.2% of hospital admissions and 7% of hospital deaths and can lead to long term complications of pulmonary hypertension and post thrombotic chronic venous insufficiency. Mechanical and pharmacological prophylactic modalities have been shown to be effective in randomised control trials, with hip and knee joint replacement surgery providing a useful model for testing of efficacy of pharmacological agents for VTE prophylaxis. Anticoagulants are associated with bleeding risk which may compromise surgical outcomes in the short and long term. Bleeding is a much more readily apparent complication for the surgeon whose experience of fatal PE will be limited due to the overall low incidence of clinical VTE and with the majority of VTE events occurring after hospital discharge. There are abundant guidelines on VTE prophylaxis such as those from the American College of Chest Physicians (ACCP), the International Union of Angiology (IUA), the UK National Institute of Clinical Excellence (NICE) and the American Academy of Orthopaedic Surgeons (AAOS), with each guideline interpreting extensive data and assigning grades of recommendations according to level of evidence. Patients entered into randomised control trials do not necessarily reflect everyday clinical practice and newer interventional techniques are increasingly being used where there is limited or no information on VTE risk. Nevertheless, rational guidelines are needed for clinicians who have to make judgements for individual patients, weighing up the risk of development of VTE against the risks of available prophylactic modalities. Prevention of VTE remains one of the most important safety interventions in the management of surgical and medical patients.

Sunday 18 October
1400-1530
HSANZ BMTSAA Nurses Combined Symposium: Transplantation 1. Which alternative donor to use?
Hall C
1400

Choosing The Optimal Donor - Going Beyond Siblings

Sergio Giralt
Department of Stem Cell Transplantation and Cellular Therapies of the University of Texas M.D. Anderson Cancer Center, USA

Over the last 5 years the field of allogeneic stem cell transplantation has undergone major changes. The availability of reduced intensity conditioning regimens have expanded the pool of potential patients, while the advent of molecular typing and increases in the size of the volunteer unrelated donor banks, increases in cord blood transplantation and the use of post transplant cyclophosphamide as GVHD prevention post haploidentical allograft has vastly increased the pool of potential donors. This means that for patients without an identical sibling donor various alternatives exist: 1) Matched volunteer donor; 2) cord blood transplantation; 3) mismatched related transplant. The optimal source of stem cell will depend on patient age, urgency of stem cell transplant and donor availability. No prospective studies comparing different sources of stem cell have been performed, however, registry studies suggest a benefit for cord blood transplantation in paediatric patients when compared to unrelated donor marrow or peripheral blood stem cells. Prospective trials are urgently needed to provide guidance to patients and physicians.

Sunday 18 October

HSANZ BMTSAA Nurses Combined Symposium: Transplantation 1. Which alternative donor to use?

1400-1530

Hall C

1445

Cord Blood

Jeff Szer

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Umbilical cord blood (CB) was shown in to contain sufficient haemopoietic progenitor cells (HPC) to engraft in an appropriately conditioned individual more than a decade ago. First applied clinically in the paediatric population, the increasing availability of donor units, understanding of histocompatibility requirements for reliable engraftment and the use of multiple simultaneous CB units has led to increasing use in the adult population. In 2007, 59 patients received CB transplants 19 of whom were over the age of 15 years. Intriguingly, the total nucleated cell numbers required for engraftment are approximately one log lower for CB when compared with bone marrow and this observation, combined with differences in the immunological characteristics of CB have resulted in a different portfolio of conditioning and immunosuppressive regimens than those usually used in bone marrow or peripheral blood stem cell transplantation. Nevertheless, there are many common factors common to all three stem cell sources of prognostic importance: most importantly, cell dose and histocompatibility. Cell dose is of particular importance in larger adult recipients and the observation that ultimate engraftment is derived from only one of the multiple CB units infused and the biological characteristics of the "dominant" unit may help to further improve selection of donors. A major barrier to more widespread adoption of CB transplantation in the adult population of Australia and New Zealand is delayed engraftment and the downstream clinical and resource implications of this. CB is rapidly becoming a mainstream alternative cell product for transplantation which will result in almost all patients requiring a donor being able to find one. Current studies with ex-vivo expansion will, if ultimately successful, help to abrogate the engraftment issue and further increase the clinical use of CB transplantation.

Sunday 18 October

HSANZ BMTSAA Nurses Combined Symposium: Transplantation 1. Which alternative donor to use?

1400-1530

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1505

Haploidentical

Ken Bradstock

Abstract not received at time of going to print

Sunday 18 October
ANZSBT Symposium: The Universal Blood Cell

1400-1530
Hall B
1400

Towards a Universal Blood Supply – Fact or Fiction?

Martin L Olsson

Dept. of Clinical Immunology and Transfusion Medicine, University and Regional Laboratories, Skåne, Sweden

Dept. of Laboratory Medicine, Lund University, Lund, Sweden

Eliminating the risk for blood-group-incompatible transfusion errors and simplifying blood logistics by creating a universal blood inventory is a challenging idea but not a new one.

This lecture will summarize current concepts that have been proposed and tested to generate universally transfusable red blood cells (RBCs) for administration to patients. Several principally different lines of thinking have been applied to achieve this goal, for instance antigen masking, antigen stripping, humanised animal RBCs, siRNA-silenced blood group genes and *ex vivo* RBC production. These efforts will be described and their *pros* and *cons* mentioned briefly.

One of the projects will be discussed in more detail: An international team of collaborators explored the possibility to use a newly discovered family of bacterially-derived exoglycosidases to cleave off the A- and B-defining sugars in a specific manner on RBCs to convert them enzymatically into cells that type as group O. These RBCs were characterized extensively by immunohaematological and biochemical methods. In addition, storage parameters were measured and toxicological tests performed with the most promising enzyme candidates. Finally, clinical trials involving healthy volunteers have been started for the first time with group A RBCs converted to type as group O. The presentation will review the current status of this technology and its potential for introduction in the clinical component preparation laboratory.

Of all the strategies envisioned to make universal blood, only the enzyme-converted RBCs have yet been tested in humans. The other principles have shown promising initial results and some were tested in animal models. However, each methodology faces major challenges including difficulties to scale up production, regulatory issues, high costs for clinical trials and production. Thus, it will be several years before any of these products can become available to patients in need for blood. In the meantime, matching efforts and group O shortages will continue.

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Kodecytes – Designer Red Cells Created with KODE™ Biosurface Engineering Technology

Stephen Henry

AUT University & KODE Biotech Ltd, Auckland, New Zealand

KODE™ Biosurface Engineering Technology comprises the use of specifically designed constructs to introduce functional moieties (including antigens) into the surface of living cells without affecting their vitality or innate functionality. Previously this attachment technology has been used to create ABO sensitivity controls, by adding carbohydrate A and B antigens onto group O red cells and creating A_{weak} and/or B_{weak} kodecytes (a generic term used to describe cells modified by KODE™ cell surface engineering). Additionally kodecytes bearing Lewis, Galili, fluorophores and biotin have also been created.

Recently the repertoire of constructs able to be attached to cells has been expanded to include blood group peptides and other diagnostic markers. This has allowed for the creation of specialised red cells for antibody identification and expansion of the antigenic profiles of antibody screening and identification panels. The ability to also use this technology *in vivo* has opened up new possibilities for the study of incompatible transfusion reactions and the tracing and targeting of circulating cells.

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1505

Use of Modified Red Cells as Diagnostic Tools

Damien Heathcote

CSL Bioplasma Immunohaematology, Melbourne, Australia.

Human red blood cells have been in routine use for diagnostic purposes ever since Landsteiner bled his colleagues in 1900 and discovered the ABO blood group system. During this time they have remained essentially unchanged although their use has been extended into newer platforms such as Column Agglutination Technology and solid phase. Donor units destined for reagent manufacture have traditionally been washed and resuspended into diluents without any alteration to their antigenic makeup.

In 2005 CSL produced the first example of a diagnostic human red cell reagent that had blood group antigens added to it *in vitro* in the form of blood group A and B antigens. These cells have enabled a closer control of the ABO blood grouping process as well as allowing the user to routinely experience weak ABO reactions. The design and manufacture of the samples prevents predictive result interpretation which also makes them ideal for staff competency assessment and monitoring.

In late 2008 CSL released into international markets the first example of a human red cell reagent for antibody screening that has the *in vitro* addition of peptide-based antigens. These cells are capable of detecting IgG examples of antibodies directed against the MNS antigens MUT and Mur. These antibodies are more commonly seen in Asian populations and have been shown to be a common cause of haemolytic disease. Donors with glycophorin variants exhibiting these antigens do exist in Asian populations but these cells will also detect non-clinically relevant IgM examples of these antibodies which are more common than the IgG examples, and thus create unwanted testing. The successful addition of both carbohydrate and peptide-based antigens heralds a new age for the use of red cells in the diagnostic arena and opens up numerous possibilities for the creation of cell phenotypes not seen in nature.

Sunday 18 October

ASTH Symposium: Anti-platelet Therapy and Arterial Thrombosis

1400-1530

Hall D

1400

Overview of Anti-platelet Therapy

Huyen Tran

Abstract not received at time of going to print

Sunday 18 October

ASTH Symposium: Anti-platelet Therapy and Arterial Thrombosis

1400-1530

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1445

Measuring Resistance to Anti-Platelet Drugs – Methods and Clinical Utility

Marco Cattaneo

Clinica Medica, Ospedale San Paolo, Università degli Studi di Milano. Milano. Italy

The definition “resistance to anti-platelet drugs” should be limited to situations in which failure of the drug to hit its pharmacological target has been documented by specific laboratory tests. Aspirin resistance, as determined by specific tests (e.g., serum thromboxane B₂), appears to be rare (1-2%) and, in most instances, is caused by poor compliance. In contrast to aspirin, studies that used specific tests to measure the pharmacological effect of thienopyridines (e.g., VASP phosphorylation) showed a wide variability of responses to these drugs, with significant proportions of subjects (about 30%) who are very poor responders. Inter-individual differences in the extent of metabolism of thienopyridines to their active metabolites is the most plausible mechanism for the observed inter-individual variability in platelet inhibition. The demonstration that some patients may be “resistant” or “poor responders” to the pharmacological effect of anti-platelet drugs, has prompted the need of laboratory monitoring of anti-platelet therapy. However, many published studies have been performed using unspecific tests of platelet function, which identify patients on anti-platelet treatment with high residual platelet reactivity, which is not necessarily due to resistance to anti-platelet drugs. Despite this drawback, identification of patients with high residual platelet reactivity may be useful to predict their risk of atherothrombotic events. However, many studies still need to be done to identify the ideal laboratory test and to answer basic questions on its clinical utility and cost-effectiveness, before monitoring anti-platelet therapy can be recommended in the clinical practice. Until then, monitoring of anti-platelet therapy should be considered for investigational purposes only.

Sunday 18 October
HSANZ Symposium: Multiple Myeloma

1600-1730
Hall C
1600

A Guide for Clinicians on the New Therapies and How They Line Up Against Autologous Transplant

Thierry Facon
CHU Lille, France

The treatment of multiple myeloma (MM) has undergone significant developments in the recent past. The availability of the novel agents, thalidomide, bortezomib and lenalidomide, has expanded treatment options and has improved outcomes for patients. Following the introduction of these agents in the relapsed/refractory setting, they are also undergoing investigation in the initial treatment of MM. A number of phase 3 trials have demonstrated the efficacy of novel agent combinations in the transplant and non-transplant settings and based on these results, standard induction regimens are being challenged and replaced.

In patients not eligible for transplantation, MP-thalidomide (MPT) and MP-Bortezomid (MPV) have been found superior to the traditional MP regimen. Several studies investigating MP-Lenalidomide (MPR) or Lenalidomide-Dexamethasone (Rd) are ongoing (or have been recently completed). Of note MPT and MPV have resulted in median PFS/TTP and OS times around 2 and 4 years respectively, and these results are similar to those achieved in the early 1990s using conventional chemotherapy followed by a single transplant (IFM90)

In the transplant setting, a number of newer induction regimens are now available that have been shown to be superior to VAD (Bortezomib-Dexamethasone, VTD, PAD) For the last 15 years, high-dose therapy(HDC/ASCT) has been a standard therapy for MM in younger patients. The superiority of HDC/ASCT compared to conventional dose therapy is due to a higher very good partial response rate or better (\geq VGPR), which is correlated with prolonged survival (both progression-free and overall survival).Over the past 4 to 5 years, the arrival of novel agents (specifically, thalidomide, bortezomib and lenalidomide) has radically changed treatment regimens, as the use of these new drugs has improved the results of HDC/ASCT to such a degree that the timing and need for HDC/ASCT as first-line treatment of younger patients is currently a key question. This is the rationale of the question raised by the upcoming IFM/DFCI 2010 protocol (VRD + HDC/ASCT vs VRD alone). Additionally, the important issue of maintenance treatment is being investigated.

Sunday 18 October
HSANZ Symposium: Multiple Myeloma

1600-1730
Hall C
1600

Australian Studies in Myeloma

Andrew Spencer

Malignant Haematology & Stem Cell Transplantation Service, Alfred Health, Melbourne, Victoria, Australia 3004

Over the past 20 years a number of multicentre investigator-initiated clinical trials evaluating new therapies for multiple myeloma (MM) have been undertaken in Australasia. The Australian Leukaemia Study Group (ALSG) MM2 trial (1990-1992, n=113) evaluated the impact of Interferon-alpha (IFN) as both a co-induction agent and maintenance strategy. Consistent with other data from this era the positive effect of IFN was marginal and had to be weighed against IFN-induced toxicities. The single arm study of oral induction therapy with cyclophosphamide, idarubicin and dexamethasone (CID) (1997-2000, n=36) suggested equivalent efficacy to VAD and the approach has now been widely adopted in routine clinical practice. A randomised trial of amifostine cytoprotection (1999-2000, n=90) in patients undergoing HDT demonstrated a significant reduction in severe mucositis (33% versus 12%, $p=0.02$) with the use of amifostine and informed a pilot study (ALLG MM5) (2000-2001, n=10) of very high dose melphalan (220mg/m^2) in combination with amifostine that again confirmed the ability of the agent to abrogate melphalan-induced mucositis. Two early multicentre Phase II trials of thalidomide (1999-2003, n=141) undertaken by the group at the Peter MacCallum Cancer Centre demonstrated the lack of feasibility in combining thalidomide with either IFN or celecoxib but did confirm the efficacy of thalidomide in the setting of relapsed MM (ORR 30-40%). Subsequently the Phase III ALLG MM6 trial (2002-2005, n=269) demonstrated that post-HDT consolidation with thalidomide and corticosteroids significantly prolonged both progression free ($p<0.001$) and overall survival ($p<0.004$) compared to corticosteroids alone. The results of the study generated significant interest thus stimulating further research activities including the ongoing collaboration between the ALLG (ALLG MM11) and GIMEMA exploring the efficacy of delaying HDT in favour with lenalidomide and alkylating agents and the ALLG MM12 study designed to explore the utility of a variety of measures of post-HDT tumour burden.

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HSANZ Symposium: Multiple Myeloma

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1710

Pathophysiology of Bone Disease in Multiple Myeloma

Andrew Zannettino

SA Pathology-RAH, University of Adelaide, South Australia, Australia

Multiple Myeloma (MM) is an incurable haematological malignancy characterised by the clonal proliferation of malignant plasma cells (PC) within the bone marrow (BM). MM accounts for approximately 1% of all cancers and is the second most common haematological malignancy after non-Hodgkin's Lymphoma (NHL). Each year in Australia, approximately 1,100 people are diagnosed with MM, almost 80% of whom are over the age of 60. Alarming, in the period between 1993 and 2003, there was a 44% increase in the number of Australians diagnosed with MM. The main clinical manifestations of MM are the development of devastating osteolytic bone lesions, bone pain, hypercalcaemia of malignancy, renal insufficiency and increased BM angiogenesis. MM is the most common cancer to metastasize to bone, with up to 90% of patients developing bone lesions. The bone lesions in MM are purely osteolytic in nature, and up to 60% of patients develop a pathologic fracture over the course of their disease. The MM-induced bone lesions are a result of increased osteoclast (OC) activity in areas adjacent to MM PC. This increase in OC activity is also accompanied by a MM PC-mediated suppression of osteoblast (OB) differentiation and activity, resulting in severely impaired bone formation. This presentation will cover our current understanding of the pathophysiology underlying bone disease in MM and will review emerging therapies designed to target MM bone disease.

No conflict of interests to disclose

Sunday 18 October
ANZSBT ASTH Combined Symposium: Critical Bleeding

1600-1730
Hall B
1600

Transfusion Medicine in Acute Bleeding

Dafydd Thomas

Abertawe Bro Morgannwg University NHS Trust, Swansea, Wales, UK

As with many other emergency medical situations, acute bleeding needs to be dealt with in a calm and organised fashion. The parallels between a cardiac arrest situation or dealing with suspected anaphylactic shock are well made and the handling of acute bleeding can be optimised by the use and implementation of a massive haemorrhage protocol. There are a significant number of such protocols in practice but these are usually developed for local use as so many of the hospitals have rather unique features. When reviewing the various examples it becomes obvious that there are a number of similarities. It could therefore be possible to compile a protocol that can be used as a rather basic generic example to be customized by those hospitals that do not already use such emergency protocols.

The protocols produced in this way can contain the latest advice and evidence allowing personnel not familiar with such recommendations to follow an algorithm which helps them to immediately deliver best care.

These protocols need not be considered to be limiting the use of blood components but rather helping the overwhelmed and often nervous clinician to use such a valuable resource effectively and within guideline.

If timely use of blood components can be achieved together with concurrent monitoring of coagulation and physiological variables, the net result may be an improved clinical outcome and more rational use of blood components.

Sunday 18 October

ANZSBT ASTH Combined Symposium: Critical Bleeding

1600-1730

Hall B

1645

Damage Control Resuscitation - Coagulopathy of Trauma

Lena Napolitano

Abstract not received at time of going to print

Sunday 18 October
ANZSBT ASTH Combined Symposium: Critical Bleeding

1600-1730
Hall B
1710

The Role of Fibrinogen Concentrates in Managing Massive Transfusion

Benny Sørensen

Haemostasis Research Unit, Centre for Haemophilia and Thrombosis, Guy's and St Thomas' NHS Trust & King's College London School of Medicine, London, UK

Fibrinogen is a 300 kDa large protein produced in the liver. The average plasma concentration is 1.8-4.3 g/L. Fibrinogen plays a crucial role for haemostasis by i) facilitating platelet aggregation by bridging of glycoprotein IIb/IIIa receptors, and ii) fibrin polymerization trigger via thrombin generation. In addition, fibrinogen is an acute phase reactant. In principal, deficiency of fibrinogen can be inherited or acquired. Congenital fibrinogen deficiency is a miscellaneous coagulation disorder resulting in either hypo-fibrinogenemia causing bleeding symptoms or dys-fibrinogenemia resulting in bleeding or thrombosis tendency. Acquired deficiency of fibrinogen develops in conjunction with other disorders, such as liver disease, disseminated intravascular coagulation, and excessive bleeding.

The role of levels and function of fibrinogen as a haemostatic agent in management of peri-operative and traumatic haemorrhage are grossly underestimated. There may be several reasons for fibrinogen concentrate being overlooked as potent haemostatic intervention. For decades, haematologists have set the lower threshold level of fibrinogen at 1 g/L. Unfortunately, this level has never been clinically validated, yet mentioned again and again, although a series of publications have indicated that the critical level of fibrinogen may be significantly higher. A majority of patients experiencing excessive bleeding are treated with colloid plasma expanders for volume substitution. The presence of colloid plasma expanders or high levels of fibrin degradation product have been reported to induce artificial false high levels of fibrinogen when measure by the Clauss method. These phenomena may further have masked the recognizing and understanding the importance of fibrinogen in management of peri-operative/traumatic bleeding. Finally, the use of plasma expanders such as colloids, gelatine, or dextrans, is now known to induce a coagulopathy characterised by acquired hypo-fibrinogenemia and abnormal fibrin polymerisation. Moreover, experimental studies, animal studies, retrospective clinical surveys, as well as prospective randomised clinical studies have demonstrated excellent haemostatic effect of substitution with a fibrinogen concentrate.

The present presentation review laboratory aspects in evaluation of fibrinogen, the mechanisms of dilutional coagulopathy, and summarize clinical efficacy and safety outcome following intervention with fibrinogen concentrate.

Sunday 18 October**Nurses – Free Communications 1: Apheresis****1600-1730****Hall D****1600****O001****Trouble shooting the way through Therapeutic Plasma Exchange in a 2 year old boy with Thrombotic Thrombocytopenic Purpura (TTP)****Grainne Dunne***Clinical Nurse Consultant Apheresis SCH*

Thrombotic Thrombocytopenic Purpura (TTP) is a complex disease process where the first line of management is apheresis, i.e. Therapeutic Plasma Exchange. To add to this complex disease is the challenge of managing an apheresis patient who is only 2 years old!

How do we fine tune apheresis issues such as fluid volumes and blood product replacement in a small body who is already so sensitive to these issues? How can we perfect the apheresis procedure so as to allow this young boy his greatest chance at survival?

There is not a lot of literature on paediatric Therapeutic Plasma Exchange. We therefore need to learn what we can from our own experiences and from our apheresis colleagues with regards to issues such as appropriate blood product replacement and maintaining suitable fluid balances when the body is at high risk of renal failure. Endeavouring to reduce or prevent additional complications such as fluid overload while using apheresis machines designed primarily for adult patients who are less sensitive to fluid changes.

These are some of the complex problems we encountered and examined during the management of this particular patient. Apheresis operators should constantly examine their apheresis practice and should continuously strive towards improved techniques when managing young children in apheresis. This case study also demonstrates the need for apheresis vigilance together with high quality apheresis training and experience.

No conflict of interest to disclose

Sunday 18 October
Nurses – Free Communications 1: Apheresis

1600-1730
Hall D
1615

0002

Managing the Complexities of Renal Patients during Therapeutic Plasma Exchange

Tracy Clarke

Prince of Wales Hospital, Randwick, NSW

Aim

Renal patients pose a unique set of complexities that need to be considered during apheresis procedures. There is an increasing number of renal patients undergoing apheresis particularly pre and post renal transplantation to reduce the risk of acute rejection. Our purpose is to explore these issues and identify safe management strategies for this patient population.

Method

170 procedures in 14 patients were performed for patients undergoing or having undergone renal transplantation or Haemolytic Uraemic Syndrome (HUS). Clinical condition pre and post procedure, complications experienced during the procedure, management strategies and response to treatment was documented and compared. Fluid balance requirements, urine output and dialysis requirements were considered in identifying procedural parameters and individual patient management strategies were developed to reduce the risk of adverse events and improve patient outcomes.

Result

Common toxicities experienced during apheresis were citrate toxicity and hypotension. The rate of citrate toxicity for this patient population was 9.4% compared to 8.1% overall. Interestingly, hypotension defined as a drop in BP of greater than 10% from baseline or systolic BP less than 100mmHg was documented in only 2.4% compared with 12% overall. Of greater significance was the additional adverse event related to transfusion of fresh blood products which was documented in 7 of the 14 patients and a reaction to promethazine was noted in 3 patients. Procedures were performed at varying fluid balances ranging from 80% to 100% fluid balance depending on patient needs and changes to individual patients run parameters were made following an adverse event with success in preventing further events.

Conclusion

Renal patients pose unique and different clinical challenges when undergoing apheresis procedures compared to other patient populations. Careful consideration of individual patient parameters, in particular electrolyte and fluid balance is required to successfully undertake apheresis while minimising adverse events.

No conflict of interest to disclose

Sunday 18 October
 Nurses – Free Communications 1: Apheresis

1600-1730
 Hall D
 1630

O003

Plasma Exchange for treatment of Chronic Intractable Urticaria – A Single Case Experience

Fran Owen¹, Pauline Warburton¹, Robert Loblay²

¹ *Haematology Department, Wollongong Hospital, Wollongong, NSW, Australia*

² *Allergy Unit, Royal Prince Alfred Hospital, Sydney, NSW, Australia*

Aim

Chronic idiopathic urticaria is defined as recurring episodes of hives lasting more than 6 weeks. One third of patients have an autoimmune mediated urticaria, often experiencing more severe symptoms. Quality of life can be markedly affected. Management includes minimizing stress, overheating and alcohol. H1 and H2 antagonist drug therapies may be of benefit. Oral steroids may be used for severe exacerbations. Third line therapies including immunosuppressive drugs, intravenous immunoglobulin and plasmapheresis have been used in refractory cases. The use of plasmapheresis is rare and there is limited information in the literature regarding this treatment.

A 36 year old woman with an 18 year history of urticaria including angioedema was referred to our unit for a trial of plasmapheresis. Symptoms had improved during pregnancy and it was felt that hormonal factors were involved. Current treatment includes a progestogen implant and regular promethazine and doxepin. She presented intermittently to Emergency for management with adrenaline and steroids.

Method

Plasmapheresis was commenced using a one plasma volume exchange and Albumex 4% for replacement fluid. Treatments were given twice weekly for 4 weeks, weekly for 6 weeks, then second weekly (four months to date) with the aim to extend to monthly treatments if possible. An apheresis Hickman's catheter was inserted early due to poor venous access.

Result

Our patient has had a good response to plasmapheresis. She has stopped all oral medication. She experiences occasional mild urticaria/angioedema which is controlled by doxepin prn. Her quality of life has improved and she is keen to continue this treatment.

Conclusion

The use of plasmapheresis has resulted in a good clinical response in this patient to date. Chronic intractable urticaria is not an uncommon condition but treatment with plasmapheresis is rare as other measures are usually effective. Randomized trials are not feasible and reports of its use are generally anecdotal.

No conflict of interest to disclose

Sunday 18 October
Nurses – Free Communications 1: Apheresis

1600-1730
Hall D
1645

O004

Use of the Fresenius COM.TEC Cell Separator for PBOC Collection: Ongoing Need for Apheresis Operator Interventions

Jennifer Leutenegger, Kari L Mudie, Cheryl J Hutchins, Maree Bransdon, Alanna Geary, Glen A Kennedy
Royal Brisbane and Women's Hospital, Brisbane, Australia

Background

Historically, many transplant units have used the COBE Spectra (Version 6.1MNC) for collection of peripheral blood progenitor cells (PBPC). Recently, Pharmatel Fresenius Kabi has introduced a new technology, the COM.TEC autoMNC (Version 4.02) for PBPC collection. The Fresenius COM.TEC cell separator aims to decrease Apheresis Operator variability in PBPC collection via the autoMNC software programme, which predicts estimated CD34+ cell yield. The challenge for the Apheresis Operator is to adapt to this new technology without impacting on the quality of the PBPC product.

Aim

To compare the PBPC product collected on the Fresenius COM.TEC and the COBE Spectra.

Methods

A prospective study of 48 patients undergoing autologous PBPC collection following mobilisation with cytotoxic treatment and G-CSF. Collection was commenced when circulating peripheral CD34+ cell count was greater than or equal to $10 \times 10^6/\text{kg}$ body weight. Patients were alternatively assigned to have the 1st day of PBPC collection on either the Fresenius COM.TEC or the COBE Spectra cell separator.

Results

Overall, 20 patients had PBPC collected on the Fresenius COM.TEC and 28 on the COBE Spectra. Even though the Fresenius COM.TEC cell separator is a highly automated device, several variables were still found to influence the collection efficiency, including equipment performance, operator technique, institutional requirements and patient variables. Overall, the product collected using the Fresenius COM.TEC yielded similar CD34+ cell yields though a higher total red blood cell count, red blood cell volume and larger total collection volumes in comparison to collections performed on the COBE Spectra. This significantly impacted on laboratory processing post collection. Apheresis Operator interventions to overcome suboptimal product collection on the COM.TEC included manipulation of centrifuge speed, spillover volumes and buffy coat volumes. These interventions appeared to somewhat reduce cellular contamination and overall volume of the Fresenius COM.TEC collections.

Conclusion

Our experience highlights the need to formally assess collection efficiency of new technologies introduced in PBPC collection. Despite the aims of the Fresenius COM.TEC cell separator to decrease Apheresis Operator variability in PBPC collections, we still found that several factors influenced collection efficacy of these machines, and indeed an ongoing need for Apheresis Operators to manipulate machine parameters to reduce cellular contamination and collection volume on the Fresenius COM.TEC.

No conflict of interest to disclose

Sunday 18 October

Nurses – Free Communications 1: Apheresis

1600-1730

Hall D

1700

O005

Calcium Replacement in Apheresis

Andrew Scullion

Calvary Mater Hospital, Newcastle NSW, Australia

Aim

Citrate toxicity and the related effects of hypocalcaemia are an often experienced side effect of apheresis procedures.

The aim of my research was to develop an easy to navigate and functional tool for the apheresis operator to utilise that would aid in maintaining a safe apheresis environment for a haemodynamically stable patient free of or with minimal effects of citrate toxicity.

Method

A literature review of research papers relevant to this specific area of apheresis was undertaken to gain a comprehensive understanding of methods and dose ranges used in the intervention, treatment and management of side effects attributed to citrate toxicity in the apheresis setting. Reference material relating to the pharmacological aspects of treating hypocalcaemia was also reviewed to ascertain correct dosages for treatment pathways consistent with patient parameters.

Result

The result of the literary review formed the basis for the development of a flow chart using mechanical and pharmacological intervention as well as utilising prophylactic means to combat apheresis related citrate toxicity and the effects of hypocalcaemia. See flow chart as attachment.

Conclusion

The development of this flow chart for calcium replacement in apheresis gives the apheresis operator safe, research derived options and guidance for the treatment of citrate toxicity, from it's mild forms to more severe reactions, taking into account individual patient variances and the changing nature of an apheresis procedure.

No conflict of interest to disclose.

Sunday 18 October

Nurses – Free Communications 2: Discharge and Outpatient Care

1600-1730

Hall A

1600

0006

The Development and Implementation of a Nurse-Led Follow-Up Clinic for Patients Who have Undergone an Autologous Stem Cell Transplant (ASCT)

Trish Joyce¹, Linda Clark¹, Sharna Moloney¹, Meinir Krishnasamy¹, David Ritchie^{1,2}, Michelle Fleming³, Kirsten Herbert¹, John Seymour¹, Jenny Byrne³

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Introduction

The needs of individual patients following ASCT are highly varied. Patients commonly experience ongoing adverse effects such as fatigue, poor appetite, difficulties in resuming work, alterations in body image and sexuality issues. Evidence indicates that traditional medical models of follow-up care may fail to meet patients' psycho-social needs and are sub-optimally effective at management of late effects. Nurse led clinics (NLCs) have been shown to offer patients increased continuity of care, more individualised follow-up and improved psycho-social outcomes. In response to this, we obtained funding to undertake a pilot study to test a nurse-led follow-up clinic for patients who have undergone an ASCT.

Aim

This study set out to test the acceptability, feasibility and safety of the NLC. The aim of the paper is to describe the development of the NLC from its conceptualisation to implementation.

Method

A quasi-experimental pilot study was developed. Phase 1 involved the collection of baseline data to gain an appreciation of problems experienced by this group of patients at Peter Mac. This data was used to inform evidence-based interventions to be delivered in the NLC. Algorithms were developed to support the nurses working in the clinic to ensure safe and consistent practice. Standardised nursing assessment documentation was developed to record the issues presented by patients, the interventions delivered by nurses in the NLC and their efficacy. Caseload and quality assurance data will also be monitored. Phase 2 involves testing the acceptability, feasibility and safety of the NLC and this is now underway.

Conclusion

We believe this innovative model of care has the capacity to enhance the current model of follow-up for patients following an ASCT. We are testing this assumption using evidence, wherever possible, to inform the design, content and evaluation of our initiative.

No conflict of interest to disclose

A42

Sunday 18 October

Nurses – Free Communications 2: Discharge and Outpatient Care

1600-1730

Hall A

1615

0007**An Integrated Outpatient Autograft Program: The Royal Hobart Hospital Model of Care****Gillian Sheldon-Collins**, Louise Nicholson

Royal Hobart Hospital, Hobart, Tasmania, Australia

Aim

The Royal Hobart Hospital is the tertiary treatment facility for Tasmania. The Outpatient Oncology/Haematology Service at this hospital has managed an outpatient autologous transplant program for 11 years for patients with a variety of diagnoses. This presentation demonstrates that this model of care is safe and sustainable within the context of Australia's rapidly changing health care environment.

Method

A literature search was conducted and contact made with other Australian hospitals to investigate outpatient models. The Tasmanian Bone Marrow Transplant Data Base was investigated to reveal the number of patients selected for inpatient versus outpatient care, the distribution of transplant related deaths and length of hospital inpatient stay. Protocols supporting the outpatient model of care were reviewed including daily assessment by nursing, medical and allied health staff.

Result

The literature review reveals few published articles regarding models of outpatient care especially in Australia, although several hospitals provide outpatient programs. The Royal Hobart Hospital model demonstrates that patients can be safely supported in the community when ease of access to the tertiary treatment centre is assured. The review of the data base demonstrates the safety of outpatient care by drawing comparisons between transplant related deaths in the inpatient versus the outpatient group. The practice of accurately selecting outpatients without increasing risk is supported by the data.

Conclusion

The Royal Hobart Hospital demonstrates that patients can be carefully selected and safely managed in an outpatient autograft program. This model is adaptable and sustainable as the available human and material resources change over time. This presentation contributes to the available body of knowledge and growing trend toward outpatient services.

No conflict of interest to disclose

Sunday 18 October

Nurses – Free Communications 2: Discharge and Outpatient Care

1600-1730

Hall A

1630

0008

An Evaluation of a New Model Of Nursing Care in Queensland's Leading Haematology and Bone Marrow Transplant Unit

Emily Russell, Annette Barnes, Kylie Hilford

Royal Brisbane and Women's Hospital, Queensland, Australia

Aim

A 'New Model of Nursing Care' (NMNC) project was undertaken at the Bone Marrow Transplant and Haematology Unit, Royal Brisbane and Women's Hospital. This project included the implementation of three new leadership roles (two clinical Team Leaders (TL) one organisational Shift Coordinator (SC)). The aim of this evaluation was to determine if the new leadership roles arising from the NMNC enhanced patient care delivery, staff support and professional development.

Method

Forty-seven permanent nursing staff on the unit were invited to complete the NMNC questionnaire. Twenty three (49%) staff completed and returned the questionnaire. Both qualitative and quantitative data were collected to examine the perception of benefits associated to the introduction of NMNC. Results were categorised into three themes; organisational, clinical and educational.

Results

Organisational – The SC role aimed to manage organisational tasks such as patient flow and human resources. Staff generally reported feeling well supported by their SC, and felt the SC role allows other roles to focus on clinical tasks. However, 4 staff (17%) did not feel they had a good understanding of the SC role and suggested that succession management into the role would improve their understanding.

Clinical - The TL role was designed to lead a group of nurses in the delivery and coordination of patient care. A majority of staff agreed that they were more clinically supported by the TL (87%), and 91% believed the role improved basic nursing care. The presence of the TL in direct patient care was appreciated by 83% of nursing staff despite some variance in the perceived effectiveness of the specific individuals fulfilling the role.

Educational – Ninety-six percent of all staff agreed or strongly agreed that the clinical facilitators, clinical nurses and TL have been readily available to assist and facilitate their education needs. The improvement of staff education through the implementation of NMNC was evident through staff's ability to attend in-services, complete assessments, and attend ward meetings.

Conclusion

Overall, the response to the introduction of the NMNC has been positive. All staff agreed that their ability to provide holistic nursing care has improved and would like to continue working within the framework. Of note, 96% of staff stated that it has encouraged more effective teamwork.

Areas for improvement were identified as communication between the two TL and SC, discharge planning processes, and succession planning of staff into the SC role.

There is no conflict of interest to disclose

Sunday 18 October
Nurses – Free Communications 2: Discharge and Outpatient Care
1600-1730
Hall A
1645
O009
Cancer Outreach Team: Taking the Hospital Out of Myeloma
Kerrie Murphy
CNC Cancer Outreach Team, Prince of Wales Hospital, Randwick Sydney NSW 2031

With recent advances in the field of myeloma treatment patients are living longer with better health outcomes. Also treatment options such as Thalidomide provide the patient with the potential for less hospital stays. However the side effects of these treatments coupled with regular biphosphates infusions, venous access device care and the need for regular pathology tests ensures that the patient is required to attend the hospital setting.

The Cancer Outreach Team (COT) established in 2001 provides community based care for patients with oncological or haematological malignancies who are undergoing treatment at the Prince of Wales Hospital. Due to the changing nature of myeloma management COT has been able to provide a unique home based service that enables the patient to remain at home for the majority of their treatment. Services provided by COT in the home include:

- Venous access device care
- Zoledronic acid infusions
- Bortezomib administration
- Monitoring of oral therapies such as Melphalan, Thalidomide, Prednisone and Lenalidomide
- Triage service for febrile episodes
- Supportive care
- Allied health referral
- Pathology service
- Neulasta and GCSF administration
- Post chemotherapy and peripheral stem cell transplant support

This presentation will outline the unique home based care provided for patients with myeloma by COT at Prince of Wales Hospital.

No conflict of interest to disclose

Sunday 18 October

Nurses – Free Communications 2: Discharge and Outpatient Care

1600-1730

Hall A

1700

O010

The Haematology Step-Down Unit – A Novel Approach to Early Discharge and The Continuity of Care Through Care Co-Ordination

Sandra Liddell & Douglas Joshua

Institute of Haematology, Royal Prince Alfred Hospital, Camperdown, NSW Sydney, Australia

Aim

The Haematology Step-Down Unit (HSDU) was opened in February 2005 to promote and facilitate the early discharge of haematology patients that have undergone Bone Marrow Transplantation and/or high dose chemotherapy.

Method

The unit is a small ambulatory care area adjacent to the haematology inpatient unit. It comprises of 5 chairs and 1 bed. It is run by the Haematology Care Coordinator and supported by one other registered nurse. Its hours of operation are 0800hrs to 1700hrs on week days only. The HSDU provides a continuum of care through the transitional processes of inpatient to outpatient. It offers a haematology specific service: central line care, blood product support, bisphosphonate therapy, monoclonal antibody therapy along with counselling, education, support and pre chemotherapy assessments. The service allows for patients to be discharged before count recovery after treatment. The service also provides efficient and prompt triaging of the un-well haematology patient.

Results

The majority of patients (~ 50%) triaged in the HSDU were for febrile neutropenia. Other conditions included hypercalcaemia, nausea and vomiting post chemotherapy, symptom management, pain control and central line complications. In 2008 the unit saw approximately 180 patients per month. In 2008 76 patients avoid casualty by coming through the HSDU. The unit is cost effective returning about \$180 000 last financial year through Medicare.

Conclusions

The future direction is one of growth due to the ever increasing demands on our already strained public health system. The philosophy of Care Coordination aids in effective follow up and the early discharge of patients. The HSDUs capacity to provide these services could be enhanced by the addition of a nurse practitioner.

No conflict of interest to disclose

Sunday 18 October
BMTSAA Symposium

1600-1630
Meeting Rooms 1/ 2
1600

Cell Therapy Infrastructure: Development and Subsidized Access

Stewart Hay

Research Infrastructure Support Services, Level 6, 464 St Kilda Road, Melbourne, 3004

Research Infrastructure Support Services (RISS) is funded by the Federal Government to facilitate access for researchers to cGMP facilities for the manufacture of human cells for transplant, as well as supporting facilities to develop and maintain TGA licensing.

RISS supports cell therapy facilities at The Queensland Institute of Medical Research, The Children's Hospital at Westmead, The Royal Prince Alfred Hospital, Cell Therapies, The Institute of Medical and Veterinary Science and the Royal Perth Hospital. In the last two years RISS has provided approximately \$1.4 million to support cGMP related cell therapy research. This research has involved a variety of cell based approaches for treatment of cancer, inflammatory disorders, and infections.

The project administered by RISS is funded through the National Collaborative Research Infrastructure Strategy (NCRIS) and will run until 2011. The goals of NCRIS are to improve collaboration and to avoid unnecessary duplication of infrastructure.

Sunday 18 October
BMTSAA Symposium

1600-1630
Meeting Rooms 1/ 2
1610

Report from Australian Regional ISCT Meeting

John Rasko

Australian Regional Vice President, ISCT, on behalf of the Organising Committee

Following in the successful footsteps of the full International Society for Cellular Therapy meeting in Sydney in 2007, we have initiated a regional one-day ISCT meeting in Adelaide on Saturday 17 October 2009. Our program entitled "Cellular Therapies –Translational Medicine" was designed to emphasise the theme of providing a forum for national and international speakers to update us on the breadth and depth of clinical cellular therapies. Importantly we included a regulatory workshop and series of case studies for participants involving good manufacturing practices. Speakers include the President of ISCT, Mary Laughlin (Cord Blood Cells); Robert Deans (MSCs); Leslie Wolfe (chondrocytes); David Connell (tendon repair); Ineke Slaper-Cortenbach (ISBT-labeling standards); John Greenwood (skin); Stan Gronthos (bone); Giles Plant (neuronal repair) as well as talks on pluripotent stem cells and immunotherapies.

We hope to encourage all scientists and clinicians involved in the burgeoning field of cellular therapies to get involved and support this peak industry body.



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ABSTRACTS - Monday 19

0715-0815
Meeting Room 4

Aplastic Anaemia/Aplastic AML

Neal Young

Notes:

O011

Instability of FLT3 Mutations In Relapsed Acute Promyelocytic Leukemia (APL): A Comparative Analysis Of Paired Diagnostic And Relapse Bone Marrow Samples

0830

Amanda Hugman, Juliet Ayling, Albert Catalano, Shane Supple, Harry Iland
Institute of Haematology, Royal Prince Alfred Hospital, Sydney, NSW, Australia

Aim
 FLT3 mutations are common in APL and are associated with an adverse prognosis. To determine their role in relapse, we compared the FLT3 status of patients enrolled in the ALLG ϕ APML3 protocol at diagnosis and at first molecular or haematological relapse.

Method
 Total RNA from marrow mononuclear cells was tested for FLT3 mutations by fragment analysis of fluorescently-labeled RT-PCR products using high resolution capillary electrophoresis and Peak Scanner analysis software. PCR products larger than the normal FLT3 product, and distinct from previously known and newly identified alternative transcripts, were indicative of a FLT3 internal tandem duplication (ITD). Their FLT3 specificity was confirmed by digestion with Dral \pm cloning and sequencing. D835 kinase domain point mutations (PM) were detected by amplification and EcoRV digestion. Patients with undigested products >10% were sequenced to confirm D835 PM.

Results
 Paired diagnosis and relapse samples were available from 19 patients.

Diagnosis	Wild type (WT)			Internal tandem duplication (ITD)			Point mutation (PM)		
	8			9			2		
Relapse	WT	ITD	PM	WT	ITD	PM	WT	ITD	PM
	7	0	1	4	5	0	1	0	1

Four patients with ITDs at diagnosis expressed only WT FLT3 at relapse. In patients with ITDs at diagnosis and relapse, there was evidence of clonal selection and/or evolution. One patient relapsed with multiple ITDs of differing sizes to the diagnostic clone. Another with 2 ITDs relapsed with only one (the major clone at diagnosis), and one with 4 ITDs relapsed with only one (a minor clone at diagnosis). In one patient serial relapse samples gave discrepant results which were reconciled by the design of patient-specific primers oriented to exclusively amplify the original ITD. With this system, ITD-specific PCR products were detected in the diagnosis and both relapse samples, but not in two intervening remission samples.

Conclusion
 In the context of APL, instability of FLT3 mutations with gains and losses at relapse suggests that FLT3 mutations are secondary events in leukemogenesis. They are not reliable markers of minimal residual disease, and are unlikely to serve as useful therapeutic targets.

This research was supported by the Cancer Institute NSW Clinical Fellowship and the Haematology and Oncology Targeted Therapies (HOTT) Award 2008 (Roche Oncology & Haematology in conjunction with COSA, MOGA and HSANZ). Roche had no role in analyzing the data or preparing the abstract.



O012

0845

Arsenic Pharmacodynamic Factors Impact on Efficacy of Treatment with Arsenic Trioxide (ATO) in Combination with Idarubicin plus All-Trans Retinoic Acid for Acute Promyelocytic Leukaemia (APML)

Frank Firkin¹, Lisa Lincz², Shane Supple³ and Harry Iland³ for the ALLG APML4 Study
¹ St Vincent's Hospital, Fitzroy, Vic; ² Mater Hospital, Newcastle, NSW; ³ Royal Prince Alfred Hospital, Camperdown, NSW

Aim

To examine relationships of blood arsenic levels and genetic status of rate-limiting enzymatic steps in methylation of inorganic arsenic to Molecular Complete Remission (MCR) and adverse events during treatment of APML with ATO plus Idarubicin and All-Trans Retinoic Acid (AIA regimen).

Methods

32 patients where blood arsenic levels were determined at the end of Induction/ First Consolidation in the APML4 study were evaluated. Arsenic was assayed by atomic absorption spectrophotometry. Polymorphisms of Glutathione S-Transferase (GST) M1, T1 and O1, and Methylene Tetrahydrofolate Reductase 677 were determined by PCR of leukocyte DNA. MCR was determined by RT-PCR of aspirated bone marrow.

Results

MCR was achieved in 19 of 29 evaluable subjects after Induction with AIA, and in all 29 after Consolidation with AA. Blood arsenic levels did not differ between subjects who achieved, or failed to achieve MCR after Induction. 12 of 14 subjects (86%) with the null GST-M1 polymorphism achieved MCR after Induction, in contrast to 7 of 15 (47%) with the positive GST-M1 polymorphism ($p=0.027$, chi square test). No relationship was detected with status of other enzyme polymorphisms. Adverse reactions during Induction were common and unrelated to the final pre-dose blood arsenic level that indicated total arsenic retentive capacity. Most Induction adverse reactions appeared to be multifactorial and were not replicated during ATO re-exposure in Consolidation. Mild-moderate neutropenia (43%) and LFT abnormalities (14%) in consolidation were not related to blood arsenic levels or GST-M1 polymorphism status.

Conclusion

Presence of the null polymorphism of GST-M1 that converts inorganic arsenic to methylated catabolites more slowly than the positive polymorphism is associated with more rapid achievement of CMR in APML treated with AIA. More rapid responsiveness is consistent with consequences of the resulting greater proportion of arsenic in its therapeutically active inorganic form, and also indicates ATO materially contributes to efficacy of AIA Induction therapy.

This research was supported by a grant from Roche Australia. The company had no role in analysing the data or preparing the abstract.



O013

0900

Predictors of Early Mortality in Adult AML After ICE Induction Chemotherapy: A Sub-analysis of the Australasian Leukaemia and Lymphoma Group (ALLG) AML M7 Study

Annerie van der Jagt¹, Ken Bradstock², John F. Seymour³ and **Andrew Wei¹**

¹ Department of Clinical Haematology, The Alfred Hospital, Melbourne, Victoria, Australia.

² Department of Haematology, Westmead Hospital, Westmead, NSW, Australia. ³ Department of Haematology and Medical Oncology, Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia

Introduction The ALLG AML M7 study conducted between November 1995 and May 2000 treated 293 patients with *de novo* AML between the ages of 15 and 60 years. Induction was with ICE, consisting of cytarabine (3 g/m² 12-hourly on days 1, 3, 5, and 7); idarubicin (12 mg/m² by on days 1-3; modified to 9 mg/m² after patient 44) and etoposide (75 mg/m² days 1-7 inclusive). Fungal prophylaxis was with fluconazole. Although the reported early death rate was 10% in this study, the potential for sub-groups with a higher risk of ICE induction-related mortality has not been examined.

Methods 293 AML patients were analysed for the risk of early death (< 42 days) according to the following variables: age, WCC, cytogenetic risk category, ECOG performance status, body surface area, haemoglobin, platelets, peripheral blast count, bone marrow blast count, serum creatinine, bilirubin and LDH. Logistic regression was performed using SPSS. **Results** Overall, 35 patients (11.9%) died within 6 weeks of commencing induction and prior to receiving consolidation chemotherapy. The early death rate was 9% in the 44 patients receiving 12mg/m² idarubicin prior to the study dose modification. The initial 114 patients were randomised to receive or not receive G-CSF. Early death rates were 6/53 (11%) in those receiving ICE+G-CSF compared to 5/61(8%) in those not receiving G-CSF. 4/35 patients had documented persistent AML prior to death. Deaths were associated with septicaemia or pneumonia (77%), multiorgan failure (26%), fungal infection (23%) and intracerebral haemorrhage (9%).

The induction death rate for patients according to age strata was 0% (<20 yo; n=23), 2.1% (20-30 yo; n=48), 5% (31-40 yo; n=60), 14.7% (41-50 yo; n=75) and **23.3% (51-60 yo; n=86)**. The induction death rate for patients according to WCC strata was 13% (WCC<10; n=138), 4.8% (WCC 10-30; n=62), 7.1% (WCC 31-50; n=28), 11.1% (WCC 51-70; n=18), 11.1% (WCC 71-100; n=18) and **27.6% (WCC>100; n=29)**.

The strongest univariate predictors of early death were WCC>100 (p=0.017), Age 51-60 (p<0.000), ECOG 3 (p=0.08), poor risk karyotype (p=0.04), and creatinine >upper reference range (p=0.03). In multivariate analysis, age 51-60 (p<0.000; odds ratio 4.26) and WCC>100 (p=0.008; odds ratio 3.75) emerged as significant independent variables for early death. Creatinine >upper reference range (p=0.05; odds ratio 3.5) and poor risk karyotype (p=0.07; odds ratio 3.01) were of borderline significance. Of patients aged 51-60, the early death rate was 22% for those with ECOG 0-1 and 31% for those with ECOG 2-3 performance.

Conclusion This study has identified a number of baseline variables significantly associated with early ICE induction related mortality. The risk /benefit of ICE induction in patients over the age of 50 warrants further investigation. Analysis of the ALLG AML12 study population will be relevant to confirm these findings and quantify the potential benefit of enhanced spectrum anti-fungal prophylaxis.

No conflict of interest to declare

A52

O014 Elderly AML Patients (>60 years) with Poor Cytogenetic Features Treated with Investigational Agents have Superior Outcomes Compared to Those Receiving Intensive Chemotherapy

0915

Yee-May Victoria Ling, Jenny Muirhead, Sharon Avery, Sushrut Patil, Andrew Spencer and Andrew Wei.

Department of Clinical Haematology, The Alfred Hospital, Melbourne 3004, Australia.

Introduction

Outcomes for older patients diagnosed with acute myeloid leukaemia (AML) remain unsatisfactory, even with intensive chemotherapy (IC). Although these patients are candidates for investigational treatments, it is unclear whether specific cytogenetic (CG) sub-groups are more likely to benefit. This study compared survival of older AML patients treated with investigational agents with those who received IC or best supportive care (BSC) within cytogenetic categories.

Methods

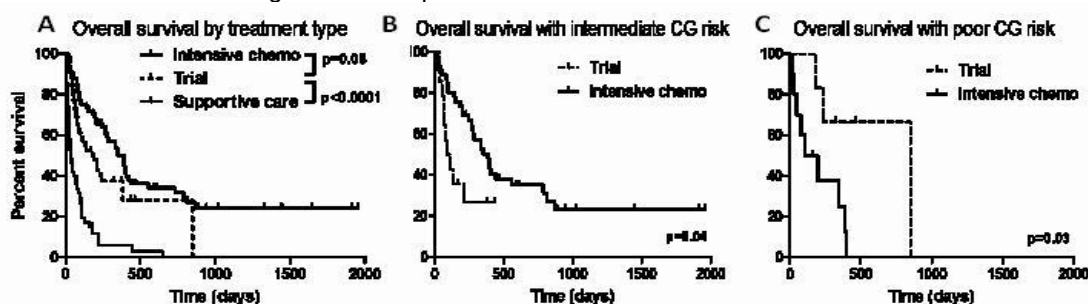
158 patients over the age of 60 diagnosed with AML from February 2001 to April 2009 at a single tertiary institution received IC [Cytarabine 100 mg/m² for 7 days + Idarubicin 12 mg/m² for 3 days], trial therapy (TT) [mTOR inhibitors, histone deacetylase inhibitors or aptamer-based therapies] or BSC. CG risk was based on the MRC classification (Grimwade 1998) and Kaplan-Meier analyses were plotted using Graph-Pad Prism.

Results

65 patients received IC (av. age 66), 26 received TT (av. age 72) and 67 patients received BSC (av. age 76). Overall survival (OS) with TT was superior to BSC (Fig A) and not significantly worse than with IC. Accounting for cytogenetic risk, OS in the intermediate CG risk group was greater with IC (median 333 days) than with TT (median 100 days) (Fig B). Conversely, OS in patients with poor-risk cytogenetics was higher with TT (median 322 days) than with IC (median 131 days) (Fig C).

Conclusions

In older AML patients, treatment with investigational therapies was superior to BSC and in those with poor-risk CG, superior to IC. Thus, elderly AML patients with poor-risk cytogenetics should be considered for investigational therapies.



No conflict of interest to disclose



O015

0930

Clinical, Immunological and Haematopoietic Responses in a Phase-II Study of Lenalidomide (len) and Stem cell Factor (SCF) in Low IPSS Myelodysplastic Syndrome (MDS)

David S Ritchie^{1,2,3}, Paul Neeson², Kellie Tainton², Karen Chen², Mandy Shin², Angela Chan³, Stuart Berzins³, Melita Kenealy¹, Hang Quach^{1,2}, H Miles Prince^{1,2,3}.

¹.Department of Haematology and Medical Oncology, Peter MacCallum Cancer Centre.

².Haematology Immunology Translational Research Laboratory, Research Division, Peter MacCallum Cancer Centre, ³.University of Melbourne, Melbourne, VIC.

Introduction

Pathogenesis of MDS includes haematopoietic stem cell (HSC) dysfunction and immune suppression of haematopoiesis. Lenalidomide has efficacy in MDS with or without del(5q). Lenalidomide effects include enhanced haemoglobin production, modulation of NK cell and DC function, Treg inhibition and T cell proliferation/IFN- γ production. We describe the safety and clinical, immunological and hematopoietic effects of len in combination with rhSCF in 13 patients (pt).

Patients and Methods

The trial was approved by University of Melbourne (#050409) and Peter MacCallum Cancer Centre Ethics Committees. ClinicalTrials.gov registration: NCT00434239.

Eligibility

Symptomatic anemia (Hb<100g/L), ECOG \leq 2, ANC \geq 0.5 \times 10⁹/L, platelets \geq 25 \times 10⁹/L. Pt received len 10mg/d, d 1-21 of each cycle (C). Pt not in CR by C3, received rhSCF 10-20 μ g/kg sc for the first seven days of C3. Bone marrow (BM) was assessed for blast forming unit (BFU-E) morphology and peripheral blood (PB) was analysed for T, NK, NKT and B cell numbers at baseline, 8w, 4m and 10m.

Results

n=13, F=6, median age=61 (45-89), IPSS<0.5=3, 0.5-1=10, del(5q)=6. Twelve pts completed \sim 1 cycle. One withdrew prior to C1 and one at C10 due to intolerance. Of 12 evaluable pt, 6 achieved CR and 5 transfusion independence (TI). Median time to best response = 4m. Five have sustained responses, 3 CR and 2 TI, at 11, 26, 27m and 11, 17m respectively. Baseline CD3+, Treg and NKT cell number were normal and did not alter following len. Durable responses were associated with higher baseline BFU-E and PB B cells and normalization of BFU-E morphology in response to len. BM dysplasia and abnormal BFU-E persisted however even in CR, suggesting improved productivity from abnormal HSC rather than re-establishment of normal haematopoiesis.

Conclusions

Len and rhSCF combination is safe and effective MDS, without altering PB immunology or removing underlying dysplastic haematopoiesis.

No conflict of interest to declare

A54



O016

0945

WT1 Expression Levels in Bone Marrow and Peripheral Blood of Patients with Acute Myeloid Leukaemia (AML)

James X Gray, Lyle McMillen, Russell Saal, Steven Lane, Peter Mollee, Robert Bird, Devinder Gill and Paula Marlton
Princess Alexandra Hospital, Brisbane, Queensland.

Background

WT1 is over expressed in the majority of AML patients and has been used as a marker of minimal residual disease (eg. Cilloni *et al.*, 2008).

Aim

To analyse WT1 expression in patients with AML when measured at diagnosis and post-chemotherapy; and to correlate with patient characteristics and outcome.

Method

This is a prospective, longitudinal study in which the levels of WT1 expression from patients with AML are measured from bone marrow and peripheral blood specimens at the time of diagnosis and at various time-points along their treatment and clinical courses. RNA was extracted from 10^7 leukocytes purified from blood and bone marrow using TRIzol® (Invitrogen) extraction. Multiplex RQ-PCR was performed using standard techniques (Superscript®, Invitrogen). Copy number of WT1 was normalised to 10^4 copies of ABL.

Results

67 AML patients were studied: median age 51years, 52% male. 18%, 72% and 10% had good, intermediate and poor risk cytogenetics. WT1 expression levels were determined from bone marrow (bm-WT1) and peripheral (pb-WT1) blood in all patients at the time of diagnosis and again following induction chemotherapy in 55 patients (82%). Median bm-WT1 levels at diagnosis were 3889 and varied significantly according to: cytogenetic risk group (good risk 13536, intermediate risk 2631, poor risk 1685 ($P_{bm} = 0.06$, $P_{pb} = 0.01$); blast percentage ($P_{bm} = 0.01$, $P_{pb} = 0.01$) and age ($P_{pb} = 0.05$). Diagnostic pb-WT1 showed similar correlations with baseline parameters. The WT1 expression levels were reduced following induction chemotherapy with a mean of 2.4 log reduction (range, 0 to 4.2) seen in marrow aspirate and 2.8 log reduction (range, 0 to 4.3) seen in peripheral blood. Data collection and analysis is ongoing with diagnostic and post-induction WT1 levels to be correlated with leukaemia-free and overall survival.

Conclusion

This study demonstrates a correlation between WT1 expression and known risk factors for early relapse and analysis is ongoing to validate its utility as an independent risk factor.

Reference

Cilloni *et al.*, 2008. Early prediction of treatment outcome in acute myeloid leukemia by measurement of WT1 transcript levels in peripheral blood samples collected after chemotherapy. *Haematologica*, **93**(6):921-924.

No conflict of interest to disclose



0017

0830

High Dose Dexamethasone Counteracts the Immunostimulatory Effects of Lenalidomide

Hang Quach¹, Andy Hsu¹, David Ritchie¹, Paul Neeson¹, Kevin Lynch², Simon Harrison¹, H Miles Prince.¹

¹Peter MacCallum Cancer Centre, East Melbourne, VIC, Australia. ⁴ Celgene Pty Ltd, Melbourne, Australia

Background

Dexamethasone (dex) and lenalidomide (len) is a potent treatment for multiple myeloma (MM). Len-induced immune-stimulation enhances anti-tumour responses. Conversely, dex-induced immunosuppression may subvert the full capacity of len to act via immune mechanisms against MM. We undertake a prospective and systematic analysis of NK-cell number and function in len-dex treated MM patients, and evaluated the mechanisms by which dex downregulates len-induced NK activation in *in-vitro* assays using patients' and normal donors' blood samples

Method:

NK-cell numbers and function were assessed in 25 relapsed MM patients receiving low-dose len (15mg d1-21q28) and dex (20mg/d, d1-4, 9-12, 17-20) at serial time points. NK function (assessed by % lysis of ⁵¹Cr labelled K562 target cells at 50 (effector):1 (target) ratio) on normal donors and paired patient PBMCs (baseline and cycle 6 or 9) were assessed after 72h *in-vitro* treatment with len, dex, len+dex, and dex+IL2 combination. The extent of NK-cell function recovery after dex washout was assessed after 3, 6 and 9 days.

Results: After a median of 9 (2-19) len-dex cycles (C), 22 of 25 patients responded (16% CR/VGPR, 64% PR). Despite a clinical response, NK function progressively decreased compared to baseline at C6 (n=15, p=0.005) and C9 (n=11, p=0.03). *In-vitro*, len increased NK function in PBMC from healthy donors (mean 54% K562 cell lysis from len-treated PBMC vs. 38% lysis in untreated PBMC, p=0.04) and MM patients at baseline. Dex decreased NK function [mean 7.6% K562 cell lysis (dex treated) vs. 38% (untreated) p=0.01], even in the presence of len [mean 7% K562 cell lysis (len+dex) vs. 38% (untreated), p=0.002]. This could be rescued by IL-2 in normal PBMCs and some myeloma patients at baseline, but not after prolonged len-dex treatment. Dex-induced NK inhibition was reversible as NK-function recovered 3 days after dex washout.

Conclusion:

The decrease in NK function after prolonged len-dex treatment is due to the antagonistic effects of dex on len-induced NK activation. We suggest that alternative dosing schedules of dex, after initial induction with len-dex co-therapy may optimise len-induced immunostimulation of NK cells and subsequent sustained disease control via anti-MM immunity.

Conflict of interest: "This research was partially supported by Celgene corporation. The company had no role in analysing the data or preparing the abstract."

A56



O018

0845

Heat Shock Protein 90 Inhibitor Induces Apoptosis in Human Multiple Myeloma Cell Lines and Synergises with Azacytidine

Tiffany Khong and **Andrew Spencer**

Myeloma Research Group, Alfred Hospital, Melbourne, Victoria, Australia

Aim

Heat shock protein 90 inhibitors (HSP90i) have been shown to inhibit cell growth and induce apoptosis in some cancer cells. We studied the impact of a novel orally bioavailable HSP90i on a panel of human myeloma cell lines (HMCLs) and primary myeloma samples.

Method

Dose responsiveness of HMCLs to HSP90i was determined via MTS assay at a range of HSP90i doses (0.1nM-10 M) for 72 hours. FACS and cell cycle analysis were used to evaluate the profile of the cells post HSP90i treatment. Western Blot analysis using PARP, IL6-R, AKT, Mcl-1, p-MEK, MEK, p-Stat3 and Stat3 antibodies were performed to evaluate the mode of action of HSP90i. Addition of exogenous IL-6, a cytokine essential for myeloma survival, was analysed to determine if IL6 abrogated HSP90i-induced apoptosis. Primary samples from relapsed/refractory myeloma patients were also studied utilising Apo 2.7-PE staining of CD45^{neg}CD38^{pos} cells. Combination studies with azacytidine (AZA), known to inhibit JAK/Stat and NF B signalling pathways, was investigated in HMCLs to ascertain synergy and schedule dependency with HSP90i.

Results

HSP90i demonstrated IC₅₀ of 50-500 nM against all HMCLs at 72 hours. This was associated with accumulation of cells in the G₀/G₁ phase indicating cell cycle arrest. Evidence of apoptosis was supported by PARP cleavage which occurred within 24 hours. HSP90 client proteins (eg. AKT, MEK and Stat3) decreased rapidly following HSP90i treatment. Exogenous IL-6 failed to rescue HMCLs from HSP90i-induced apoptosis or HSP90i-induced inhibition of Stat 3 phosphorylation. In the HMCL NCI-H929, combining HSP90i with AZA demonstrated synergy at all schedules tested. Maximal synergy was observed with HSP90i + AZA contemporaneously [combination index (CI) = 0.409] followed by HSP90i treatment for 24 hours prior to AZA for 24 hours (CI= 0.478) and subsequently the reverse schedule, AZA then HSP90i (CI = 0.655).

Conclusion

Inhibition of HSP90 shows potential for future myeloma therapy.

This research was supported by Novartis. The company had no role in analysing the data or preparing the abstract.



O019

0900

Bortezomib May Overcome Adverse Risk Associated with Increased Tumor Burden, but not Metaphase-Defined High Risk Cytogenetics in Multiple Myeloma

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Introduction

Multiple myeloma (MM) is clinically heterogeneous and risk stratification is vital for prognostication and informing treatment decisions. As bortezomib is able to overcome several high-risk features of MM, the validity of conventional prognostication systems needs to be reevaluated. We sought to evaluate survival data in MM, identify important prognostic factors and study the impact of bortezomib on the overall survival (OS).

Methods

We study the survival data of 261 previously untreated MM patients managed at our institution, where bortezomib became available for relapsed disease from 2004. Patient and disease characteristics, and survival data were evaluated overall, and with respect to bortezomib exposure.

Results

The median age of all patients was 62 years. 20%, 45% and 34% of patients presented with ISS stages I, II and III respectively. Conventional karyotyping on 204 patients detected abnormalities in 49% (del 13 [17%], hypodiploidy [18%], hyperdiploidy [22%], pseudodiploidy [7%] and near tetradiploidy [2%]). Overall, the international staging system (ISS) and metaphase cytogenetics were discerning of survival where the median for the entire cohort was 5.2 years. However, when stratified by bortezomib exposure, only metaphase cytogenetics was still discriminating of the long-term prognosis. The presence of an abnormal non-hyperdiploid karyotype overrides all other clinical and laboratory parameters in predicting a worse outcome on multivariate analysis (median survival 2.7 years, Hazard Ratio 2.4, $p=0.007$). The presence of a normal diploid karyotype correlated with longer survival.

Conclusion

Our study suggests that bortezomib may overcome adverse risk associated with increased tumor burden (as measured by the ISS), but not adverse genetics on conventional karyotyping. The absence of any abnormality on metaphase cytogenetics may act as a surrogate for low plasma cell proliferation, thereby conferring a better prognosis.

No conflict of interest to disclose



O020

0915

Elevated Serum Levels of CXCL12 is Associated with Increased Osteoclast Activity and Osteolytic Bone Disease in Multiple Myeloma Patients

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Aim

The Plasma cell (PC) malignancy, multiple myeloma (MM), is unique among haematological malignancies in its capacity to cause osteoclast (OC)-mediated skeletal destruction. We have previously shown that elevated plasma levels of PC-derived CXCL12 are associated with presence of X-ray detectable osteolytic lesions in MM patients. The aim of this study was to investigate the role played by CXCL12 in the recruitment and activation of OC in MM.

Methods

The OC-activating potential of MM PC derived CXCL12 was examined in vivo using a mouse model of MM-mediated focal osteolysis. In this model, MM PC lines, such as RPMI-8226, are injected in the tibiae of Balb/c nude mice. The osteoclast activating potential of MM PC-derived CXCL12 was assessed by micro-CT, histological analyses and serum markers of bone loss.

Results

Implanting RPMI-8226 gave rise to osteolytic lesions proximal to the tumour, resulting in a 5% decrease in bone volume (BV) compared with vehicle control. Importantly, bone loss was significantly inhibited with systemic administration of the CXCL12/CXCR4 antagonist T140. Furthermore, implanting CXCL12-overexpressing RPMI-8226 cells resulted in a 13% decrease in BV and was associated with increased OC recruitment proximal to the tumour, increased serum matrix metalloproteinase activity and increased levels of collagen I degradation products.

Conclusion

These findings confirm our hypothesis that MM PC-derived CXCL12 stimulated the recruitment and activity of OC, thereby contributing to the formation of MM osteolytic lesions. Therefore, inhibition of CXCL12-CXCR4 may be an effective modality to inhibit osteolysis in MM patients.

No conflict of interest to disclose



0021

0930

Severity of Osteoporosis in Multiple Myeloma and the Prognostic Role of Lumbar Spine QCT

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Aim

To determine the frequency and severity of osteoporosis in patients with multiple myeloma and its relationship with disease activity.

Design

Prospective single centre study.

Methods

108 consecutive patients with biopsy-proven multiple myeloma were evaluated over a 10-year period. The data collected comprised of clinical demographics (age, sex, smoking history, alcohol intake and medications), serum biochemistry (calcium, 25-hydroxyvitamin D, parathyroid hormone), paraprotein estimations, markers of bone resorption (urinary deoxypyridinoline), spinal radiography and bone densitometry (DXA: dual-energy X-ray absorptiometry and QCT: quantitative computed tomography). Individual patient BMD T-scores were derived from normative database comprising of 250 % healthy+men and women age 20-40 years. The patient data was expressed as mean \pm 1 SD. Relationship between BMD and variables of myeloma activity was assessed using regression analysis.

Results

There were 56 men and 52 women with a mean age of 69 years. 35% (n=36) presented with stage III disease. Patients were treated with various chemotherapeutic regimens (melphalan, prednisone, VAD, interferon and in latter years with thalidomide). 13% (n=14) underwent stem cell or bone marrow transplantation. 80% (n=86) died during the course of the study period, with a mean survival of 48 months. 43% (n=46) were vitamin D deficient (25OHD < 50nmol/L). 24% (n=26) had evidence of high bone turnover (uDPYD > 6.5 nmol/mmol creatinine). 56% (n=61) had X-ray or CT evidence of lytic lesions. 54% (n=58) had skeletal fractures, with 42% (n=45) of these involving the thoracolumbar spine. The mean lumbar spine QCT T-score = . 3.3, lumbar spine DXA T-score = . 2.1 and femoral neck DXA T-score = -1.4. 57% (n=62) had osteoporosis defined by lumbar spine QCT (T-score \leq -2.5). There was no correlation between the lumbar spine QCT and either the serum calcium, paraprotein or Hb. In the multivariate analysis, the lumbar spine QCT was a major independent variable contributing to myeloma-related deaths (chi square=12.63, P=0.02).

Conclusions

Although osteoporosis is frequent in myeloma, the value of lumbar spine QCT in this condition is not fully appreciated. This study suggests that lumbar spine QCT T-score values are markedly reduced in patients with myeloma and may contribute independently to both the morbidity and mortality of the disease.

No conflict of interest to disclose

A60



O022

0945

TWEAK Contributes to Osteolytic Disease in Multiple Myeloma Through the Induction of CXCL12

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2. *Department of Orthopaedics and Trauma, Royal Adelaide Hospital, Adelaide, AUSTRALIA*

Aim

We compared the gene expression profiles (GEP) of purified plasma cells (PC) from patients with multiple myeloma (MM) and the non-malignant precursor condition, monoclonal gammopathy of uncertain significance (MGUS) in order to identify genes with roles in myeloma bone disease (MBD). One candidate gene identified in this screen was the TNF-like weak inducer of apoptosis, or TWEAK. The aim of this study was to determine the role played by TWEAK in the pathogenesis of MBD.

Methods

ELISA was used to measure serum levels of TWEAK and CrossLaps in MM and MGUS patients. The expression of TWEAK and its receptor, Fn14, in primary patient cells and myeloma cell lines was examined using real time PCR and flow cytometry. The effects of TWEAK on stromal cell expression of osteoclast activating factors (RANKL, CXCL12) were investigated using real time PCR following culture of human mesenchymal stromal cells (MSC) in the presence of recombinant TWEAK.

Results

MM cell lines and primary patient CD138⁺ MM plasma cells were found to express TWEAK mRNA and protein, but not its receptor, Fn14. In keeping with previous GEP analysis, serum levels of TWEAK were found to be elevated in patients with MM compared to MGUS patients. Of note, there was a significant correlation between serum TWEAK levels and levels of serum CrossLaps (a measure of bone breakdown), suggesting that TWEAK may play a role in MBD. While unable to stimulate osteoclast formation directly, TWEAK dose-dependently stimulated MSC to produce CXCL12, a factor that we and others have shown previously to be a major contributor to MM-induced osteoclast recruitment and bone loss.

Conclusion

MM PC produce TWEAK which induces CXCL12 expression in MSC, thereby contributing to MBD. TWEAK is thus a potential therapeutic target for prevention of the bone pain and pathologic fractures in MM.

No conflict of interest to disclose



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Day 19

HAA
2009
HSANZ ANZSBT ASTH

0830-1000
Meeting Rooms 10/11

0023

0830

The Impact of Regular Erythrocytapheresis on Acute and Chronic Complications of Sickle Cell Disease in Adults

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Aim

A retrospective study of the impact of a regular erythrocytapheresis (ECP) program on the acute and chronic complications of sickle cell disease (SCD) in adult patients at a single institution.

Methods

Thirteen patients aged 22 to 63 years (median 30 years) (3F: 10M) with homozygous sickle-cell anaemia (HbSS) (n = 8) or sickle β thalassemia (HbS/ β) (n = 5) were enrolled between December 1998 and November 2008. Patients were exchanged at a median frequency of 5 weeks (range 3-6) with mean 5.5 red cell units/exchange (4.1-6.3). Endpoints evaluated: the incidence of SCD related acute events (requiring hospitalization) following and prior to initiation of regular ECP, the progression of pre-existing related end-organ damage and development of new end-organ damage (with MRI brain, TTE, assessment of liver and endocrine function), the effectiveness in reducing HbS levels acutely and prior to the next exchange, the development of significant alloantibodies and iron overload. Data were retrieved from the patient medical records, computerized blood bank records and the ARCBS.

Results

The indications for commencement were recurrent painful crises (PC) (n=7), acute chest syndrome (ACS) (n=3), silent cortical ischaemia (n=1), pulmonary hypertension (n=1), multi-organ crises (n=1) and pregnancy (n=1). Two patients had failed hydroxyurea therapy. 8 patients did not have any acute events following commencement of ECP. A total of 16 acute events occurred in five patients in 846 months of patient follow-up. Events were PC (n = 13) and ACS (n = 3); 10 events occurred in 2 patients and 6 events in the remaining 4 patients. The annual rates of PC/ACS pre-ECP compared to post-ECP in the 6 patients with comprehensive data available pre-ECP were 4/0.48, 3.2/0.85, 0.4/0, 1/0.14, 2/0 and 1.6/0. None of the 3 patients commenced on ECP due to ACS experienced any acute events. No patient experienced stroke or multi-organ crises whilst on the ECP program. No patient demonstrated evidence of new end-organ dysfunction or progression of end-organ dysfunction (pulmonary hypertension and silent cortical ischaemia), including those who experienced acute events. Regular ECP reduced HbS levels to the target level of less than 30% immediately post-exchange and, in most patients to less than 50% immediately prior to the next exchange. Alloimmunisation rates were comparable to the literature (clinically significant Ab incidence 23%), and ECP was confirmed to be effective preventing progressive iron overload, particularly when initiated early.

Conclusion

Regular ECP was demonstrated to be an effective, well-tolerated therapy for both acute and chronic complications of SCD in adults.

No conflict of interest to disclose

A62



O024

0845

Cytomegalovirus Scoring Index (CSI): A Clinical Score for Predicting the Likelihood of Early Cytomegalovirus Reactivation Following Allogeneic Stem Cell Transplantation

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Aim

This is a retrospective study aimed at establishing a clinical score to help stratify patients at risk of early CMV reactivation following allogeneic stem cell transplantation (HSCT).

Methods

335 patients undergoing HSCT were divided into a training set (n = 235) and validation set (n = 100). Logistic regression analysis on the training set identified recipient and donor CMV sero-positivity, presence of acute graft versus host disease (GVHD) requiring corticosteroid therapy and use of antithymocyte globulin (ATG) or alemtuzumab as significant risk factors for CMV reactivation and weighted scores were assigned for each risk factor. A weighted pre-transplant score (CMV scoring index or CSI) was calculated for each patient using the scores of the risk factors (excluding GVHD). The CSI was collapsed into 3 groups . low risk (score of 0-3), intermediate risk (score of 4-5) and high risk (score of 6 -7) and CMV reactivation rates calculated.

Results

In the training set, CMV reactivation rates were 5.8% in low risk, 44.8% in intermediate risk and 67.7% in high risk groups. In the intermediate risk group, presence of GVHD was associated with higher reactivation rates (57.8%) compared to its absence (24.5%) (p = 0.002). No impact was seen in the low or high risk groups. In the validation set, reactivation rates were 0% in low risk, 46% in intermediate risk and 68.4% in high risk groups. Again, the presence of GVHD was associated with higher reactivation rates (64%) in the intermediate risk group only [28% in absence; p = 0.022)].

Conclusion

The CSI is useful in identifying 3 groups of transplant recipients with widely varying risks of early CMV reactivation. Specific approaches to reducing CMV reactivation needs to be designed and tested within each risk group to maximise the benefits of prophylactic and pre-emptive strategies in reducing CMV following HSCT.

No conflict of interest to disclose



O025

0900

Adoptive Immunotherapy for Prophylaxis of Cytomegalovirus Infection in Allogeneic Haemopoietic Stem Cell Transplant (HSCT) Recipients – An Update

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Introduction

Cytomegalovirus (CMV) reactivation post haemopoietic HSCT continues to present a management problem due to increasingly immunosuppressive transplant regimens. Cellular therapy with donor-derived CMV specific cytotoxic T cells (CTL) is effective for controlling established CMV infection and probably also for prophylaxis of infection after HSCT. We present an update of our clinical experience using CMV CTL for prophylaxis after HSCT.

Aim

To assess the safety and efficacy of prophylactic infusion of CMV specific, donor-derived CMV CTL to prevent CMV infection in HSCT recipients.

Methods

Donor-derived CMV CTL were isolated *ex-vivo* via stimulation with mature monocyte-derived dendritic cells (DC) expressing CMV pp65 antigens. After 2 antigen stimulations on days 1 and 7, donor T cells were expanded with exposure to increasing concentrations of IL-2 until day 21 of culture.

Recipients of HSCT were infused with CMV CTL at a target dose of 2×10^7 cell/m² on or after day 28 and monitored for a period of 12 months. CMV PCR was performed weekly to day 100 post transplant and monthly thereafter. Immune reconstitution was assessed by tetramer staining and IFN- γ ELISPOT.

Results

A total of 28 patients have been infused with CMV CTL since 2005 (9 under protocol 1, 17 under protocol 2). Post-infusion follow-up is complete in 22/26. There were no infusion related adverse events. Eight of 28 patients experienced CMV reactivation as determined by at least one positive PCR during the follow-up period. Three patients experienced 2 or more positive PCRs. No patient was treated with CMV specific therapy (ganciclovir or foscarnet) and in all cases CMV PCR tests became negative during follow up. There were no cases of CMV reactivation related organ damage. Graft vs host disease developed in 7/26 patients (26%), while 4/26 relapsed (15%). During the follow-up period, 5/26 patients died due to relapse (2 patients), graft failure and GVHD after second transplant (1 patient), TTP (1 patients) and GVHD (1 patient). CMV specific immunity was detectable post-infusion in all of 24 patients for whom immunological follow-up data is available.

Discussion

Adoptive immunotherapy with CMV-specific CTL is a safe therapy for prophylaxis of CMV infection after HSCT. Our ongoing data suggest efficacy in controlling reactivations of CMV occurring after transplant and preventing tissue infection without the need for specific anti-viral pharmacotherapy. Randomised studies currently underway in Europe will clarify the role of CMV-specific adoptive immunotherapy in this clinical setting.

There are no conflicts of interest to disclose

A64



0026

0915

Catheter-Related Bloodstream Infection Incidence and Risk Factors in Adults With Cancer: A Prospective Cohort Study

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Aim

Central venous catheter-related blood stream infections (CR-BSI) cause considerable morbidity in adult patients with cancer. A range of central venous access devices (CVAD) may be used across differing patient diagnoses and for varying indications. The aim of our study was to determine the incidence and risk factors for CR-BSI.

Methods

A prospective observational cohort study of consecutive adult patients requiring a CVAD in a haematology-oncology unit was performed. All CVADs were inserted under radiological guidance in a dedicated facility. Standardised surveillance methodology for CR-BSI was applied and data on CVAD complications including symptomatic venous thrombosis was recorded.

Results

1119 CVADs were assessed in 723 patients over 50,478 line days. The rate of CR-BSI per 1,000 line days was 2.63. Factors associated with CR-BSI included: type of CVAD (greatest for non-tunnelled lines (HR 3.67, $p < 0.0001$) and tunnelled lines (HR 1.83, $p = 0.0062$) compared to PICC lines); patient diagnosis (greatest for aggressive haematological malignancies (HR 2.95, $p = 0.0031$) and least for oesophageal, colon and rectal cancers (HR 0.26, $p = 0.014$) compared to other solid tumours); side of insertion (greatest for right sided lines (HR 1.61, $p = 0.024$)); and number of prior line insertions (HR 1.2, $p = 0.016$). Symptomatic line related thrombosis was less common in patients with oesophageal, colon and rectal cancer (HR 0.43, $p = 0.027$) and in lines inserted in the internal jugular vein (HR 0.2, $p = 0.0083$) compared to PICC lines.

Conclusions

CR-BSI in an Australian adult cancer population are comparable to other reports in the literature. This study highlights the utility of a standardised CR-BSI surveillance strategy in adult patients with cancer, provides further data to support the use of PICC lines in such patient populations and, suggests that the side of line insertion may influence CR-BSI.

No conflict of interest to disclose



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Day 19



0830-1000
Meeting Rooms 10/11

O027

0930

Haemophagocytic Syndrome Associated With Tuberculosis – Early Recognition, Early Management

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² *Australian Centre for Blood Diseases, Prahran, VIC, Australia*

Aim

Haemophagocytic syndrome (HPS), a non-neoplastic disorder resulting from dysregulation of histiocyte activation, is often heralded by progressive cytopenia, hyperferritinaemia, and hypercytokinaemia potentially culminating in fatal multi-organ failure. Secondary cases may occur in the setting of infection or malignancy. We report a successfully treated case of HPS secondary to tuberculosis and review similar cases in the literature.

Methods

A PubMed search using keywords haemophagocytic syndrome, haemophagocytic lymphohistiocytosis, macrophage activation syndrome and tuberculosis was undertaken. Note was made of initial presentation, time to diagnosis, initial management and overall outcomes. A similar case at our institution was reviewed.

Results

Since 1980s, approximately 13 cases of tuberculosis-related haemophagocytosis have been reported. A universally dismal outcome is seen in cases in which antituberculous treatment was not initiated due to delays in diagnosis. Of those that received treatment, only 50% made a complete recovery.

Our index case is a 60yo Phillipino man who presented with fevers, cough, sweats, anorexia and progressive pancytopenia. A diagnosis of HPS was subsequently made based on bone marrow biopsy findings and a hyperferritinaemia of 11000ug/l. Dexamethasone and cyclosporine were initiated. After extensive search for secondary causes, a left apical lung mass was identified on CT. Although initial investigation with a bronchoscopy was non-contributory, excision of the lung mass confirmed acid-fast bacilli histologically and TB on PCR. Anti-tuberculous therapy was commenced. The patient made a full recovery and was discharged approximately one month later.

Conclusion

Although similarities exist, reactive (secondary) haemophagocytic syndrome is a distinct entity from the familial childhood form. Limited awareness of this type and underrecognition, in the absence of clinical guidelines is the reason for delayed diagnosis and poor outcome. Our case demonstrates the importance of searching for an underlying cause in adults who present with HPS, particularly TB, as many cases can be successfully treated by the timely initiation of treatment of the underlying infection.

No conflict of interest to disclose

A66



0028

0945

Development of a Protocol for Auditing Invasive Fungal Disease in High Risk Haematology and Stem Cell Transplantation Patients

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Aim

To develop a protocol for prospectively auditing invasive fungal disease (IFD) rates and antifungal usage in high risk patients. The audit will allow comparison to be made between institutions/units, monitor changes over time (eg provide an early alert to any increase in infections) and assess the clinical and economic impact of any changes in strategy aimed at reducing fungal disease.

Method

A literature review was performed as well as liaison with key stakeholders including Haematologists, Stem Cell Transplantation clinicians, Infectious Diseases clinicians, clinical governance committees, pharmacy and nursing staff.

Results

Inclusion criteria were designed to capture high risk patients that would represent a stable group over time. Criteria include: new allogeneic stem cell transplantation recipients and newly diagnosed patients with acute leukaemia receiving high dose chemotherapy.

The data collected is readily available and includes: pharmacy dispensing data of antifungals, pathology database review and clinical data from medical records, ward staff and clinical meetings.

Clear definitions for cases of IFD were developed. Proven, probable and possible definitions are used as described by EORTC/MSG¹ guidelines. This allows a %reportable IFD+rate (proven plus probable IFD) that can be compared across institutions. In addition, further clinically useful definitions and economic analysis methodology are defined. Audits are conducted prospectively and reports generated regularly to allow timely decision making.

IFD audits have been conducted at Alfred Health for two years. Using this protocol IFD rates were objectively quantified and as used to justify a number of changes to clinical practice and patient care. Subsequently the audit has observed a 50% reduction in the IFD rates and tracked the clinical and economic impact.

Conclusions

Following a protocol for invasive fungal disease audits can lead to a generation of robust and clinically relevant data. Thus this quality improvement activity can have real impact on improvements in patient care and outcomes.

1: De Pauw, B et al. Revised Definitions of Invasive Fungal Disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. *Clinical Infectious Diseases* 2008; 46:1813. 21

No conflict of interest to declare

A67



0029

0830

Utility of Flow Cytometry (FC) in the Assessment of Therapeutic Efficacy in Myelodysplastic Syndromes (MDS) Treated with 5-azacitidine (AZA)

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² *University of Melbourne, Parkville, Victoria, Australia*

³ *Celgene Pty Ltd, Melbourne, Australia*

Background & Aim

The availability of effective therapies for patients with MDS such as AZA means standardised criteria for diagnosis, classification, prognostication and evaluation of treatment response are increasingly important. This has been achieved in part by the 2008 WHO classification. FC is now included as a co-criterion for diagnosis. Our aim was to assess whether FC may represent a more objective tool for assessment of therapeutic efficacy.

Method

We prospectively and sequentially assessed the utility of FC during therapy, as part of two Phase II clinical trials of AZA. Four-colour FC analysis was performed on bone marrow samples using a defined antibody panel to assess blast and myelomonocytic populations. Antigen maturation patterns were evaluated by two independent assessors, comparing to healthy volunteers. A Wells FC score (WFCS) (Blood 2003) and Stachurski-FC score (SFCS) - positive, intermediate or negative were assigned (Leuk Res 2008).

Results

31 patients, 20 males, median age 65 (range: 43-89) were included. WHO categories comprised: RA (n=1), RCMD (7), RAEB-I/II (3), CMML-I/II (4), 5q- (3), MDS-U (1), FAB RAEB-t (2). Karyotyping showed, 21 good prognostic, 4 intermediate, 4 poor and 3 non-assessable. All were transfusion requiring at enrolment (30 red cell, 1 platelet), median haemoglobin 92g/L (range 61-118). Pretreatment median WFCS was 6 (range: 3-9); Stachurski-FSC 22 positive, 6 intermediate and 1 negative. 29 (94%) have been re-evaluated; median time to best clinical response (IWG-2008 criteria) was 89 days (range: 31-511), consistent with previous clinical observations (Fenaux et al, Lancet Oncol 2009). Eighteen (58%) achieved at least a PR. Of these, 15 (83%) FCS were concordant with an improvement in either WFSC +/- SFSC. 6/15 (35%) have subsequently progressed, with 83% showing concordant FSC deterioration.

Conclusion

FC offers a more subtle and dynamic objective assessment for sequential disease monitoring, and may aid MDS treatment response evaluation.

No conflict of interest to declare

A68



0030

0845

p53 Immunohistochemistry (IHC) and High-resolution Melting (HRM) Analysis Appears to Aid Classification, Define Adverse Biology and Improve Sensitivity of Residual Disease Detection in Acute Myeloid Leukaemias (AML) with Over 50% Marrow Erythroblasts

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Background

The differential diagnosis of AML with >50% marrow erythroblasts includes acute erythroid leukaemias (AEL), therapy-related AML (t-AML) or AML with multilineage dysplasia (AML-MD) with an erythroid-dominant phenotype (EDP). The World Health Organisation classification (WHO-2008) improves discrimination between these subtypes primarily through greater emphasis on cytogenetics. Detecting residual disease (RD) and remission evaluation in these subtypes remains challenging in daily practice and largely unaddressed.

Aims

To reclassify patients with AEL or AML-EDP according to WHO-2008 and assess biologic characteristics, response and outcomes utilising p53 over-expression by IHC and mutation detection by HRM.

Methods

All cases of AML with >50% erythroblasts at our institutions from 2003-2009 (n=24) were re-evaluated morphologically and reclassified with known clinical and cytogenetic data. p53 IHC and HRM were performed on pre- and post-treatment specimens.

Results

24 cases were evaluated. 8/10 cases previously labelled AEL were reclassified as AML-EDP (t-AML n=3; AML-MD n=5). Final classification included 2 AEL and 22 AML-EDP (t-AML n=6; AML-MD n=16). Median age 62 (22-79), 15 male. 15/22 (68%) AML-EDP were p53 positive (p53+) by IHC and/or HRM. p53+ AML-EDP was characterised by complex cytogenetics (92%, 11/12 evaluable karyotypes) and poor outcome (median overall survival (OS) 7.9 months, (range 1.9-14.9), 4/12 patients receiving induction chemotherapy had RD detected by conventional methods (morphology, immunophenotyping and cytogenetics). When p53 studies were incorporated into post-induction assessment, 10/11 evaluable patients were found to have RD (8 IHC+, 3 HRM+).

Conclusions

The majority of cases previously diagnosed as AEL were reclassified as AML-EDP by WHO-2008. p53 overexpression or mutation is common in AML-EDP and defines a subset of AML with complex cytogenetics, induction resistance, and poor prognosis. p53 overexpression by IHC and mutation by HRM appear to improve the sensitivity of RD detection when added to conventional methods.

No conflict of interest to disclose



O031

0900

Incidental Monoclonal Lymphocyte Populations in the Bone Marrow Assessed with Eight-Colour Diagnostic Flow Cytometry

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Aim

Monoclonal B-lymphocytosis (MBL) in the peripheral blood is a relatively new diagnostic category that has been increasingly detected with wider use of flow cytometry. A similar entity can also be recognised in the bone marrow and this study aims to characterise the incidence and phenotype of these populations using sensitive flow cytometry in a tertiary hospital diagnostic laboratory.

Method

The study is a 12-month retrospective review (July 2008 to July 2009) of all initial diagnostic bone marrow aspirates evaluated by eight-colour flow cytometry with a BD FACSCanto II. An incidental monoclonal lymphocyte population was defined when an identified phenotype comprised at least 2% of marrow lymphocytes. Patients were excluded from analysis if there was a previously diagnosed or suspected lymphoproliferative disorder, peripheral blood monoclonal lymphocytosis, or evidence of an IgM paraprotein. Demographic, indication for performing marrow investigation, aspirate, trephine, cytogenetics and flow cytometry data were collected.

Result

248 bone marrow aspirates were identified as the patients' initial diagnostic marrow. In 12 patients (5%), an incidental monoclonal lymphocyte population was detected. Median age was 77 years (63-85). The indications for investigation were for evaluation of paraprotein (other than IgM) (3), thrombocytosis (2), anaemia (2), cryoglobulinemia (1), vertebral lesion (1), PUO (1), neutrophilia (1) and neutropenia (1). A monoclonal B-cell population was identified in 9/12 patients and comprised of 2-15% of all lymphocytes. The phenotypes were either CLL (4) or B-NHL (5). A monoclonal T-cell population was identified in 3/12 patients and comprised of 20-85% of all lymphocytes. The phenotypes were either T-NHL (2) or T-LGL (1).

Conclusion

Incidental monoclonal lymphocyte populations are not uncommon in the bone marrow and are readily characterised with sensitive flow cytometric methods. The clinical significance of these populations is unknown and may be assessed with extended follow-up.

No conflict of interest to disclose

A70



O032

0915

Relationship Between the Single and Dual Platform CD34+ Cell Assays

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Aim

The CD34+ surface antigen is expressed by pluripotent haemopoietic stem cells with the number of CD34+ stem cells infused at the time of autologous transplantation being of importance in predicting timing of neutrophil and platelet engraftment. The methodology of counting CD34+ stem cells has changed recently from a dual platform (Sutherland et al 1996) to a single platform method (Keeney et al 1998), with many institutions changing to the single platform method because of its simplicity, though more technically demanding on the scientist performing the procedure. Prior to consideration changing methodology to a single platform method at Liverpool Hospital, an assessment was performed to ensure comparability between the two methods. Additionally, if a difference was found it would be necessary to establish a model of the relationship between these two methods so that previously collected harvests could be converted from a dual platform CD34+ count to a single platform CD34+ count.

Methods

From October 2008 until April 2009, 28 stem cell collection procedures had the number of CD34+ cells collected by single and dual platform methods. Firstly the dual platform CD34+ counting was performed as described by Sutherland et al (1996) using a FITC labelled anti-CD34+ antibody. Secondly, the single platform method (Keeney et al 1998) using TruCount or Flow-Count beads with a PE labelled anti-CD34 antibody, was used to count the PBSCs on these 28 harvest procedures. Statistical analysis (t-test, sign rank and regression analysis) was performed using Stata 10 software.

Results

A t-test and sign rank test was performed on the log of the dual platform (ldp) and log of the single platform (lsp) CD34+ counts. This showed a significant difference between the paired results with the sp method giving higher results than the dp method with the mean difference of 0.6 [95% CI(0.44,0.76), t=7.5, p<0.001]. A plot of log single platform (lsp) and log of dual platform (ldp) results demonstrated a linear relationship, with the regression model was developed for conversion of dual platform to single platform CD34+ counts being,

$$\begin{aligned} sp &= [\exp(0.69)] * [dp^{(0.78)}] \\ &\approx 2 * [dp^{(0.78)}] \end{aligned}$$

Conclusions

It was demonstrated that the dual platform method of measuring CD34+ cells produced significantly lower CD34+ cell count results than the single platform method. Others have found this not to be the case (Piedras-Ross et al 2003; and Barbosa et al 1999). The difference identified may relate to the fluorochromes used in the flow cytometry method at Liverpool Hospital which will require further evaluation.

No conflict of interest

A71



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Day 19



0830-1000
Meeting Room 3

O033

0930

Implementation of a Northern Territory Wide Digital Microscopy System at Royal Darwin Hospital and Northern Territory Regional Hospitals for Blood Film Morphology Review

Tina Noutsos, Merianne Wardle, Ferenc Szabo

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Introduction

Digital microscopy (DM) utilises information technology (IT) to simulate the functions traditionally performed by a physical microscope. One aspect of DM includes telepathology, which transmits moving live digital images of a glass slide between multiple remote sites. We describe the first Australian DM telepathology system, which interfaces laboratories in the Northern Territory.

Methods

The laboratory information systems of Darwin, Alice Springs, Katherine, Tennant Creek and Nhulunbuy hospitals were interfaced, and a DM telepathology system established. Olympus BX45 microscopes were fitted with CC12 cameras, linked to a PC with internet connection. Olympus analySIS intraScope® software was installed for viewing and transmission, via webcam, of live moving images. The DM system was used for real time review of blood films with simultaneous teleconference, between remote central Darwin laboratories. Feedback questionnaires regarding the utility of the DM system were completed by regional scientists.

Results

The average time for glass slides to be couriered from remote laboratories to Darwin for review is an estimated 24 to 48 hours. The established DM system resulted in a clinically significant reduction in the turn-around-time for diagnostic second opinion. This improved confidence in scientists working in isolation and enhanced patient safety. Some critical conditions, like acute and chronic leukaemias could be timely diagnosed. In addition, all RCPA external QAP slides were reviewed real time and used as an education tool. Feedback regarding the utility of the DM telepathology service was overall extremely positive with respect to image quality, use of software, real time+diagnostic support, supervision and continuing professional development. Minor limitations included the inability of the central supervisor to navigate the slides.

Conclusion

The NT DM telepathology system was an excellent tool for real time diagnostic support, supervision, education and training of remote scientists. Future directions include the purchase of motorised microscopes to address the minor limitations of the current DM telepathology system.

No conflict of interest to disclose

A72



0034

MEG3 and DLK1 expression in patients with Acute Myeloid Leukemia (AML)

0945

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Aim

The human 14q32.2 locus encodes a cluster of imprinted genes including MEG3, DLK1 and a large microRNA gene cluster. Deregulation of these genes in several malignancies suggests a central role for this locus in oncogenesis. The expression of MEG3 is down regulated in non-functioning pituitary tumours whereas DLK1 is highly up regulated in stem cells from myelodysplastic syndrome patients, with expression also detected in selected patients with AML. We and others have also shown that the 14q32 microRNA cluster is deregulated in acute promyelocytic leukemia (APL) relative to other subtypes of AML [1-2]. In this study we have developed an RT-PCR assay for MEG3 and DLK1 mRNA to determine their expression levels in AML.

Method

Total RNA was extracted from diagnostic bone marrow aspirates from 34 patients with AML (21 APL and 13 non-APL AML) and cDNA generated using standard methods. Primers were designed to amplify DLK1 and all MEG3 isoforms. PCR products were resolved on 4% agarose gels, visualized with ethidium bromide, and the bands were scored based on intensity. RNA quality was verified by parallel assessment of transferrin receptor expression.

Results

The number of patients with different patterns of MEG3 and DLK1 expression is shown in the following table.

		Positive	Weak or Negative	p-value (Fisher's exact test)
MEG3	APL	17	4	< 0.005
	AML	4	9	
DLK1	APL	3	13	> 0.3
	AML	3	10	

Conclusion

These preliminary data suggest that MEG3 expression is upregulated in APL relative to other AML subtypes, analogous with the pattern seen for the microRNA cluster at 14q32. DLK1 expression is found in selected APL and non-APL AML, though it does not differentiate the two groups. We have now developed quantitative RT-PCR for MEG3 and DLK1 to extend the current analysis to a larger patient group and to directly correlate MEG3 expression with the 14q32 microRNA cluster in individual patients.

This research was supported by the Haematology and Oncology Targeted Therapies (HOTT) Award 2008 (Roche Oncology & Haematology in conjunction with COSA, MOGA and HSANZ). Roche had no role in analyzing the data or preparing the abstract.

1. Brown C et al. Up regulated expression of a large microRNA gene cluster in acute promyelocytic leukemia. HSANZ Annual Scientific Meeting, Adelaide, 2009.
2. Dixon-McGiver et al. Distinctive patterns of microRNA expression associated with karyotype in acute myeloid leukaemia. PLoS ONE, 2008. 3(5):e2141.



O035

0830

Transfusion is Associated with Adverse Clinical Outcomes in Cardiac Surgery: An Analysis of a Large Patient Cohort

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Background

Allogeneic red cell transfusion has been reported as associated with adverse post-operative outcomes in cardiac surgery. There is conflicting evidence on potential adverse effects of platelets in this setting. We investigated whether peri-operative transfusion was independently associated with clinical outcomes.

Method

Data were prospectively collected on 9363 cardiac surgery patients at six major Melbourne hospitals from January 2005 -- December 2008 through the Australasian Society of Cardiac & Thoracic Surgeons Cardiac Surgery Database. The independent association of transfusion with a range of clinical outcomes was determined by stepwise logistic regression analysis. Patient factors (including co-morbidities and medication use) and surgical factors were included in the analysis.

Results

Procedure types were: coronary artery bypass graft (CABG) surgery in 60%, valve surgery 14%, CABG + valve 10% and other 16%. Transfusion of red cells, platelets and plasma were each associated with all outcome measures. In multiple logistic regression analysis, red cell transfusion was independently associated with multi-system failure, peri-operative myocardial infarction, stroke, prolonged ventilation, pulmonary embolism, pneumonia, deep sternal wound infection and septicaemia. Platelet transfusion was independently associated with multi-system failure and pneumonia. Plasma transfusion was independently associated with in-hospital and 30 day mortality, multi-system failure, prolonged ventilation and septicaemia.

Conclusion

Peri-operative transfusions of red cells, platelets and plasma were independently associated with increased risk of adverse events. Although identified confounding factors were controlled for, number of transfused units and laboratory investigations were not available for analysis, however a project is underway to link hospital laboratory data (including transfusion history) with this database. Whilst it cannot be concluded from this study that transfusion plays a causal role in these adverse clinical outcomes, these findings from a large cohort of patients support the need for further research into the effects of transfusion on patient outcomes in cardiac surgery to inform practice.

No conflict of interest to disclose

A74



0036

Duffy Blood Group Molecular Typing - Complementing Serology to Manage Emerging Transfusion Needs in Clinical Practice

Jenny Morrison, Jacqui Martin, Naomi Roots, Robert Flower, Catherine Hyland
Australian Red Cross Blood Service

0845

Background

The frequency of red cell blood groups varies in different ethnic populations. The Fy(a b⁻) phenotype occurs in approximately 70% of people of African origin and may arise from a homozygous mutation in the GATA-1 promoter region which prevents Fyb expression on red cells but not other cells. Blood recipients with this mutation should be able to safely receive the more common Fy(a b⁺) blood for transfusion whereas those without this mutation will need the rare Fy(a-b⁻) blood type for transfusion.

Hypothesis

Molecular DNA blood group typing can complement serological typing to resolve ambiguous results and meet clinical needs in transfusion medicine.

Aim

The aim of the project is to establish a PCR test for molecular Duffy blood group typing to classify people with the Fy(a b⁻) phenotype into 2 groups, those with and without the GATA-1 mutation.

Methods

Two PCR methods were established and evaluated using a panel of samples (n=70) supplied by the Red Cell Reference Laboratories and DNA Laboratory, ARCBS. All samples were tested using an hydrolysis probe method for mutation detection. To date 26/70 samples have been tested by a second, custom designed HRM assay with raZor probe by PrimerDesign Ltd, UK.

Results

All Duffy genotype assessments, excepting one, correlated with known phenotype, Table. The one exception phenotyped Fy(a-b⁻) but was only heterozygous for the GATA-1 mutation. Further testing on an automatic high throughput platform (BLOODchip) confirmed our results. AS-PCR confirmed its genotype to be FY/FYA but the FYA allele is apparently not expressed.

Known phenotypes (serology)	Assessed genotype*	Number tested	
		Hydrolysis Method (n=70)	HRM Method (n=26)
Fy(a-b ⁺)	FYB/FYB	20	6
Fy(a+b ⁺)	FYA/FYB	23	8
Fy(a+bw)	FYA/FYX	N/A	2
Fy(a+b ⁻)	FYA/FYA	14	4
Fy(a-b ⁺)	FY/FYB	1	Test pending
Fy(a-b ⁻)	FY/FY	11	8
Fy(a-b ⁻)	FY/FYA	1	Test pending

*FY = GATA-1 mutation preventing FYB expression

Conclusions

Molecular typing was accurate using both methods however, more testing by HRM is required. In total, 11/12 Fy(a-b⁻) individuals were found to be homozygous for the GATA-1 mutation and therefore should be able to receive Fy(a-b⁺) blood in transfusion. A novel mutation may account for the Fy(a-b⁻) individual heterozygous for the GATA-1 mutation and is being followed up. In the future molecular typing for Duffy, in conjunction with phenotyping, may guide clinicians in decision making to select blood types for transfusion.

No conflict of interest to disclose

A75



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Day 19



0830-1000
Hall B

0037

0900

DNA Typing on BLOOD-chip v1: Pilot Study to Classify Problematic Serology Samples

C Hyland¹, G Millard¹, J Condon¹, R Flower¹, M Sierra², N Avent³

¹ Australian Red Cross Blood Service. ² Progenika Biopharma SA. ³ University of Plymouth

Background

This pilot study was conducted as a component of a travel grant funded by CSL Bioplasma in 2008.

Hypothesis

DNA typing for blood groups using DNA array based systems can complement serology typing to resolve samples with ambiguous typing results.

Aim

To test whether an automated DNA typing platform could resolve samples remaining problematic after testing in ARCBS red cell serology and DNA reference laboratories.

Methods

Panel comprised nine samples with unresolved serology either due to RhD status (n=6), or prior transfusion confounding grouping (n=2), or antibody apparently related to MNS (n=1). Genomic DNA isolated from white cells was stored at -20°C prior to shipment and testing on the BLOOD-chip v1 array (Progenika Biopharma S.A, Bilbao, Spain). The system is CE- marked for diagnostic purposes in the European Union. One sample was tested further by exon sequencing.

Results

Phenotype predictions by DNA typing matched known phenotypes with one exception. Review of the apparent exception showed a sample derived from maternal genomic DNA was sent when the intent was to send the corresponding amniotic DNA. Therefore all DNA typing matched known phenotypes. DNA typing classified 5 of the 6 RhD variants definitively and further sequencing suggests the remaining sample is a novel DIIIb variant. One of the two samples confounded by prior transfusion history typed RhD negative. The cause for the MNS related antibody remains unknown although a full MNSsU & GYPB genotype was assigned.

Conclusions

This small study supports a role for DNA typing to supplement serology to resolve problematic samples. Further systematic studies are required to assess the needs and strategies for integrating DNA typing as part of reference serotyping. An interesting aspect of this study is the identification of a novel RhD IIIb variant and this is under further review. Finally the unresolved nature of an antibody in one case is an instructive signal that DNA typing is complementary to, and not an alternative to, serology.

This research was supported by ARCBS R & D Division and a CSL travel scholarship awarded through ANZSBT. Progenika provided a complimentary testing service. The test platform provided an automated analysis and accompanying print out of test results. These were collated and analysed by Progenika R & D scientists, MS and ML, and provided to CH, GM and NA for independent review and further analysis. MS and ML agreed to but did not prepare the abstract. NA was a consultant for Progenika at the time the work was performed.

A76



0038

Preliminary Findings from an Ovine Model of Blood Component Therapy Developed to Investigate Transfusion-Related Acute Lung Injury

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¹ Australian Red Cross Blood Service, Brisbane, Queensland, Australia. ² The University of Queensland, Brisbane, Queensland, Australia. ³ The Critical Care Research Group, The Prince Charles Hospital, Brisbane, Queensland, Australia. ⁴ The Prince Charles Hospital, Brisbane, Queensland, Australia. ⁵ The Princess Alexandra Hospital, Brisbane, Queensland, Australia. ⁶ Bonfils Blood Centre, Denver, Colorado, USA. ⁷ Queensland University of Technology, Brisbane, Queensland, Australia

Aim

To develop an ovine model of blood component therapy (BCT). The model will be used to study adverse reactions including transfusion-related acute lung injury.

Methods

Sheep were first infused with either saline (healthy sheep) or lipopolysaccharides (LPS) (sepsis sheep). An hour after the first infusion was completed sheep were infused with BCT of either saline (control), day-1 pool of heat-treated supernatants from human whole-blood platelets (d1-S/N) or day-5 pool (d5-S/N).

Results

LPS-infusion resulted in decreased circulating neutrophils (PMNs) corresponding with pulmonary sequestration of PMNs. Infusion of d1- and d5-S/N into healthy sheep resulted in increased circulating PMN counts. Infusion of human BCT caused only mild pulmonary injury which was most severe when d5-S/N was infused into septic sheep. D5-S/N also caused mild injury (average injury - 1) more often than d1-S/N.

1 st infusion	2 nd infusion	n	Circulating PMNs (x10 ⁹ /L)				Histology (semi-quantitative score from 0-4)					
			Pre-infusions	Post-1st infusion	Post-2nd infusion	Pre-euthanasia (3-4hrs)	Oedema	Haemorrhage	Microvascular congestion	Bronchiole pathology	Average injury	Sheep with mild pulmonary injury (avg injury - 1)
saline	saline	1	5.04	5.78	6.40	6.72	0	0	3	0	0.80	0/1
	d1-S/N	3	1.99	2.77	3.69	3.54	1.33	0.67	2.67	0.83	1.40	1/3
	d5-S/N	3	2.62	3.75	7.50	6.74	1.67	0.00	0.33	2.00	0.93	2/3
LPS	saline	2	2.45	0.08	0.11	0.14	0.00	0.00	1.50	0.00	0.40	0/2
	d1-S/N	2	2.53	0.83	0.10	0.39	1.25	1.50	2.50	1.00	1.55	1/2
	d5-S/N	3	3.68	0.04	0.14	0.58	1.50	1.67	2.33	1.33	1.70	3/3

Conclusion

These preliminary data indicate that sheep tolerate the infusion of human platelet supernatants, and therefore are potentially a suitable large animal model to study the effects of BCT. Mild pulmonary injury seen more often with stored blood product (d5-SN) may represent a mild TRALI, however further study is required to confirm this.

No conflicts of interest for the authors to disclose.

A77

0915



O039

0930

Mannose Binding Lectin Deficiency is Associated with Altered Myeloid Blood Dendritic Cell Function

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¹Australian Red Cross Blood Service, QLD, ²Mater Medical Research Institute, QLD, ³Royal Melbourne Hospital, VIC.

Background

Mannose binding lectin (MBL) is a circulating plasma pattern recognition molecule that facilitates pathogen killing via direct opsonisation, activation of the complement cascade and enhancing phagocytic uptake. Genetically driven MBL deficiency is found in 25% of the population and is associated with increased frequency and severity of infection. Dendritic cells (DC) play a central role in linking the innate and adaptive immune response. MBL deficiency may influence DC function and have important outcomes following an infectious challenge.

Aims

- To investigate whether MBL deficiency results in altered dendritic cell function.
- To investigate if addition of MBL (purified from human plasma) could change, or improve the response to pathogen.

Methods

Following zymosan stimulation in a whole blood infection model, CD11c⁺ myeloid blood (M)DC were isolated from MBL-Deficient (MBL-D, n=10) and MBL-Sufficient (MBL-S, n=6) individuals and characterised for cytokine production, surface phenotype and ability to induce allogeneic T cell responses. Total RNA was isolated from sorted MDC and investigated using expression microarray to compare the genes and signalling pathways induced in MBL-D and MBL-S individuals.

Results

MDC from MBL-D individuals displayed unique functional characteristics, including higher production of proinflammatory cytokines IL-6 (P<0.01) and TNF- α (P<0.01), but poor capacity for allo-T cell effector cell induction. Addition of MBL significantly reduced elevated IL-6 production by MDC in MBL-D individuals (P<0.05). Expression microarray analysis demonstrated MBL-S individuals had greater capacity to induce T and NK cell signalling pathways than MBL-D individuals. Further, MBL acted as a regulator of important inflammatory molecules, including T-cell receptor zeta (CD247), IFN- and perforin 1.

Conclusions

MBL deficiency was associated with altered dendritic cell function, highlighting a potential mechanism of increased frequency and severity of disease in MBL-D individuals. Addition of MBL to MDC of MBL-D individuals reduced differences in pathogen induced immune responses between MBL-D and MBL-S individuals. Therapeutic administration of MBL may modify the immune response and reduce infectious episodes in MBL-D individuals.

No conflict of interest to disclose

A78



O040

0830

GRP78 Exerts Novel Anti-thrombotic Activity and Localises to the Endothelium by Interacting with the Lectin-like Domain of Thrombomodulin

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¹Immunology Research Centre St Vincent's Hospital Melbourne, ²St Vincent's Institute of Medical Research, ³University of Melbourne

Aim

To identify novel mechanisms by which thrombomodulin exerts anti-thrombotic and anti-inflammatory activities.

Method

The endothelial cell surface maintains an anti-thrombotic microenvironment. Thrombomodulin (TM) is multi-domain modular transmembrane glycoprotein expressed primarily on vascular endothelial cells. The principal function of TM is the capacity to bind thrombin and facilitate the generation of activated Protein C (APC), which mediates anticoagulant anti-inflammatory effects. There is recent evidence that the N-terminal lectin-like domain (LLD) of TM exhibits anti-inflammatory activities independent of APC. However the molecular mechanisms that mediate the anti-inflammatory properties of the LLD remain unresolved.

The One-STrEP-tag system and tandem MS/MS Spectrometry were used to identify the LLD-interacting proteins. Interactions were validated by co-immunoprecipitations and confocal microscopy. Recombinant proteins were made using prokaryotic expression and purified by presence of His-tag and use of affinity chromatography. Anti-coagulant functions were analysed by PT, APTT and platelet aggregometry.

Results

We have identified a novel interaction between LLD of TM and a specific ~80 kDa polypeptide, which was demonstrated to be the 78 kDa glucose response protein (GRP78). TM and GRP78 were demonstrated to co-localize on cell surface as well as intracellularly by confocal microscopy. The interaction between LLD and GRP78 was confirmed by immunoprecipitation experiments using cell lysates. Moreover, LLD was shown to recognise endogenous GRP78 present in plasma of normal donors. Recombinant GRP78 prolonged tissue factor-initiated clotting (PT) as well as TF-independent coagulation (APTT). We demonstrated that the anticoagulant effect was most likely secondary to the ability of GRP78 to bind calcium, ATP and ADP. We also showed for the first time that GRP78 inhibited TRAP-mediated, collagen-mediated and ADP-mediated platelet aggregation. Recombinant GRP78 prolonged mouse tail-bleeding times in a dose-dependent manner.

Conclusion

GRP78 was demonstrated to contain anticoagulant and anti-platelet activities. We hypothesise that the LLD anchors GRP78 and thereby concentrates anti-thrombotic potential of GRP78 on the endothelial surface. This mechanism may serve to maintain the antithrombotic profile of endothelium and may also regulate the developing thrombus

No conflict of interest



O041

0845

Thromboprophylaxis During Pregnancy – A Survey of Haematologists and Obstetricians

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³ Australian Centre for Blood Diseases, Prahran, VIC, Australia

Venous thromboembolism (VTE) is the leading cause of maternal death in the western world. Pregnancy increases the risk of embolic disease by 4- to 10-fold resulting in an overall risk of 1.72 per 1000 births. The risk of maternal death has been estimated to be approximately 1 per 100,000 births and therefore it is important that we consider the diagnosis of VTE and role of thromboprophylaxis from early pregnancy. Not every woman warrants thromboprophylaxis but there are several risk factors, including both inherited and acquired conditions, which can significantly increase the risk of VTE. In view of the increasing spectrum of differing clinical situations and a lack of evidence to guide us, it is becoming ever more challenging to determine which pregnant woman need thromboprophylaxis, choosing the intensity and type of anticoagulation, and determining duration of treatment. The aim of our survey was to assess our clinical practice in line with the Royal College of Obstetricians and Gynaecologists guidelines and to establish if, we as specialists have reached a consensus on management of these difficult patients. A web-based survey was designed to explore as many of the different clinical situations regarding thromboprophylaxis in pregnancy and following vaginal delivery. The survey was mailed to members of the Australian Society of Thrombosis and Haemostasis and Royal College of Obstetricians and was available from our website between September 2008 and June 2009.

There were a total of 25 questions covering the following areas: thromboprophylaxis regimens used; management of women with and without associated risk factors; management of women receiving long-term warfarin; and the role of heparin (anti-Xa) monitoring. From the surveys mailed out we obtained 140 responses (42 haematologists and 98 obstetricians) and 42 doctors completed all 25 questions. The results will be presented.

No conflict of interest to disclose

A80



0042

0900

Understanding Hospital Physicians Perspectives and Attitudes towards the Implementation of Venous Thrombosis Prophylaxis

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¹ The University of New South Wales, ² St. George Hospital, ³ University of Technology, Sydney, Sydney, New South Wales, Australia

Aim

Venous Thromboembolism (VTE) is responsible for up to 10% of hospital mortality, making it the most preventable cause of death in a hospitalised setting. Despite the well-established need for prophylaxis, major multinational and national observational studies highlight suboptimal and inappropriate application of VTE prophylaxis. We have explored the facilitators, barriers, and attitudes of medical professionals towards VTE prophylaxis in a hospitalised setting.

Methods

Twenty physicians from a wide array of specialties and hierarchical levels at a major metropolitan teaching hospital (St George) were selected for the study. Consenting junior medical officers were randomly selected using the hospital database paging system. Senior consultants were contacted and recruited using snowball and convenience sampling. Semi-structured, open-ended questions were dictated in causal conversation and participants were given the opportunity to respond freely. The digitally audiotaped interviews were transcribed verbatim. Transcripts were coded for common themes and phrases using a grounded method approach.

Results

Physicians regularly and admittedly deviate from best practise guidelines and rely on their art of medicine or mind lines. Mind-lines are uniquely formulated through personal experience, particularly as a result of adverse effects with VTE prophylaxis. The power associated with senior staff was observed to result in organisational learning where junior staff frequently alter their practice despite being against best practice medicine. Fragmentation of Care was also observed with disagreement on which individual/specialty was responsible for conducting VTE risk assessment and instituting prophylaxis. Physicians rated the absence of reminders as being the primarily reason to oversight. Contrary to speculations within the literature, lack of VTE knowledge was not evident within this group of physicians.

Conclusions

Our results indicate that at least at this hospital the attitudes of physicians are such that VTE is felt to be important and should be prioritised. It was also clear that there was at least a minimal awareness of the existence of guidelines and the need to individually risk assess patients. Thus rather than trying to change prescribing habits by trying to improve levels of awareness and education as has been tried in the past, it would seem that our focus needs to be on instituting systems and processes that will facilitate VTE risk assessment and reporting. Whether this facilitation takes the form of reminders, templates or assigning responsibilities to particular roles remains to be tested.

No conflict of interest to disclose



0043

0915

Risk Factors Contributing to High INR Results in Patients Receiving Warfarin Therapy in the Community

Basia Diug¹, Judy Lowthian¹, Sue Evans¹, Ellen Maxwell², Michael Dooley³, Alison Street³, Peter Cameron¹, Leon Piterman⁴, John McNeil¹

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Aim

This study examines the impact of psychosocial characteristics on susceptibility to high INR results, a surrogate marker for warfarin induced bleeding. Previous studies have examined the impact of disease states, concomitant medication, older age and genetic factors on warfarin stability. There is limited information on the impact of individual psychosocial factors such as depression, cognition, health literacy and social isolation.

Methods

We undertook a case control study of 149 patients with an elevated INR (≥ 6.0) and 300 controls with an INR persistently within their therapeutic range. Cases and controls were recruited from a large metropolitan pathology provider and interviewed at either a recruitment centre or at their home by a trained researcher. Standardised measures were employed to identify potential psychosocial risk factors, including the Montreal Cognitive Assessment (MOCA), Geriatric Depression Scale (GDS-5), Duke Social Support Index (DSSI) and the Short Test of Functional Health Literacy in Adults (S-TOFHLA).

Results

One hundred and forty nine patients: (mean age 75.4 yrs, range 25-96, 46.2% female) and 300 controls (mean age 75.5 yrs, range 36-92, 41.3% female) were recruited. Mean duration of treatment was 7.3 (0.5-30) years in cases and 6.8 (0.6-30) years in controls. Atrial fibrillation was the most common indication for warfarin (44.8%). Psychosocial results are tabled below:

Variable	Cases n=149(%)	Controls n=300(%)	Unadjusted Odds Ratio (95% CI)
MOCA Mild cognitive impairment (Score <26)	117(78.5)	170(56.7)	2.79 (1.77-4.39)*
GDS-5 Possible depression (Score ≥ 2)	63(42.3)	57(19.0)	3.12 (2.02-4.82)*
DSSI Self-reported social isolation (Score ≤ 80)	45(30.2)	44(14.7)	2.52 (1.57-4.04)*
S-TOFHLA Inadequate functional health literacy (Score <67)	100(67.1)	120(40.0)	3.17 (2.09-4.81)*

*(p<0.0001)

Conclusion

This study demonstrated that impaired cognitive function, depression, reported social isolation and low health literacy were strongly associated with warfarin instability. Patients should be assessed in a holistic manner at initiation and during chronic therapy with oral anticoagulants to identify those at risk of over anticoagulation.

This research was supported by Melbourne Pathology. The company had no role in analysing the data or preparing the abstract

A82



O044

0930

Thrombin Generation and Vitamin K Dependent Procoagulant Factors in Warfarinised Adult Patients

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Aim

Immediate reversal of warfarin can be achieved by FFP with or without prothrombin complex concentrates (PCCs-Prothrombinex. VFTM, marketed in Australia). Prothrombinex-VFTM is a three factor concentrate which lacks Factor VII; it is recommended that FFP be given in conjunction. Given the potential side-effects with plasma usage, it would be preferable for Prothrombinex-VFTM to be used on its own. Recently this approach has been used successfully to fully reverse warfarin anticoagulation, however the mechanism by which this is achieved remains to be explored. In addition there is limited data on the level of suppression of Vitamin K dependent procoagulant factors in stable (INR 2-3.5) and supra-therapeutic (INR>3.5) patients. Measuring the Vitamin K dependent factor levels in these patients and in patients before and after reversal with Prothrombinex-VFTM used alone could provide an explanation why reversal occurs with a three factor PCC.

Methods

Twenty patients with stable therapeutic INRs and twenty with supra-therapeutic INR had Vitamin K dependent factor levels measured using one stage clotting assays and thrombin generation (TG) using a calibrated automated thrombinogram (ThrombinoscopeTM). Control samples not on warfarin and with normal coagulation profiles were used to establish normal TG values. Patients who were given Prothrombinex-VFTM alone for warfarin reversal had pre and post-TG and factor levels. Some of these had serial measurements of INR for up to 72hrs to ascertain the presence of a rebound effect on INR.

Results: In both stable and supra-therapeutic warfarinised patients, the factor X level was lowest, followed by factor II and factor VII. Factor IX was least suppressed. The supra-therapeutic group showed a deeper degree of suppression. The mean factor VII level was 30% (normal 50-150%) in the stable group and 12% in the supra-therapeutic group. Prothrombinex-VFTM achieved smooth reversal with no significant rebound effect. Prothrombinex-VFTM used alone for reversal can restore TG to varying degrees. There was poor correlation between thrombin generation and individual INRs.

Conclusions

The observation that factor VII is not significantly reduced in patients receiving warfarin may explain the success of a three factor PCC used alone in the reversal of warfarin anticoagulation. Prothrombinex-VFTM appears to be a simple, safe and attractive method for warfarin reversal especially in those in whom a prolonged withdrawal from anticoagulation therapy is deemed to be harmful.

No conflict of interest to disclose



0045

0945

Thrombin Generation In Inherited Protein S Deficiency

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Aim

To examine the pro-thrombotic effect of inherited and acquired protein S (PS) deficiency by performing a cross-sectional study comparing thrombin generation (TG) in controls, combined oral contraceptive pill (OCP) users, and patients with inherited PS deficiency.

Methods

TG was determined with and without 15 nM thrombomodulin (TM) using the Calibrated Automated Thrombogram ® (CAT). 40 controls, 18 OCP users, and 11 patients with inherited PS deficiency were enrolled.

Results

Mean free PS levels in controls, OCP users and PS deficient patients were 1.02, 0.79 and 0.32 U/ml respectively. Mean endogenous thrombin potential (ETP) and peak thrombin levels in the absence of thrombomodulin (2162 vs 2835 nM.min, $p < 0.001$; 380 vs 510 nM, $p = < 0.001$) were significantly higher in OCP users in comparison to controls. No difference in the results of TG without TM was observed between PS deficient patients and controls. Both OCP users and PS groups had significantly higher mean ETP [1410 (OCP) / 1364 (PS) vs 790 nM.min (controls), $p < 0.001$] and peak (287 / 302 vs 154 nM, $p = 0.001$) than controls in the presence of TM. The absolute and percentage difference in ETP and peak values with and without TM were significantly reduced in PS deficient patients in comparison to other groups, and was highly correlated with free protein S levels (R value 0.93 and 0.89) with weaker correlation seen in OCP users (R value 0.50 and 0.60).

Conclusion

A lack of inhibition of ETP and peak values with TM correlating closely to free protein S levels was observed in protein S deficient patient. This differed from OCP users where high baseline ETP and peak values without TM were observed, and in whom inhibition of TG by TM was less influenced by protein S levels, suggesting a separate pro-thrombotic mechanism in this patient group.

No conflict of interest to declare

A84



O046

0830

Undergoing Peripheral Blood Stem Cell Collection: The Patients' Experiences

Renee McMullen, Dominique Yokowo, Maryanne Hargraves
Haematology and Oncology Clinics of Australasia, Brisbane, Australia

Aim

To gain an insight into the experiences of patients undergoing peripheral blood progenitor cell (PBPC) collection.

Method

A qualitative approach was used to describe the experience of people undergoing PBPC collection. The main source of data was in-depth conversation. This study was carried out in two phases. Firstly, a cohort of seven (7) patients participated in a focus group. The aim of the focus group was to draw out a broad range of issues and themes pertaining to the experience of PBPC collection. These themes were used to develop an in-depth interview guide. The second part of the study involved researchers carrying out in-depth interviews with ten (10) participants who had recently undergone PBPC collection. The focus group and in-depth interviews were taped and transcribed verbatim. Data analysis involved systematically sorting and classifying information into representational groups and pattern.

Results

Anxiety was the central theme uncovered during the focus group. Participants described anxiety in relation to the blood cell separator, pain and effectiveness of the PBPC collection. Other themes identified from analysis of the focus group data related to issues concerning the knowledge and skill of nurses and the environment in which the PBPC collection was performed. These broad themes were used to develop an in-depth interview guide. Analysis of data from the in-depth interviews revealed eight (8) interrelated main themes. Themes which directly related to the experience of PBPC collection included; anxiety, physical symptoms and lifestyle disruption. In addition, themes which influenced the participants' experience were identified. These include; previous experience, supportive networks, nursing response, confidence in health professionals and perceptions of the environment and equipment.

Conclusion

The results of this study demonstrate that PBPC collection is a significant, often anxiety producing event for patients. PBPC collection has the potential to impact patients on a physical, emotional and psychosocial level. There are numerous potential stressors which the patient may be exposed to during PBPC collection. Nurses play an important role in helping patients cope with stressors through the provision of empathetic care which addresses the physical and emotional needs of patients.

This research was supported by the Queensland Nursing Council. The Queensland Nursing Council had no role in analysing the data or preparing the abstract.



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Day 19

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Patient Experiences & Expectations

0830-1000
Hall A

O047

0845

Upload, Download, Unload: LifeBloodLIVE - A New Zealand Online Forum for Haematology Patients

Amy Munro¹, Lisa Speedy², Christine Kerr³, Debbie Moore¹

¹ Leukaemia & Blood Foundation, Auckland, New Zealand. ² Leukaemia & Blood Foundation, Wellington, New Zealand. ³ Leukaemia & Blood Foundation, Christchurch, New Zealand

Introduction

Imagine you have been diagnosed with a haematology condition. You may never have heard of the condition, let alone know the spelling. But that won't stop you, or your family, from googling it. Just as nurses search the internet for information, so do patients. In this technological age, patients use this medium for both instant results and to seek experiential information: information that describes the experiences of fellow cancer patients [1].

Background

Helft et al have shown that approximately 30% of cancer patients use the internet to obtain cancer information [2]; only 30% of these patients discuss their findings with their doctors [2]. We need to be aware of their search patterns, and the resulting effects. Research has shown that treatment preferences are strongly influenced by fears, misconceptions and anecdotes rather than by population-based information[1]. Experiential information is found in case studies, opinions, stories, peer mentoring, online forums etc. Experiential information can help normalise patients' experiences, address isolation issues and portray the treatment and recovery experience, thereby enhancing self-efficacy for coping and promoting involvement [1].

Methods

To address the need for a New Zealand internet resource, the Leukaemia & Blood Foundation developed LifeBloodLIVE, an online forum for patients to share experiences, find links to reputable websites and gain accurate information. Users are encouraged to keep a degree of anonymity and candour. The site is moderated daily for inaccuracies by haematology nurses.

Results

Launched in 2008, the site now has more than 160 active users from diverse backgrounds, locations and haematology conditions, especially benefitting those in rural communities.

Conclusion

We must provide patients with current and easily accessible information and support. Traditional communication methods through conversations or written material are not the only options. By acknowledging and facilitating the exchange of experiential information in a non-threatening environment, patients are assisted in their cancer journey.

No conflict of interest to disclose

1. Rini et al, Peer Monitoring and Survivors' Stories for Cancer patients: Positive Effects and Some Cautionary Notes. JCO, 25:1 2007.
2. Helft et al, Use of the Internet to Obtain Cancer Information Among Cancer Patients at an Urban County Hospital. JCO, 23:22 2005.

A86



O048

0900

The Development of a Haematology Late Effects Nurse Consultant Role with a National Scope

Priscilla Gates¹ Greg Wheeler¹, John Seymour¹, David Ritchie¹, Lisa Brady¹, Samantha Schembri²

¹Haematology Late Effects Service. Peter MacCallum Cancer Centre. Melbourne Australia. ²Leukaemia Foundation of Australia.

Background

Long-term survivors of haematological malignancy are an expanding patient group with unique survivorship issues. With advances in multimodality therapy, overall survival rates now exceed 80% for many diseases, resulting in a large cohort of survivors who are at risk of developing long term Late Effects (LE) related to treatment including second malignancies, cardiac dysfunction, endocrine dysfunction, infertility and psychosocial sequelae.

Aim

The LE unit at the Peter MacCallum Cancer Centre identified the need for a new Haematology LE Nurse Consultant (LE NC) role. The aim of this initiative was to address the needs of haematology survivors focusing on screening for LE; increasing knowledge of risk factors; providing psychosocial support and counseling regarding lifestyle modifications that may reduce the incidence and/or severity of LE.

Method

Supported through funding from the Leukaemia Foundation of Australia, the dedicated Haematology LE NC role with a national scope was established. Resources developed include individualized education packages, survivorship care plans and surveillance guidelines all created by the multidisciplinary team of the Haematology LE clinic.

Results

156 patients attend the Haematology LE clinic, 23 (15%) of whom have developed second malignancies. The education packages and surveillance guidelines have been disseminated. Networks have been established and continue to be expanded throughout Australia.

Conclusion

As this is one of only three known haematology dedicated adult LE clinics in Australia, the LE NC has developed specialist knowledge and advanced practice skills from focusing on the distinct needs of this patient population. The haematology LE NC has been able to provide support and advise other nurse clinicians throughout Australia. Core functions include information provision, education for patients and families, psychosocial support, screening for LE and developing individualized lifestyle modification programs. This exciting initiative has been instrumental in supporting the unique issues faced by this population of survivors and initiating much needed research in this area.

No conflict of interest to declare



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Day 19



Patient Experiences & Expectations

0830-1000
Hall A

O049

0915

'It's Just A Flesh Wound': Battling Hodgkins Lymphoma

Cassandra Reid

Royal North Shore Hospital, Sydney

Early stage Hodgkins Lymphoma (HL) is curable in a majority of cases, with treatment regimens focusing on how best to deliver appropriate doses of chemotherapy /radiotherapy whilst balancing the risk of exposure to long term treatment sequelae such as secondary malignancies and pulmonary toxicity. For those with relapsed early stage disease who had an initial complete response to induction, salvage chemotherapy may give durable remissions in more than 60% of patients. Further, good response to second line treatment also indicates a survival advantage when proceeding to Autologous transplantation- with up to 60% long term remission. However long term survival rates are poor for patients who fail to achieve a CR or have a short duration of CR.

This case report presents the story of a 25yr old man diagnosed in 2005 with Stage 11B nodular sclerosing HL who had recurrent refractory disease. Despite undergoing three transplants and grade IV skin aGVHD to induce GVL he remained steadfast in his pursuit of cure with very little time spent as an inpatient and a willingness to try any treatment that may extend his life. What was remarkable about this case was the armamentarium of drugs and regimens that were used, pushed to the limit, and ultimately exhausted. Despite our questioning the limits of treatment and when is quality of life compromised, caring for this patient over a 4 year period was a unique lesson in family support, resilience, hope, and personal control.

No conflict of interest to disclose

A88



O050

0830

The Role of a Care Coordinator for Lymphoma Patients - Our Hospitals Initiative

Jacqueline Wykes

Hunter Haematology Unit, Calvary Mater Newcastle, NSW, Australia

Background

Lymphoma is the fifth fastest growing cancer in the western world and to better streamline and coordinate the influx of patients at our institution, Calvary Mater Newcastle (CMN), the Hunter Haematology Unit piloted and funded a nursing care coordinator titled, The Lymphoma/Myeloma Care Coordinator (LMCC).

Role/Aim

The LMCC position was designed to accommodate patients undergoing treatment regimes and involves; educating patients, carers and families regarding disease, treatment and particular regimes; scheduling appointments and investigations; collating results; documentation of treatment plans; monitoring recovery and/or complications; problem solving ; organising scripts, follow up blood tests and patient specific certificates as required; offering and referring to allied support services; acting as a resource person for staff and patients; attendance at the twice weekly multidisciplinary meeting and organising the presentation of patients at the Lymphoma Multidisciplinary Meeting.

Evaluation

A patient survey was attended twelve months post the introduction of the role and the results were positive, especially from patients who had relapsed and not been offered this service previously. The development of the position and follow up evaluation has lead to the development of new patient information packages; that encompassing written information on regimes, treatment schedules, neutropenic card, information on allied staff and support services, and information on the role of the Lymphoma Care Coordinator. To further consolidate the role and streamline patient care, patient and nurse education days in the past two years have been run to coincide with World Lymphoma Day. Also at the yearly mark the role was reviewed and with the number of patients involved, it was felt that Myeloma should be taken out of the role.

Conclusion

This pilot position was introduced in April 2007 to streamline and coordinate the care of patients undergoing treatment regimes in the outpatient setting. Since inception this pivotal clinical role has improved over 250 patient directed outpatient care at the CMN. This fluctuates from 2 to 4 newly diagnosed patients weekly, 5 - 20 patients on treatment, the ongoing organization of scans/investigations, and the numerous phone calls received and associated trouble shooting for outpatient lymphoma patients. The position profile and title has been modified to reflect the patient load with Myeloma having been taken out of the equation. All disciplines and patients, associated with the position have deemed the position invaluable in helping the patient journey; however the position is ongoing subject to funding.

No conflict of interest to declare

A89



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Day 19



Topic: Transplantation 2 GvHD/GvL

1030-1130
Hall C
1030

GVL - Non-myeloablative Transplants

Sergio Giralt

*Department of Stem Cell Transplantation and Cellular Therapies of the University of
Texas MD Anderson Cancer Center, USA*

Abstract not provided by speaker . please refer to abstracts of other talks on pages
A23 and A122

A90



The Control of Antigen Presentation by NKT Cell Activation After BMT

Geoff Hill

The Queensland Institute of Medical Research, Brisbane, QLD

Granulocyte-colony stimulating factor (G-CSF) is often used to hasten neutrophil recovery after allogeneic bone marrow transplantation (BMT) but the clinical and immunological consequences evoked remain unclear. We examined this in mouse models and found that administration of both standard G-CSF and pegylated-G-CSF early after BMT significantly increased graft-versus-host disease (GVHD). This effect was dependent on total body irradiation (TBI) rendering host dendritic cells (DC) responsive to G-CSF by up-regulating their expression of the G-CSF receptor. Stimulation of host DC by G-CSF subsequently unleashed a cascade of events characterized by donor natural killer T cell (NKT cell) activation, interferon- γ (IFN- γ) secretion and CD40-dependent amplification of donor cytotoxic T lymphocyte (CTL) function during the effector phase of GVHD. Critically, detrimental effects of G-CSF were only present when administered following TBI conditioning and at a time when residual host antigen presenting cells (APC) were still present, perhaps explaining the conflicting and somewhat controversial clinical studies from the large European and North American BMT registries. We have also investigated the effect of NKT cell activation following the administration α -GalCer molecules after BMT. The administration of KRN7000 induced hyper-acute GVHD and early mortality in all models tested and was found to result in down-stream IL-12 and DC-dependent NK and conventional T cell activation. Specific depletion of host DC, IL-12 or donor NK cells prevented this pathogenic response and the induction of hyper-acute GVHD. These data have major implications for the therapeutic use of molecules that may activate NKT cells and thus modulate antigen presentation during disease states.

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Kuns R, Morris ES, MacDonald KP, Markey KA, Morris H, Raffelt N, Banovic T, Olver S, Don A, Rowe V, Burman AC, Clouston AD, Farah C, Besra GS, Illarionov PA, Smyth MJ, Porcelli SA, Hill GR. Invariant NKT . NK cell interactions dictate transplant outcome following α -galactosylceramide administration. *Blood* 2009, 113;5999-6010



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Day 19



1030-1130
Hall B
1030

Blood Supply

Transfusion Transmitted Infection – An International Perspective

Peter Flanagan

New Zealand Blood Service, Auckland, New Zealand

The provision of sufficient safe blood products is an ongoing challenge for blood services internationally. Many developing countries continue to experience problems in the provision of adequate numbers of blood donations that are consistently tested for the major transfusion transmissible infections, HIV and Hepatitis B and C. The challenges faced by blood services in developed countries are very different indeed. Efforts continue to be devoted to further reducing the small residual risks for major blood borne viruses with tested blood and in addition a new range of emerging infections are being identified.

New threats to the safety of donated blood can arise in a number of ways. Firstly novel infectious agents can emerge. This include infections such as SARS and novel influenza infections. Novel infections can quickly establish themselves due to a combination of increased travel, migration and globalisation of commerce. Old foes can also re-emerge with climate change and the impact of developing technologies. Concerns relating to arthropod borne infections such as Dengue fever, malaria and Chikungunya infection.

New and changing threats can impact on blood service provision either because of concerns over possible transfusion transmission or by reducing the number of available donors. The recent emergence of novel influenza A H1N1 09 provides a good example of the latter concern.

Blood services can respond to new challenges in a number of ways. The introduction of new deferral criteria and introduction of selective or universal testing of donated blood. Pathogen reduction technologies might also play an important role in maintaining a safe blood supply. In all instances benefits must however be carefully balanced against the possible introduction of new risks.

No conflict of interest to disclose



Transfusion-transmitted Infection – An Australian Perspective: Are We At Risk?

Robert Flower, Helen Faddy, Catherine Hyland and Stuart Behncken
*Research & Business Development, Australian Red Cross Blood Service (ARCBS),
Brisbane, Queensland, Australia*

Any pathogen that can be transmitted to a recipient of donated blood can cause a transfusion-transmitted infection (TTI). Many TTIs have an asymptomatic blood-borne phase when there is a sufficient pathogen load to transmit the infection via transfusion. The risks associated with TTIs are traditionally managed using a combination of donor deferral and pathogen or antibody screening technologies . tools that have been used effectively to ensure a safe blood supply for Australia.

The global emergence/re-emergence of such disease agents as dengue virus, West Nile Virus, chikungunya virus, vCJD, Leshmaniasis and Chagas disease may require implementation of new management strategies to protect the Australian blood supply. Furthermore, new variants of TTIs, such as HIV and Hepatitis B virus may escape current mitigation strategies and deserve close monitoring.

The approach to management of these threats depends on a variety of factors including a risk/cost/benefit analysis. For example some arboviruses are only found in regions where the specific vector is located and introduction and establishment of these vectors may modify the risk pattern in Australia. Some viruses are transfusion transmitted, but do not cause disease (eg TT-circovirus and Borna Disease Virus). Where the disease is potentially serious, the lack of availability of screening tests may determine the approach. In the case of fresh products, the only management approach available for managing the risk related to dengue or vCJD has been donor deferral, which has had an impact on donor recruitment and collections. In the case of bacterial contamination, a combination of measures has recently been introduced that has already reduced the risk associated with this group of TTIs.

The ARCBS continually evaluates the risks to the blood supply and assesses new technologies and approaches that may be implemented to minimise these risks. These include new screening tests and pathogen reduction technologies.



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Day 19



1030-1130
Hall B
1110

Blood Supply

Management of Blood Shortage in a Tertiary Hospital Setting – A Regional Canadian Model

Meer-Taher Shabani-Rad, Doris Hawkins, Donna Thompson, Cathie Beal, Adnan Mansoor

Division of Hematology/Transfusion Medicine, Calgary Laboratory Services/University of Calgary, Calgary, AB, Canada

Background: The development of a contingency plans is critical to ensuring transfusion support during blood shortages. These plans provide outlines for the administration, patient risk assessments and different phases of blood shortage. However, not many practical modules present to address the daily issues of blood utilization and implementation of contingency plans. Therefore this regional module was designed to manage critical blood shortages.

Design: Annual blood utilization data for Calgary health region (2007-8) were analyzed and integrated into a module. The transfused patients/blood utilizers ranked based on clinical services. The impact of cancellation of elective surgeries during blood shortages was evaluated. The percentage of life-saving/massive transfusions relative to total blood utilization was determined. The Blood Utilization indices were defined and employed to create this module. The module was used to forecast the blood inventory based on supply/utilization and different phases of shortage (Green-Amber-Red).

Result: The top blood utilizers were Internal medicine (25%), orthopaedics(11%) and cardiac surgery (11%), oncology (10%) and critical care (8%). The elective general surgery patients had used 3% of the total red cells. Life-saving/massive transfusions (≥ 3 units/24hrs) were 30% of total red cells. Based on blood indices, regional inventory, daily usage and supply/utilization a module created which is able to predict the different phases of blood shortage.

Conclusion: The elective surgery only account for 3% of total blood utilization therefore cancellation of elective surgeries has no significant impact on saving blood during amber phase. Access to a module designed based on blood utilization/inventory indices will help to implement the recommendations of contingency plans in appropriate time and manner.

No conflict of interest to disclose



Recent Results From Trials of New Anticoagulants

John Eikelboom

McMaster University, Hamilton, Ontario, Canada

Venous thromboembolism (VTE) affects an estimated 17,000 Australians each year and is a major cause of morbidity and mortality. Rapidly-acting parenteral anticoagulants, such as heparin, are used for the prevention and initial treatment of VTE, whereas the slower-acting vitamin K antagonists (VKAs) are used for long-term therapy. The introduction of low-molecular-weight heparin (LMWH) and fondaparinux has simplified parenteral anticoagulant therapy and these agents have replaced heparin for many indications. Development of new oral anticoagulants to replace VKAs has been slower than that of parenteral agents. Ximelagatran, an oral thrombin inhibitor, was briefly licensed in Europe but was withdrawn in 2006 because of potential hepatic toxicity. Although this set the field back for several years, the situation has changed with the recent introduction of dabigatran etexilate, a new oral thrombin inhibitor, and rivaroxaban, a new oral factor Xa (fXa) inhibitor. Licensed in Europe and Canada for prevention of VTE in patients undergoing hip or knee arthroplasty, dabigatran etexilate and rivaroxaban streamline out-of-hospital thromboprophylaxis because the drugs can be given once-daily in fixed doses without coagulation monitoring. The greater unmet medical need, however, is to find a replacement for VKAs for long-term therapy. The results of long-term trials of dabigatran etexilate will be available first, while those with rivaroxaban, apixaban and edoxaban will soon follow. Despite the optimism, the transition from VKAs to new oral anticoagulants is likely to be gradual because VKAs are effective and entrenched in clinical practice and because the acquisition cost of the new agents is likely to be higher. New oral anticoagulants will be least affordable in developing countries where the need to replace VKAs is highest because of the growing burden of thromboembolic disease and the lack of resources and infrastructure to monitor VKAs.



Venous Thromboembolism Prophylaxis Guideline Implementation

Harry Gibbs

Princess Alexandra Hospital, Brisbane, QLD

Most cases of venous thromboembolism (VTE) are caused by hospitalisation. Prophylaxis reduces this risk of VTE by up to 90%. Prophylaxis levels however remain unacceptably low worldwide. Strategies to improve VTE prophylaxis are required.

Quality improvement strategies shown to improve VTE prophylaxis include active education, reminders and audit and feedback. At the Princess Alexandra Hospital, a full time VTE prevention Nurse Consultant position was created to oversee a guideline implementation program. At 12 months, the absolute improvement in appropriate prophylaxis was 31% with a further improvement of 17% in the subsequent 12 months. This approach has been studied in a multi-centre interventional trial.

Methods

A full time nurse practitioner implemented a six month VTE prophylaxis program. An audit of the VTE risk and prescribed prophylaxis was performed during the first month on at least 200 consecutive medical patients at each hospital. A prophylaxis program was implemented during the next four months and repeat audit performed during the final month. VTE risk was assessed using the ANZ Guidelines for the Prevention of VTE.

Results

8774 patients were audited in 15 hospitals in all Australian states. The majority of patients in both audits were at high VTE risk, requiring prophylaxis (82% at baseline, 81% post-intervention). At baseline, 38% of high risk patients were receiving appropriate prophylaxis and this improved to 54% post-intervention ($p < 0.001$). The improvement occurred regardless of baseline prophylaxis rates and was due to both an increase in the use of anticoagulants and in elastic stocking use in patients with a contra-indication to anticoagulants. There was no increase in inappropriate prophylaxis in low risk patients.

Conclusion

Implementation of a multifaceted VTE prophylaxis program by a dedicated nurse practitioner significantly improves VTE prophylaxis rates in high risk medical patients. This model of care should significantly improve patient safety.

This research was supported by Sanofi-Aventis. The company arranged statistical analysis but had no role in this abstract preparation.



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ABSTRACTS - Monday 19

1130-1230
Hall C

The Planned and the Unexpected

Luen Bik To
IMVS, SA

In the age of hypothesis-driven research, a fundable project is one that is well thought out, supported by preliminary data showing its feasibility and has a sound research plan. In life, plans do not always go smoothly. The unexpected often heralds new possibilities and innovations. In this Oration, key events that influenced the discovery of peripheral blood stem cell mobilisation and transplantation in Adelaide in the 80s and 90s will be narrated. Peripheral blood stem cell transplantation enables more diseases and older patients to be treated more safely and opens up the field of graft engineering and regenerative medicine. The discovery journey illustrates how serendipity and opportunities guide planning. Life post discovery brought new and unexpected challenges and anecdotes as a senior manager will also be shared.

A97



O051

1330

KIR Haplotype Influences Clinical Outcome Following HLA-Matched Sibling Haemopoietic Stem Cell Transplantation

Susan Heatley^{1,2}, Charles Mullighan³, Lucy Sullivan², Andrew Brooks² & Peter Bardy⁴

¹ *Australian Red Cross Blood Service, Adelaide, Australia*

² *University of Melbourne, Melbourne, Australia*

³ *St Jude Children's Research Hospital, Memphis, USA*

⁴ *Queen Elizabeth Hospital, Adelaide, Australia*

In haploidentical allogeneic haemopoietic stem cell transplants (HSCT), natural killer cell alloreactivity due to Killer cell Immunoglobulin-like Receptors (KIR) repertoire has been reported to be advantageous. Benefits include superior survival, reduced relapse and graft versus host disease particularly in patients transplanted for Acute Myeloid Leukaemia (AML). However data from HLA-matched sibling HSCT are inconsistent.

We examined the association between KIR haplotype, relapse, overall survival and acute graft versus host disease (aGvHD) in a cohort of 147 HLA matched siblings undergoing HSCT. KIR genotyping was by multiplex PCR-SSP and haplotypes were categorised as the A haplotype carrying the 2DL1, 2DL3, 3DL1 and 2DS4 genes, all other combinations were denoted the B haplotype. Combinations of KIR haplotype were assigned to each transplant according to the donor and then recipient haplotype ie AA-AA, AA-Bx, Bx-AA and Bx-Bx where Bx denotes either BB or BA.

In the entire cohort relapse, overall survival and aGvHD were not significantly associated with donor and recipient KIR haplotype. However when only AML patients (n=69) were considered AA donors-Bx recipients had superior survival rates and those with AA-AA the most inferior. Interestingly this was also true for grades II-IV aGvHD, with AA-Bx having no aGvHD and AA-AA the most (p=0.032). Furthermore, when stratified to include the patients receiving myeloablative conditioning, an AA donor-AA recipient had inferior overall survival (p=0.015) and the most severe aGvHD (p=0.054).

KIR haplotype influences the clinical outcome of HLA-matched sibling HSCT, particularly in patients diagnosed with Acute Myeloid Leukaemia.

No conflict of interest to declare

A98



O052

1345

Elasticity Preserves Primitive Murine and Human Haemopoietic Cells

Jeff Holst^{1,2}, Sarah Watson¹, Liang Ma³, Andres F. Oberhauser³, Anthony S. Weiss⁴ and John EJ Rasko^{1,2,5}

¹Gene & Stem Cell Therapy Program, Centenary Institute, Camperdown, NSW 2050 Australia; ²Faculty of Medicine, University of Sydney, Australia; ³Department of Neuroscience and Cell Biology, University of Texas Medical Branch, Galveston, Texas 77555, USA; ⁴School of Molecular and Microbial Biosciences G08, University of Sydney, Australia; and ⁵Cell and Molecular Therapies, Sydney Cancer Centre, Royal Prince Alfred Hospital, Camperdown, Australia.

Surprisingly little is known regarding the effects of the physical microenvironmental niche on haemopoietic stem cells. We have explored the effects of matrix elasticity on stem cell properties using a unique synthetic substrate, tropoelastin, which we used to show that both murine and human primitive haemopoietic cells are more efficiently maintained on an elastically extensible substrate than controls including fibronectin and collagen, during *ex vivo* culture. We have shown that culturing primitive murine haemopoietic cells on tropoelastin *in vitro* for up to 7 days led to ~3 fold increases in lineage Sca. 1⁺cKit⁺ cells. Furthermore, these cells demonstrated increased clonogenicity (~1.7 fold increase), increased proliferation (using CFSE labelling) and increased engraftment (~2.5 fold increase) following transplantation. Similar results were shown for human cord blood cells cultured on tropoelastin: there was an increase in number of lineage CD34⁺CD38⁺ progenitor cells (~1.7 fold increase), and increased clonogenicity (~2.2 fold increase). To confirm which properties of tropoelastin were required to maintain stem cells, we analysed truncated mutants of tropoelastin and crosslinked tropoelastin. These experiments confirmed the necessity to maintain elastic extensibility rather than effects related to the integrin binding domain present in its carboxy.terminus. Atomic force measurements of truncations and crosslinked tropoelastin revealed a threshold level of extensional elasticity (~125 nm) that is required for increased maintenance of progenitors. These data strengthen the hypothesis that stem cells sense the elasticity of their microenvironment and differentiate appropriately as recently shown for mesenchymal stem cells (Engler *et. al.*, *Cell*, 2006, 126:4, 677-689). We suggest that the stem cell niche is a tensegrity structure that provides the appropriate extensional elasticity for maintenance of HSCs. A consequence of this idea is that alterations to niche elasticity in disease states may contribute to abnormal haemopoiesis. Further, elastic substrates such as tropoelastin may offer a new approach to biomaterial design aimed to achieve optimal *ex vivo* culture conditions for primitive haemopoietic cells.

No conflict of interest to declare



O053

1400

CXCL12 Expression is Up-Regulated by Hypoxia in Multiple Myeloma Plasma Cells

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Aim

The CXCL12 chemokine is aberrantly over-expressed by MM PCs and circulating plasma levels correlate with BM angiogenesis in MM patients¹. Recent studies show that CXCL12 expression is regulated by hypoxia in several cell types, which is highly relevant to MM given the hypoxic nature of the BM microenvironment. The aim of this study was to examine whether hypoxia mediates aberrant CXCL12 expression by MM PCs, and the contribution of the HIF transcription factors to this response.

Methods

HIF-1 α , HIF-2 α and CXCL12 protein expression was examined in patient material using immunohistochemical staining. The hypoxic regulation of CXCL12 was assessed in MM cell lines using PCR and western immunoblotting. Transduced MM cell lines (in which HIF-1 α or HIF-2 α were stably over-expressed or silenced) were generated to examine the role of the HIFs in the regulation of CXCL12 in MM PCs. Luciferase, EMSA and ChIP assays were performed to determine whether HIF-2 binds directly to the CXCL12 promoter.

Results

HIF-1 α expression was detected in numerous cells throughout the BM, whereas HIF-2 α expression was restricted to macrophages and MM PCs. Strong co-localisation of HIF-2 α and CXCL12 expression was detected in MM PCs. Prolonged hypoxic culture significantly up-regulated CXCL12 expression in MM cells and in contrast to previously-published findings², this response was mediated predominantly by HIF-2. Using a murine xenograft model of MM, stably-transduced HIF over-expression MM cell lines stimulated a marked increase in MM-induced angiogenesis, and administration of the CXCL12/CXCR4 antagonist, T140, to the mice strongly decreased the level of HIF-induced angiogenesis.

Conclusion

CXCL12 expression is strongly up-regulated in response to prolonged hypoxia in MM PCs, and is an important contributor to MM-induced angiogenesis. We propose that targeting the hypoxic niche may represent a viable therapeutic strategy to inhibit MM angiogenesis and disease progression.

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No conflict of interest to disclose

A100



O054

1415

Prevalence of Melanoma and Non-melanoma Skin Cancer in Chronic Lymphocytic Leukaemia in Australia

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⁴ *Chronic Lymphocytic Leukaemia Australian Research Consortium (CLLARC).*

Patients with chronic lymphocytic leukaemia (CLL) are suspected to have an increased risk of both melanoma (MSC) and non-melanoma skin cancer (NMSC). Australia has one of the highest rates of skin cancer in the world. NMSC is by far the most common type of cancer in Australia. Although it is not recorded by most Australian cancer registries, it is estimated that approximately two-thirds of Australians will experience at least one NMSC during their lifetime before the age of 70 years. There have been no previous publications assessing the prevalence of either MSC or NMSC in CLL patients in Australia. We performed an audit of skin cancer prevalence (both MSC and NMSC) in two separate haematology practices in Northern Sydney and Central Coast Area Health Service, NSW, Australia over a six month period. One practice was a specialist haematology referral unit at a major metropolitan teaching hospital and the other a regional community based practice. Data from 178 CLL patients (121 regional centre, 58 metropolitan) was collected to determine the prevalence of both MSC and NMSC and any correlation with disease stage or prior therapy.

Of the 178 patients, there were 100 males and 78 females with median age 70.5 years (range 22 - 96 yrs). There were 19 patients with MSC, and 99 with NMSC. The 19 (10.7%) patients diagnosed with melanoma were 12 males and 4 females with median age 72.7 (range 47- 87). All but 2 melanoma patients also had NMSC. The prevalence of 10.7% melanoma in CLL patients is much higher than the Australian lifetime risk of developing melanoma (1 in 25 [4%] for men and 1 in 34 [3%] for women). Most patients with MSC had Binet stage A disease (14/16) and only 5/16 had received prior therapy for their CLL. 3 patients had more than one melanoma and 1 had metastatic disease. There was no significant difference between the metropolitan and regional practices with melanoma prevalence.

The prevalence of NMSC was 55.6% affecting 99 of the 178 patients. There were 56 males and 43 females with median age 74.5 years (range 47-96). An additional 9 patients had solar keratosis without NMSC. There was significantly higher prevalence from the regional centre (62.8%) compared with patients from the tertiary referral centre (36.7%). This appears to reflect both the older age and area demographics of the CLL patients managed at the regional centre. Higher sunlight exposure of patients from the regional centre is another probable factor. There was an overall trend of increased risk of NMSC with increasing age in keeping with other data suggesting that the association between CLL and NMSC becomes stronger as the population studied becomes older. There was no obvious association between NMSC and prior therapy for CLL with 65/99 patients with NMSC having had no prior therapy. In conclusion, skin cancer prevalence is high in Australian patients with CLL, especially melanoma which at 10.7% appears substantially higher than the risk for the general Australian population.

A101



O055

1430

Development of rsolCD39-PSGL as a Novel Drug with Anti-thrombotic and Anti-inflammatory Effects

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Aim

To develop a novel antithrombotic that targets endothelium and can also suppress inflammation.

Method

There is a positive feed-back loop between coagulation and inflammation and purinergic nucleotides mediate both processes. ATP stimulates inflammation and ADP is a critical activator of platelets. CD39 (NTPDase 1) is expressed on endothelial cells and leukocytes and hydrolyses ATP and ADP to AMP, which is then converted to adenosine by the ecto-5q nucleotidase (CD73). Removal of ADP abrogates platelet aggregation and adenosine demonstrates anti-inflammatory, vasodilatory and platelet-inhibitory activities. We have previously confirmed the antithrombotic potential of CD39 in transgenic mice (JCI, 113:1440-6).

We have designed a novel therapeutic (rsolCD39-PSGL) containing the extracellular ADPase motifs of CD39 coupled with a 20 amino acid tag based on the minimal peptide sequence of P-selectin glycoprotein ligand-1 (PSGL-1) necessary to recognise P-selectin. This tag targets rsolCD39-PSGL to P-selectin expressed on activated endothelial cells and platelets. rsolCD39-PSGL was expressed in CHO cell line with stable expression of the glycosyltransferases C2GnT and FucT-VII.

Results

rsolCD39-PSGL is produced as a non-covalent dimer. ADPase activity (quantified by free phosphate release) of rsolCD39-PSGL was 2.3 mmoles/ug/min compared to apyrase (6.49 mmoles/ug/min). ADPase activity (>90%) was retained after incubating up to 24h at 37°C. rsolCD39-PSGL specifically bound P-selectin on thrombin-activated (but not quiescent) platelets with no binding on thrombin-activated P-selectin-null platelets. rsolCD39-PSGL recognised both recombinant mouse and human P-selectin by ELISA. Dose-dependent prolongation of tail bleeding time was observed following iv/ip administration with in vivo activity up to 24h. Preliminary testing (rsolCD39-PSGL 0.2ug/g, dose that did not prolong template bleeding time) demonstrated significant protection against renal ischaemia reperfusion injury (p<0.001).

Conclusion

In summary, rsolCD39-PSGL is an innovative bifunctional drug with antithrombotic and anti-inflammatory effects and can be targeted to the endothelium.

Disclosure of interest: Grant Support: NIH and NHMRC

A102



O056

1445

Soluble Glycoprotein VI (GPVI) in Human Plasma: Shedding of GPVI from Platelets Induced by Coagulation

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Aim

The aim of this study is to evaluate the effect of coagulation on the surface expression of the platelet collagen receptor, glycoprotein VI (GPVI). We have previously shown that collagen, collagen-related peptide (CRP), snake toxins or anti-GPVI antibodies induce metalloproteinase-mediated ectodomain shedding of GPVI, generating an ~55-kDa soluble fragment (sGPVI), and a ~10-kDa remnant fragment that remains platelet-associated.

Methods

We used a newly-developed enzyme-linked immunosorbent assay (ELISA) to measure sGPVI levels in human plasma from healthy individuals, normal platelet-rich plasma where coagulation was experimentally induced, and plasma from patients with disseminated intravascular coagulation (DIC).

Results

Initial studies showed that plasma sGPVI levels in 192 healthy individuals were 19.5 ± 15.4 (2xs.d.) ng/mL, and levels were independent of age, gender or common GPVI polymorphisms (associated with Gln317/Leu substitution). However, sGPVI levels were markedly elevated following coagulation: First, while plasma sGPVI was independent of the anticoagulant used for blood collection (acetate-citrate-dextrose (ACD), citrate, or EDTA), collecting blood into a silica-coated coagulation tube and analysing serum from clotted blood showed markedly elevated sGPVI levels (124 ng/mL *cf.* 29 ng/mL in ACD-anticoagulated plasma). Second, inducing coagulation in normal citrated platelet-rich plasma by recalcification with or without added tissue factor resulted in increased sGPVI. This increase in sGPVI followed initial thrombin generation and peaked after 30 minutes at ~7-10. fold baseline levels. Shedding was strongly inhibited by hirudin (thrombin inhibitor) or GM6001 (broad spectrum metalloproteinase inhibitor), suggesting activated thrombin induced metalloproteinase-mediated GPVI shedding from platelets. Third, initial analysis of patients with DIC showed elevated plasma sGPVI consistent with increased GPVI shedding *in vivo* associated with coagulopathy.

Conclusions

These findings reveal that coagulation results in ectodomain shedding of platelet GPVI, and significant elevation of sGPVI levels in plasma. GPVI depletion limiting adhesion-dependent platelet activation may compensate for increased procoagulant activity in disease states.

No conflicts of interest to disclose

A103



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Day 19

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Hall B
1330

Haemolytic Anaemia – Is There A Requirement for Urgent Transfusion?

Paul M Ness

Transfusion Medicine Division, Johns Hopkins Medical Institutions, Baltimore Maryland USA

One of the most vexing problems in AIHA is handling the acute situation where all blood is incompatible and the patient has severe, worsening anaemia. Since the panagglutinin in the patient's serum typically reacts with all donor red cells, crossmatching donor blood is a difficult and time consuming process and probably of little benefit. The most pressing problem in a patient with previous pregnancies or transfusions is detecting alloantibody which may be hidden by the autoantibody. Sophisticated immunohaematology laboratories can use a combination of procedures including differential adsorption and warm autoadsorption to identify underlying alloantibodies. Another issue given much discussion among immunohaematologists is transfusion to patients whose autoantibody demonstrates relative specificity. When compatible blood cannot be found, many clinicians request "least incompatible" blood with the hopes that additional safety will be provided. Another approach to add transfusion safety is prophylactic antigen matching.

On occasion, a transfusion may be required before the serologic evaluation is completed. In these cases, serologically incompatible blood is safe for transfusion, with expected in vivo survival comparable to the patient's own red cells. Reluctance to transfuse these patients due to serologic incompatibility or an incomplete workup can be devastating. Patients with severe anaemia may appear to be haemodynamically stable, but these patients have life-threatening anaemia and should be transfused immediately regardless of the serologic evaluation or compatibility. The onset of confusion in a patient with worsening anaemia is a particularly important clinical indication that transfusion is required.

It is difficult to generalize about the appropriate transfusion trigger for a patient with severe AIHA. Factors such as the rate of onset, presence or absence of accompanying hypovolaemia, and, most importantly, the underlying health status and cardiorespiratory reserve must be taken into account. If the cardiovascular system is healthy and there is no significant degree of hypoperfusion, good tissue oxygenation can be maintained at subnormal Hb levels. In a young otherwise healthy adult or child, a haemoglobin level of 4 g/ dL can be tolerated if it has developed slowly.; in adults greater than 50 years, the presence of underlying cardiovascular pathology should be expected and a haemoglobin of 6 g/dL should be maintained. The evidence for these targets comes from animal data and cases of severe anaemia in patients who refuse transfusions.

A104



New Developments in Immunohaematology

Martin L Olsson

*Dept. of Clinical Immunology and Transfusion Medicine, University and Regional
Laboratories, Skåne, Sweden*

Dept. of Laboratory Medicine, Lund University, Lund, Sweden

The International Society of Blood Transfusion acknowledges more than 300 blood group antigens, which are polymorphic markers on the red blood cell (RBC) surface. The majority of these are classified into one of 30 blood group systems while some still belong to collections or the high- and low-frequency antigen series. Thanks to rapid developments in molecular and cell biology, the genetic basis for all but one of the systems has already been established. This mass of knowledge has allowed new directions in immunohaematology including genomic blood group typing and recombinant production of antigens for neutralization tests, for modification of test RBCs or as potential tools in screening assays for antibodies against RBCs. In this context, any blood group antigen the molecular genetic basis of which is not known constitutes a problem and a challenge. If the molecule that carries the blood group antigen in question is unknown, then the corresponding gene cannot easily be identified and the underlying polymorphism not utilised in genotyping assays. Therefore, a current development in immunohaematology is to look for emerging new antigens or go back and revisit the orphan blood groups, i.e. antigens without a family (blood group system) or a home (genetic locus). Several clinically important high-frequency RBC antigens like Vel, Lan and At^a are awaiting assignment to either existing or, more likely, new systems. We recently discovered the genetic basis of the only blood group system (P) for which no locus was known. In more than 200 samples we have shown full concordance between the P1 (presence of P1 antigen)/P2 (absence of P1) phenotypes and a single nucleotide polymorphism that opens a potential open reading frame in a newly discovered exon of the *A4GALT* gene. This locus is already known to encode an enzyme that synthesizes the P^k antigen. This finding will allow for genetic typing of the P1 antigen which is also important as a receptor for pathogens like *E.coli*.



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Day 19



1330-1500
Hall A
1330

Fungal Infections

Narin Bak

Abstract not received at time of going to print

A106



Management of Steroid Induced Hyperglycaemia

Lyn Green¹, Mitra Guha¹, Michelle Hargreaves¹, Allan Hayward^{1,2}

¹CNAHS, Royal Adelaide Hospital, Adelaide, South Australia.

²SA Pathology, Adelaide, South Australia.

A number of drugs can potentially induce or worsen hyperglycaemia, with corticosteroids (steroids) having the most potent impact. In previously normoglycaemic people, steroids can induce impaired glucose tolerance or diabetes, whilst the blood glucose control of people with diabetes usually becomes problematic and requires attention. Exogenous steroids induce insulin resistance and promote gluconeogenesis.

Patients with cancer often receive exogenous steroids as a component of their chemotherapy. Caring for people with cancer who either have existing diabetes or who develop diabetes as part of their treatment for cancer is a challenge for the person, their carer(s) and clinicians alike. Having either cancer or diabetes can be overwhelming enough. With the potential of combining the two, it is imperative that there is a pathway for detection (in the person with previously normoglycaemia) and management of hyperglycaemia (in both people with known diabetes and steroid-induced diabetes) to ensure optimal patient care.



Current Treatment Strategies in Mantle Cell Lymphoma

Martin Dreyling for the European MCL Network

Dept. of Medicine III, University Hospital Großhadern/ LMU Munich, Germany

Mantle cell lymphoma (MCL) shows an aggressive clinical course with a continuous relapse pattern and a median survival of only 3-4 years. However, recent reports have described an improved outcome and even an indolent course in about 10% of cases. The MIPI allows a risk estimation of the individual patients.

Induction

Rituximab monotherapy has only moderate activity in MCL. However, in three randomized studies, the addition of Rituximab to conventional chemotherapy has significantly improved response rates. CHOP-like regimens achieve a slight advantage in comparison to non-anthracycline containing combination. Purine analogs like Fludarabine, achieved high CR and overall response rates especially in combination with alkylators (e.g. cyclophosphamide +/- mitoxantrone). Especially in medical non-fit patients, Bendamustine might be an alternative approach. In younger patients, another promising approach is a dose-intensified regimen including high dose ara-C (HyperCVAD) which achieved remarkable survival rates. In a phase II study of a sequential CHOP/DHAP regimen, event-free and overall survival were 63% and 81%, respectively, at 4 years with a suggested long term survival plateau. Recently, molecular targeted approaches including proteasome inhibitors, IMiDs and mTOR inhibitors have achieved remarkable response rates and will be integrated in future study concepts.

Consolidation

Median duration of remission after conventional chemotherapy alone is rather short. Interferon- α maintenance results in a slightly improved progression-free survival (PFS), but its use hampered by observed side effects. In a randomized study of the *European MCL Network*, myeloablative consolidation with subsequent autologous stem cell transplantation (ASCT) resulted in a significantly improved PFS (34% vs. 11% after 5 years; $p < 0.001$) and a trend towards improved overall survival (5y OS: 65% vs. 54%). Based on these results, combined immunochemotherapy followed by ASCT represents the standard approach in patients < 65 years.

Despite these significant advantages, continuous relapses have been observed. Radioimmunotherapy consolidation and allogeneic transplantation with dose-reduced conditioning represent promising therapeutic options in relapsed disease.



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ABSTRACTS - Monday 19

1530-1630
Hall B
1530

Perioperative Blood Management

Bernd Froessler

Lyell McEwin Hospital, Adelaide, South Australia, Australia

Minimizing allogenic blood transfusion is important. Despite this widely accepted view among experts, existing strong recommendations of transfusion triggers and suggested clinical indicators, many patients continue to receive transfusions inappropriately. Blood management programs need to be established and funded at each hospital in order to provide best clinical practice.

The presentation will outline the requirements for a comprehensive blood management program, illustrate the successful management of patients and reveal common limitations.

A109



Iron Deficiency Anaemia (IDA): Pre-operative Assessment

Kathryn Robinson^{1,2,3}

1. BloodSafe Program, Adelaide, South Australia (SA)

2. Australian Red Cross Blood Service, Adelaide, SA

3. Queen Elizabeth Hospital, Adelaide, SA

There is a high prevalence of anaemia in pre-operative patients, with SA studies finding around 30% of colorectal cancer patients and 15% of joint replacement patients anaemic prior to surgery. This is not surprising given the reported incidence of anaemia in the older population of 10%, with 1/4 due to iron deficiency.

Numerous studies have documented pre-operative anaemia as a transfusion risk, but addressing it in practice is difficult. Whenever possible patients should be assessed early enough to allow for proper investigation and treatment prior to the scheduled procedure. It is important to recognise that IDA may be due to serious gastrointestinal pathology such as cancer.

Barriers to improving the identification and management of pre-operative anaemia include:

- Lack of awareness of the importance of pre-operative anaemia and transfusion avoidance
- Mildly abnormal results not seen as important
- Ill defined responsibility for follow-up and management of abnormal results
- Difficulty in interpreting the cause of anaemia and therefore management
- Lack of a clinical pathway/algorithm for identification and management of pre-operative anaemia
- Limited timely access to specialty clinics/advice (eg haematology, gastroenterology)
- Limited resources/capacity in the system to deal with anaemia
- Limited systems to partner and exchange information with primary care
- Difficulty providing a coordinated pathway for a patient's peri-operative journey

Pilot strategies to improve practice in SA are underway and include engagement with local champions, formation of multidisciplinary improvement teams, strategies to raise the profile of the problem, patient information resources, checklists and patient hand held records to support the peri-operative process.

No conflict of interest to disclose



The Hidden Costs of Red Cell Transfusion

Erica Wood^{1,2}, Linley Bielby^{1,2}, Russell Hunt³, Axel Hofmann⁴, David Westerman², David Roxby³

Australian Red Cross Blood Service, Melbourne; Peter MacCallum Cancer Centre, Melbourne; Flinders Medical Centre, Adelaide; all in Australia; Medical Society for Blood Management, Laxenburg, Austria

Major investments have been made in the safety of the Australian blood supply including for blood components and fractionated plasma products. Recently, more attention has focused on improving the safety of clinical transfusion practice. Concurrently, health costs are escalating, driven by changes in demographics, community expectations, clinical practice and technology. Costs of transfusion are generally not well documented, and we have had limited understanding of the true complexity, risks and costs. Australian data are lacking in this area.

The cost of transfusion study is a collaboration designed to capture the hospital costs of a red cell transfusion. Using data from January-December 2006 from Flinders Medical Centre and Peter MacCallum Cancer Centre, detailed clinical and laboratory process maps were constructed and validated for every step required to transfuse a single unit of red cells at each hospital site. De-identified aggregate transfusion episode data were extracted from clinical and laboratory databases. 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, assigned for each process step, and incorporated into costing software modules.

The complexities of the activities demonstrate the importance of understanding all the processes, in order to create a safe and workable system. This includes a trained multidisciplinary team, with appropriate equipment and other resources, to manage every stage of the process. Better information regarding costs and complexities will inform assessments of transfusion practice, and alternatives to transfusion, including directing where we should invest efforts to improve the transfusion process and minimise risks and costs.

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



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Day 19



**1530-1630
Hall D
1530**

Treating Patients with Inhibitors – Which Agent and How to Monitor

Nigel Key

University of North Carolina, Chapel Hill, NC, USA

The cumulative incidence of inhibitors to factor VIII (FVIII) or FIX approaches 35% in severe hemophilia A and 3-5% in hemophilia B, respectively. In 2009, the choice of hemostatic therapy for management of bleeding in these patients is limited to high dose FVIII for those with low titer inhibitors (<5 BU), or one of the bypassing agents. The latter are used in those with high titer inhibitors or with low titer inhibitors when it is judged inadvisable to risk an anamnestic or allergic response to FVIII/FIX. The only head-to-head trial of the currently available bypassing agents (FEIBA[®] and recombinant FVIIa) – the FENOC trial – demonstrated an efficacy of 80±2% at 6 hours post treatment for 1 dose of FEIBA or 2 doses of FVIIa. An important conclusion of this trial was that a proportion of patients with inhibitors respond discordantly to these therapies when used at standard doses. To complicate matters, responsiveness to a given agent may vary between bleeds at different sites for a given patient. These observations beg the question as to the physiologic reasons for these discrepancies, and some candidate explanations will be reviewed. However, these clinical inconsistencies, together with the desire to correlate clinical efficacy and safety with a measurable laboratory endpoint (such as thrombin burst or clot strength) have spurred a literature focused on this question. Global assays of hemostasis have received the most attention, particularly thrombin generation tests (TGT) and thromboelastography (TEG). However, the best approach has yet to be determined. Arguably, the question of the best laboratory measure of efficacy (surrogate endpoint(s)) cannot be resolved until there is consensus as to the clinical endpoints that truly correlate with hemostasis.



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ABSTRACTS - Monday 19

1530-1630

Hall D

1530

Treating Patients with Inhibitors – Which Agent and How to Monitor

Nigel Key

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A113



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Day 19



1530-1630
Hall D
1610

Inhibitors in Haemophilia – The Australian Perspective

Chris Barnes

Abstract not received at time of going to print

A114



O057

Moving on with Myeloma – A Novel Approach to Patient Support

Jacqui Jagger, Lesley Richardson
Cancer Care Centre, Gosford Hospital, NSW, Australia

Background

The Central Coast of NSW has a higher than average incidence of Myeloma due to its ageing population. Nurse Coordinators found that the patient/carer requirement for ongoing myeloma specific information and support was much greater in comparison to other groups with a haematological malignancy.

Aim

To develop a local support and information programme for patients and carers living with myeloma.

Method

In March 2007 a Needs Analysis survey was sent out to the myeloma patient population on the Central Coast (N=66). The survey was designed to elicit level of interest in a support group, meeting frequency, venue and what people want from a support group. 33% of surveys were returned (N=22). 100% of respondents were interested in attending a myeloma specific support group.

The first meeting of the Central Coast Myeloma Support Group (CCMSG) was held in August 2007. The Nurse Coordinators facilitated the discussion on setting groups purpose, name, group agreement, committee roles and the model of the group was agreed.

Results

A member satisfaction survey was completed after 12 months, the results confirmed a high level of satisfaction with the group. CCMSG actively participate in myeloma initiatives, current projects include a ~~F~~aces of Myeloma DVD and development of education strategies with our GP collaborative.

Conclusion

The needs of the myeloma population are unique due to the incurable nature of the disease and the longevity of the treatment trajectory. CCMSG was set up in response to direct patient/carer request and needs analysis. There is a high level of satisfaction from the members who feel supported and have been empowered to become actively involved in myeloma at a local and statewide level.

No conflict of interest to disclose



O058

Quality and Equity in Myeloma Nursing Care: Development of Evidence Based Nursing Guidelines for the Management of Myeloma

Tracy King¹ Monica Morris² & Myeloma UK Nurse Guidelines Group - Marvelle Brown³, Sophie Corderoy⁴, Sharon Dines⁵, Shirley Crofts⁶, Flora Dangwa⁷, Jeff Horn⁸, Mary Kelly⁹, Lisa Nicholls¹⁰

¹ Royal Prince Alfred Hospital Sydney/Myeloma Foundation of Australia ² St George's Hospital London, UK. ³ Thames Valley University London, UK. ⁴ King's College Hospital London, UK ⁵ Royal Marsden Hospital London, UK ⁶ Royal South Hants Hospital Southampton, UK ⁷ Royal Free Hospital London, UK. ⁸ Aberdeen Royal Infirmary Aberdeen, Scotland. ⁹ Tullamore General Hospital Tullamore, Ireland. ¹⁰ University College London Hospital, UK

Aims

The past decade has seen greater awareness of heterogeneity in myeloma and improved therapeutic outcomes. Nurses identify supportive care needs and manage toxicities of treatment often in the outpatient setting. Myeloma UK recognises the vital role of nurses and in response to an identified need, led a project to develop Nursing Guidelines.

Objectives of the project included:

- Evidence-based, nursing guidelines that complement existing medical guidelines
- Achieve a national standard of care for myeloma nursing
- Emphasize the importance of the nurse specialist in the management of myeloma and provide guidance for more junior nurses

Methods:

An expert group of myeloma nurse specialists was formed. Existing guidelines and consensus statements were reviewed and gaps identified. Principal sections were identified to include management of treatment toxicities, disease complications and psychosocial support.

Content was sourced via:

- Comprehensive literature reviews
- National policy guidelines
- Expert, consensus opinion

The group met quarterly over 1 year with regular internal review and collaborations.

Results:

It became clear that 2 separate documents would provide the most useful resource.

1. Myeloma Nursing Guidelines . key points, assessment and graded recommendations.
2. Myeloma Nursing Reference Guide . background information, policies and appendices

Accreditation of the guidelines is being sought from the Royal College of Nursing. Dissemination of the guidelines is via an existing Myeloma UK nurse program, journal publications and conference presentations. The breadth of knowledge fed into the project highlighted the benefits of Intentional nurse working groups.

Conclusions

Dissemination of specialist knowledge offers nurses the knowledge and evidence base to provide a recognised standard of care.

No conflict of interest to disclose

A116



O059

***Habitus*; the Experiences of People Living with Myeloma**

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Introduction

This is a 3-year longitudinal, Cancer-Council-funded study of the experience of living with myeloma. The main aims of treatment are to control disease, secure remission and maximise quality-of-life. The median duration of survival has doubled to 5 years over the last 10 and many patients now survive 10-15 years. Despite this, most patients die from their disease despite onerous and complex regimens involving multi-disciplinary support and treatment from a number of different modalities. Living with, and dying from, myeloma is both resource intensive and often a painful and difficult journey, there is little research examining the experiences of people with myeloma.

Methods

Ten patients, with myeloma, attending one of three hospitals, were recruited together with a carer. Participants were interviewed 3 times over 14 months. 60 interviews were generated. These data were analysed using the constant comparative method of Grounded Theory. Interviews were digitally recorded and transcribed verbatim. Data were managed using NVIVO software.

Results

Early data from this study can help to build a rich description of the myeloma experience. Concepts and understandings of *habitus*, work and time have emerged as important.

Conclusion

Increased duration of survival has implications for service delivery and future research into MM. Understanding how people manage the experience of living longer with MM can help tailor services to patients' needs. The concept of *habitus* will be used as a framework to present the complexity of experience for people living with myeloma.

This research was supported by The Cancer Council NSW. The company had no role in analysing the data or preparing the abstract.



O060

Telephone Triage – Implementation and Evaluation of a New System

Kirstin Bubner and Melissa Ellard

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Aim

Telephone triage is gaining in acceptance as a cost effective measure to reduce the demands on the current health care system. With ongoing research enabling many cancer treatments to be moved to an outpatient area, the ability to provide support to patients, their carers and other health professionals after hours is vital.

The pressure on staff working on inpatient wards to field the after hours calls and deal with the complex nature of cancer patients, their diseases and treatments requires adequate education, training and systems.

The development of the RAH Telephone triage form ensures each call is documented, an outcome is recorded and the form is filed in patients case notes where applicable. The form being in duplicate enables nursing staff to audit and evaluate each call. A flow chart was also introduced as a learning tool and to ensure staff have a protocol to follow when managing these calls.

Method

The new triage forms were introduced into practice in October 2008. Ongoing education sessions and feedback are provided to staff to ensure compliance with the new system

A Flow chart advising staff of how to manage calls after hours was put in place

Results

A six month review of the new forms was performed between October 2008 and March 2009 showing an increase of 30% in the total number of calls in comparison with the same period October 2007 to March 2009.

Conclusion

The new telephone triage system has

- provided a more streamlined approach to after hours calls with the redirection to the appropriate area
- provided a focus on gaps in education to patients and staff
- provided a safe and legal environment for nursing staff to provide advice
- improved patient outcomes as each call is followed up on and reviewed by senior nursing staff +/- medical staff
- provided evidence on the amount of time nursing staff are spending on calls
- provided the ability to share ideas with other Haem/Onc units in Australia

No conflict of interest to disclose

A118



O061

Human Immunodeficiency Virus (HIV) Lymphoma: An Overview of Disease and Treatment Regimens, a Review of the Literature

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Background Information

Research globally has revealed that the incidence of Lymphoma (Hodgkin's and Non-Hodgkin's), in HIV infected patients is significantly greater than in the non-infected population and its treatment presents a therapeutic challenge. Although, sufferers' overall survival has dramatically improved with the introduction of Highly Active Antiretroviral Therapy (HAART), their prognosis is poorer than similar lymphomas in HIV-negative patients.

Aim

To discuss why there is an increased risk of HIV patients developing lymphoma, the role of HAART in HIV lymphoma treatment and what treatment regimens are used for these patients and why.

Method

Searches of Databases .

- Medline (1980-July 2009)
- CINAHL (1980-July 2009)
- The Cochrane Library, 2009, Issue 2 (The Cochrane Database of Systematic Reviews and The Cochrane Central Registry of Controlled Trials)

MeSH terms used: HIV, Hodgkins and Non-Hodgkins Lymphoma, HAART and Acquired Immune Deficiency Syndrome (AIDS).

Reviews were restricted to randomized control trials, English language and human subjects.

Results

Various research studies have shown that a CD⁴ count of <200 significantly increases the risk of lymphoma occurring. HAART is used to raise CD⁴ counts and therefore improve immune function. The anti-lymphoma effect of HAART is associated with an improvement in the degree of host immunodeficiency. There is conflicting data on the use of HAART when giving HIV patients chemotherapy, and also on which treatment regimen should be given. DA-EPOCH, an infusional chemotherapy regimen, has recently proven to be well tolerated and effective, indicating that greater tumour cell death occurs when cells have prolonged low concentration exposure, in comparison to brief high concentration exposure, eg. when administering CHOP. Recent research has also highlighted whether the use of Rituximab would be effective in the HIV population. Stem-cell transplantation in the relapsed population is also currently being debated.

Conclusion

The optimal treatment therapy for HIV lymphoma patients is currently still being debated and researched, and therefore cannot yet be defined.

No conflict of interest to disclose

A119



O062

Catheter Related Infections and Balancing Reality with Best Practice

Tracy Clarke

Prince of Wales Hospital, Randwick, NSW

Aim

Annual review of the Central Venous Access Device (CVAD) database is undertaken in January each year to monitor associated complication rates. A review undertaken in 2009 showed a significant increase in infection rates from previous years that identified a need to review practices within the unit with the aim of reducing catheter related infections.

Method

A comparison of the literature and current practices was undertaken revealing several discrepancies. Practices within the unit are influenced by a combination of organisational difficulties, a need to meet patient comfort and psychosocial issues. Practice changes in relation to published guidelines were discussed with the multidisciplinary team. These included closer examination of device selection for individual patients including the number of lumens required, changes to routine care such as the solutions used for skin preparation, accessing lumens, and limiting the disconnection of patients from IV lines.

All CVADs inserted are entered into a database identifying device type, insertion location, proceduralist designation, dwell time and complication details. 88 devices inserted during 2008 are compared to 20 devices inserted over 4 months following implementation of changes.

Result

2 complications of note were the frequency of catheter dislodgements and catheter related infections defined as either a catheter infection with identified organism or suspected catheter infections resulting in catheter removal. There were no significant differences between device type, insertion location or proceduralist designation and dwell time to infection ranged from 2 days to greater than 1 year. Coagulase negative staphylococcus was identified as the organism in 12 (56%) of the devices which was considered significant. Results indicate a reduction in confirmed catheter related infections from 25% to 10%. No catheter dislodgements have occurred.

Conclusion

CVAD complications can be reduced through vigilance in care. Careful monitoring of devices and adherence to protocol is essential in maintaining good patient outcomes.

No conflict of interest to disclose

A120



Which AML Patients to Transplant in CR1?

Alan Burnett

School of Medicine Cardiff University, UK

Several collaborative group studies have attempted to define which patients benefit from a standard allograft (SCT) in first complete remission, without any consensus being reached, so the role of SCT in CR1 remains a matter for debate. A number of issues feed this debate. It is important that the outcome be measured in terms of overall survival, rather than disease free or relapse free survival. There is no doubt that for most patients allocated to SCT the risk of relapse is reduced irrespective of risk, but this comes at a price of excess morbidity and mortality. There is also the option of delaying the transplant until second remission.

The heterogeneity of AML means that the relapse risk varies considerably between patients, and is conventionally defined by cytogenetics into good, poor and intermediate risk groups comprising 15, 15-20 and 60% of patients. There are some differences between collaborative groups about what abnormalities fit into which category, and it is only recently that large enough numbers of rarer abnormalities have been accumulated that they can be more accurately be allocated. We have become concerned about the insensitivity of cytogenetics alone to segregate patients, and have developed a multi-parameter risk score that is validated in patients who enter remission.

Finally, the question arises as to the best methods of comparing the impact of SCT on survival with a view to selecting patients for SCT in CR1. Registry studies examine the outcome of transplants given, which has obvious limitations in relation to comparative outcomes of chemotherapy alone, due to the fact that those who receive the SCT have survived without relapse and are otherwise fit for the procedure. The traditional method is of donor vs no donor. This also has problems in that in some studies there are significant numbers of patients who do not receive the transplant, but also care must be taken in understanding the analysis and the particular question being asked. For example if the question is who should receive SCT in CR1 some of these analyses are flawed because the starting patient group are patients with donors. In the MRC AML12 trial for example several of these patients with donors did not receive the SCT until CR2 and indeed there was a growing trend for patients without sibling donors to receive an allograft from an unrelated donor. A third approach is the use of a Mantel-Byer analytical approach. This also has imperfections but works on the basis that the transplant curve starts when a transplant takes place, i.e. patients remain on the chemotherapy curve until SCT at which point they are assessed on the SCT curve. Time adjustments are made to modulate the bad actors on the chemotherapy curve. Under these circumstances the impact of SCT whether sibling or MUD, whether standard or reduced intensity, can be evaluated, capturing only patients transplanted in CR1. When this was done in patients in the MRC AML12 and 15 Trials there was no significant survival benefit overall for SCT in CR1 using the standard risk definitions. However using the multi-parameter risk score which has the effect of moving about 20% of the cytogenetically defined the intermediate risk patients into the poor risk group there is a significant survival benefit for this new poor risk patient group. This is the first demonstration in MRC data of a survival benefit, so we believe that the new risk score can define about 30% of patients who will benefit from SCT using a standard allograft in CR1, but even this needs to be prospectively validated. In the AML15 trial the survival for patients receiving Mylotarg who are standard risk is 64% at 5 years further confirming the fact that not all standard risk patients are likely to benefit from SCT. Similar prospective evaluations of reduced intensity allograft are less frequent. Preliminary analysis of the AML15 trial experience suggest a trend for benefit in patients over 45 years with standard risk but no benefit for patients with high risk disease.

This subject will continue to be debated because of the impact of molecular parameters on prognosis, but at the moment there is not enough data to be confident that mutation data should dictate the decision to transplant or not.

A121



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Day 19



1730-1830
Meetings Room 3

Choosing the Optimal Conditioning Regimen for Each Patient - Are We There Yet?

Sergio Giralt

Department of Stem Cell Transplantation and Cellular Therapies of the University of Texas MD Anderson Cancer Center, USA

Patients undergoing an allogeneic hemopoietic stem cell transplant (HSCT) are prepared with chemotherapy and/or radiotherapy to reduce tumor burden and to facilitate engraftment of donor hematopoietic cells. Traditionally major modifications in the conditioning regimen were aimed at improving disease control by increasing the dose intensity of the regimen. Increases in dose intensity did not result in major improvements in survival due to increases in non-relapse mortality. In an effort to expand access to this procedure to older and debilitated patients many investigators have explored reduced doses of radiation or alkylating agents. These regimens have been variable named non-myeloablative or reduced intensity regimens, based on the observation that many of these regimens have been given without stem cell support and the doses of agents administered is substantially less than what has been given for a traditional conditioning regimen. The advent of intravenous busulfan has also allowed for the development of a relatively non-toxic ablative regimen with fludarabine busulfan. Thus in the current era transplant physicians need to tailor the best possible conditioning regimen to the patient based on the patients diagnosis, performance status, comorbidities, and preferences. In the future the presence of specific gene polymorphisms may allow transplant physicians to make even more personalized choices regarding the optimal conditioning for each patient.



Mutation Testing and Quantitative BCR-ABL in Chronic Myeloid Leukaemia

Susan Branford

*Division of Molecular Pathology, Institute of Medical and Veterinary Science,
Department of Medicine, Adelaide University, Adelaide, South Australia, Australia*

Imatinib is now considered the most appropriate treatment for patients with chronic myeloid leukaemia (CML). However, the optimal management of CML relies on accurate long term monitoring of treatment response because not all patients are responsive to imatinib, and some patients lose response. Given the recent development of more potent BCR-ABL kinase inhibitors, which may be active in imatinib-resistant patients, early detection of suboptimal response or relapse on imatinib therapy assumes increasing importance. For these patients serial analysis of BCR-ABL peripheral blood transcript levels by sensitive real-time quantitative PCR techniques provide the most accurate and clinically relevant monitoring strategy. Over the next few years, it is anticipated that standardised molecular techniques will be widely adopted as part of the routine monitoring strategy for patients with CML. Milestone molecular measurements are now considered prognostic and provide warning signals of suboptimal response. Rising levels of BCR-ABL are an indication to test for kinase domain mutations, which are the major mechanism of imatinib resistance. More than 100 mutations have now been identified, which confer varying degrees of resistance. Furthermore, the characterisation of resistant mutations is important to direct therapy as recent *in vitro* data has confirmed that some mutations create more biologically aggressive forms of BCR-ABL. Mutation analysis at the time of commencing second-line inhibitor therapy is essential to guide the selection of the appropriate drug. The majority of mutations remain sensitive to these inhibitors, however, T315I confers complete resistance. Additionally, recent clinical studies have identified a limited spectrum of imatinib resistant mutations that confer a degree of resistance to either nilotinib or dasatinib. For patients with these mutations, careful selection of inhibitor is warranted to optimise response. Mutation analysis remains relevant during second-line inhibitor therapy as mutations appear to be the major mechanism of resistance, albeit with a limited spectrum of mutations.



Blood Group Genotyping – New Tools for Old Markers

Martin L Olsson

*Dept. of Clinical Immunology and Transfusion Medicine, University and Regional
Laboratories, Skåne, Sweden*

Dept. of Laboratory Medicine, Lund University, Lund, Sweden

Whilst agglutination has ruled the field of blood group determination for more than a century and still does, there is an exciting development towards alternative typing methodologies. Today's sophisticated level of understanding regarding the underlying biochemical and genetic bases of the molecules that carry or synthesize protein and carbohydrate blood group antigens, respectively, has made possible the emergence of DNA-based blood group prediction. Initially, this was mainly thought to be useful in the foetal-maternal setting to identify those babies at risk for haemolytic disease of the newborn by extracting DNA from nucleated cells in chorionvillus biopsies or amniotic fluid. This practice was later improved following the discovery of free foetal DNA in maternal plasma. Thus, fetal RhD prediction is now often performed by sensitive realtime PCR using DNA extracted from a blood sample. In addition, a whole array of reasons beyond pregnancy to perform blood group genotyping by various PCR- or sequence-based assays has been proposed, for instance including:

- 1) resolution of ABO or RHD discrepancies in blood donors and patients;
- 2) extended phenotype prediction in multitransfused individuals whose red cells may be difficult to distinguish from those originating from donors;
- 3) DAT positive patients when phenotyping is difficult to perform;
- 4) RHD zygosity testing in paternal samples to estimate risk of HDN in future pregnancies;
- 5) Test red cell donors whose types should be confirmed homozygous for *RHD* or *FY* markers (to avoid pseudo-homozygotes with silent *Fy* or weak *Fy^x* alleles in screen panels);
- 6) Typing or screening for antigens for which antisera are scarce, expensive or even unavailable.

Currently, genotyping approaches are developing from being manual, labour-intensive tests performed for single samples mainly by highly specialized reference laboratories, to a status where high-throughput DNA-based typing platforms are now being installed at the major centres worldwide as a real complement to serology in everyday practice. Accordingly, blood donor or patient typing and even selection/matching of the most suitable blood unit may soon be based on results from large-scale microarray-based typing.



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ABSTRACTS - Monday 19

1730-1830
Meeting Room 5

Inhibitor Management

Nigel Key

Notes:

A125



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Day 19



**1730-1830
Meeting Room 11**

Nurse Led Clinic

Sherri Ozawa

Notes:

A126

Tuesday 20 October
HSANZ Symposium: Acute Leukaemia/Acute Biology

0830-1000
Hall C
0830

Transcription Factor C/EBP alpha Regulates Hematopoietic Stem Cell Proliferation and Maintenance

Daniel Tenen

Harvard Institute of Medicine, Boston, USA

Hematopoietic stem cells (HSCs) undergo an abrupt change from an actively cycling state to largely quiescent in bone marrow 3 weeks after birth. However, little is known about how this switch is regulated. Here we report that levels of C/EBP alpha, a transcription factor that is frequently disrupted in human acute myeloid leukemia, regulate the proliferative states of HSCs. C/EBP alpha excision in adult mice results in a significant expansion of HSCs and elevated proliferation rates, indicating C/EBP alpha functions as a mitotic inhibitor in adult HSCs. Interestingly, HSCs show a rapid increase in C/EBP alpha expression 3 weeks after birth. Consistent with levels of its expression, loss of C/EBP alpha in 4-week old mice results in a large expansion of HSCs, while only a minor change is observed in C/EBP alpha deficient newborn mice. Furthermore, C/EBP alpha expression is diminished in adult cycling HSCs following cytotoxic cyclophosphamide treatment, suggesting that down-regulation of C/EBP alpha might contribute to the re-activation of quiescent adult HSCs. Gene profiling analysis of C/EBP alpha^{-/-} HSCs shows up-regulation of Notch 3 and 4 and down-regulation of their inhibitors, indicating enhanced activation of Notch signaling, a signal pathway that has been implicated in promoting HSC expansion. Finally, C/EBP alpha deficiency also causes impaired adhesion and retention of HSCs, leading to massive egress of HSCs from BM to distal organs and a repopulating failure.

Tuesday 20 October
HSANZ Symposium: Acute Leukaemia/Acute Biology

0830-1000
Hall C
0915

Insights from MRC Trials in AML

Alan Burnett

School of Medicine, Cardiff University, UK

There have been continuous MRC trials in AML since 1966. In 1994 the organisation came under the auspices of the newly formed National Cancer Research Institute (NCRI). Over the last 20 years annual recruitment has increased from 100 to >1200 with randomisations from 100 to >2500 and in that period over 10000 patients have contributed >25000 randomisations. Trials have focussed on studies in patients under 60 including children, on older patients who are fit for chemotherapy, and the elderly unfit population, and include patients with >10% marrow blasts.

In younger patients with intermediate and good risk disease, but not poor risk disease the longterm survival has improved, with around 80% achieving CR and 40-50% surviving. No more than a total of 4 treatment courses are required, and a retrospective study suggests that a total of 3 courses may be sufficient for good risk and intermediate risk patients which is a question being tested in the current AML17 trial. Of the several induction chemotherapy combinations tested none have proved to be superior to Daunorubicin/ Ara-C. The addition of Mylotarg to induction has provided significant benefit in CBF leukaemias (84% survival at 4 years) and a trend for benefit in intermediate risk but no benefit in poor risk patients. The traditional MRC MACE/MidAc consolidation is myelosuppressive and preliminary results from AML15 suggest that survival is similar with High dose Ara-C whether given at a dose level of 3g or 1.5 g. The additional contribution of stem cell transplantation to patients of intermediate risk is minimal, particularly if they have received mylotarg in induction, but adding mylotarg in consolidation produced no obvious additional effect.

Much has been learned about prognostic factors, and a new risk score can redefine a larger group of poor risk patients where we can for the first time see some benefit for transplantation. It is at the moment unclear how much additional information molecular characterisation provides with respect to who should be transplanted, and studies with FLT3 inhibition are ongoing.

In patients over 60 years who are given intensive chemotherapy the remission rate is 60%, but the relapse rate continues to be high, so in common with other collaborative groups virtually no improvement in overall survival has been seen in the last 30 years. MDR modulation with PSC-833 failed, as did an augmented dose of Ara-c, and there was no benefit in the addition of a 4th treatment course. The ongoing AML16 trial is testing the use of the novel nucleoside, clofarabine in combination with daunorubicin with or without mylotarg in induction and demethylation maintenance therapy. An important majority of older patients do not enter clinical trials and this maybe because the treatment options are unsuitable for less fit patients, who are a group of patients of current interest but difficult to precisely define. In order to cater for this group we compared Low Dose Ara-c with best supportive care and showed some superiority due to the 18% of patients who entered CR, but this was uncommon in patients with poor risk cytogenetics, however this did give a platform for a novel strategy of testing new combinations in a "pick a winner" which is ongoing.

Tuesday 20 October
ANZSBT Free Communications 1

0830-1000
Hall B

0063

0830

The Efficacy of Prothrombinex[®]-VF for Acute Reversal of a Prolonged INR With or Without Fresh Frozen Plasma. A Two Centre Prospective Audit

Louise Bobbitt¹, Jacqueline Raynes², Sue Dainty¹, Hilary Blacklock², Ross Henderson¹ and Sanjeev Chunilal¹

1. North Shore Hospital, Waitemata District Health Board, Auckland, New Zealand

2. Middlemore Hospital, Counties Manukau District Health Board, Auckland, New Zealand

Aim

To determine whether Prothrombinex-VF (PTX) is effective for acute reversal of a prolonged INR with or without concomitant use of Fresh Frozen Plasma (FFP).

Method

A prospective audit of patients who received PTX between August 2008 and May 2009 at two Auckland Hospitals. Eligible patients were all those who received PTX with or without FFP for correction of a prolonged INR. The dose of PTX and vitamin K prescribed along with the decision whether to use FFP was made by a Clinical Haematologist on a case by case basis and based on the patients actual or estimated weight and relevant clinical history and co-morbidities.

Results

A total of 122 separate doses of PTX administered to 120 patients for acute reversal of a prolonged INR. Forty two percent of patients received FFP (average 2 units per person) with their PTX. Over 80% of patients in both groups received vitamin K with average doses of 4.8mg in the no FFP group and 5.3mg in the FFP group. The mean dose of PTX given was 1487IU (no FFP) versus 1670IU (with FFP). The mean INR before reversal was similar for both groups 4.9 (no FFP) and 5.0 (with FFP). On average the post reversal INR was collected at 10 hours after PTX administration and was 1.3 for both groups irrespective of vitamin K administration.

Conclusion

Prothrombinex-VF is effective in quickly reversing a prolonged INR and was achieved with relatively low doses (20IU/kg). The efficacy of reversal was independent of the administration of vitamin K or FFP.

This research was supported by CSL. The company had no role in the design, data analysis or preparing the abstract.

Tuesday 20 October
ANZSBT Free Communications 1

0830-1000
Hall B

O064

0845

Benchmarking Transfusion Across New Zealand

Richard Charlewood

New Zealand Blood Service, Auckland, New Zealand

Aim

By comparing transfusion rates between individuals or institutions, a relative indication of the individuals or institution's performance can be obtained. This exercise, often called benchmarking, has been applied to various aspects of health care.

New Zealand Health Information Services (NZHIS) stores medical procedure codes as part of the National Minimal Dataset (NMDS) provided by all DHBs. NZBS stores the transfusion history of all patients transfused with blood issued by all but the very smallest of blood banks. By extracting data from the NMDS and NZBS databases and linking the two datasets, the aim is to derive transfusion rates per procedure for each district health board (DHB).

Method

NZHIS extracted a table of with, NHI, and date of procedure and procedure code for all patients from 1 January 2002 until 31 December 2007 undergoing abdominal hysterectomy, total hip replacement, CABG and trans-urethral prostatectomy. NZBS extracted a table of red cells transfused using the NHI and date of surgery. NZHIS provided a table of procedure codes and procedure names. Using the database, the numbers of procedures performed and numbers of units transfused per procedure performed were extracted for each DHB. The multi-region ethics committee gave ethics approval for this study prior to commencement. Opt-in was gained from 18 of 21 DHBs at the time of the first data extract.

Results

A total of 69684 procedures were identified. The mean proportion of patients transfused was 13%, 32%, 52% and 5% for hysterectomy, hip replacement, CABG and TURP respectively. The (geometric) mean number of units transfused was 3.1, 2.3, 6.6, 2.3 for hysterectomy, hip replacement, CABG and TURP respectively. Analysis of means was used to identify outlying DHBs - both relative under and over-transfusers.

Conclusion

The initial concept has been demonstrated on four procedures and some useful information about relative performance of DHBs shown.

No conflict of interest to disclose

Tuesday 20 October
ANZSBT Free Communications 1

0830-1000
Hall B

O065

0900

Transfusion Practices in Massive Haemorrhage in Intensive Care

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¹ *Flinders University, Adelaide, South Australia*

² *SA Pathology, Flinders Medical Centre, Adelaide, South Australia*

Background

Primary resuscitation for massive haemorrhage often occurs in emergency departments or operating theatres with ongoing resuscitation in intensive care (ICU).

Aim

To retrospectively review transfusion practice in the pre-ICU phase and ICU for patients with massive haemorrhage and associated mortality risk factors.

Methods

During 1998 to 2006, we developed an electronically linked database of blood and blood product usage and laboratory data with clinical outcome. All surgical patients who received ten or more units of red cells and required ICU admission were included.

Results

Two hundred and sixty three surgical patients were identified from a total of 307 patients who received ≥ 10 units of red cells. Two hundred and twenty five surgical patients were treated in both pre-ICU and ICU settings. Pre-ICU patients received a median of 11 units of red cells, 4 of FFP and 2 of platelets in a RC:FFP ratio of 1:3 and RC:Platelet ratio of 1:10.

Following ICU admission, patients received a median of 4 units of red cells, 3 of FFP and 1 of platelets in a RC:FFP ratio of 1:1 and RC:Platelet ratio of 1:4. Patients on arrival in ICU had a median platelet count of $109 \times 10^9/L$ (IQR 79-152), INR 1.6 (IQR 1.4-1.9), APTT 48.7 seconds (IQR 38.4-68.3) and base deficit of -7 mmol/L (IQR -11 to -2). Median INR decreased to 1.4 within 8 hours of ICU admission and remained constant. Median base deficit decreased to -8 mmol/L during the first 4 hours and returned to normal by 24 hours. The ICU mortality rate was 19% and was associated with acidosis and a significant base deficit ($p = 0.005$).

Conclusions

This study indicated that patients in ICU received more aggressive use of FFP and platelets to correct coagulopathy compared to the pre-ICU phase. A similar approach in pre-ICU settings may be effective in decreasing overall red cell requirements and improving mortality.

No conflict of interest to disclose

Tuesday 20 October
ANZSBT Free Communications 1

0830-1000
Hall B

O066

0915

Understanding Transfusion Outcomes Through Clinical Registries: Validation of a Linkage Technique

Louise Phillips¹, Nikita Schembri¹, Zoe McQuilten², Mark Polizzotto², Christine Akers³, Melissa Wills⁴, Susan Whitehead³, Erica Wood², John McNeil¹, Merrole Cole-Sinclair⁴

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³*Haematology Unit, Alfred Pathology Service, Alfred Hospital, Melbourne, Victoria, Australia*

⁴*Diagnostic Haematology, St Vincent's Hospital, Melbourne, Victoria, Australia*

Background

It is unclear how variation in transfusion practice affects patient outcomes. Several registries gather clinical outcomes data without transfusion information. Hospitals are required to retain information regarding blood components transfused. An opportunity exists to use these two sources to explore effects of transfusion on clinical outcomes.

Methods

Prospective validation of LIS data against individual patient records was undertaken at two major Victorian hospitals. Data regarding all transfusion episodes were compared over seven 24 hour periods. All clinical areas, fresh component types, and days 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. They were issued to: inpatient 221 (37%), intensive care 109 (18%), outpatient 95 (16%), operating theatre 45 (7.5%), emergency department 27 (4.5%) and unrecorded 99 (17%).

All products recorded issued by the LIS were transfused to intended patients. Median time from component issue to transfusion initiation could be calculated for 482 (81%) components: red cells 18 minutes (95% CI 16-20; IQR 10-33), platelets 20 (95% CI 16-24; IQR 11-38), FFP 60 (95% CI 17-96; IQR 13-163) and cryoprecipitate 44 (95% CI 3-179; IQR 7-194). Transfusion commenced within recommended timeframe for 73% of red cells, 69% platelets, 50% plasma and 68% cryoprecipitate.

Conclusions

Across a range of blood component types and destinations 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 analysis of joint data sets to determine the impact of transfusion on clinical outcomes.

There are no conflicts of interest to declare

Tuesday 20 October
ANZSBT Free Communications 1

0830-1000
Hall B

O067

0930

Appropriateness of Red Cell Usage within 7 major New Zealand Hospitals

Richard Charlewood, Suzie Rishworth & Christopher Corkery
New Zealand Blood Service, Auckland, New Zealand

Aim

There was little published information about the appropriateness of red cell usage in New Zealand, therefore a prospective audit involving three common surgical procedures (CABG, THR, TAH) at seven major NZ hospitals was undertaken to assess appropriateness of this component following surgery and to assess what alternatives to blood transfusion were employed.

Method

Transfusion nurse specialists prospectively collected data from a minimum of fifty operations at each of seven hospitals in New Zealand. Transfusion data was collected from the time of admission until the patient was discharged, reached day seven, underwent repeat surgery or died. Each transfusion was assessed by two Transfusion Medicine Specialists.

Results

416 operations involving 415 patients were identified over the 10 month period. A total of 327 red cell units were transfused to 29% (n=119) of the patients. Transfusion rates showed significant differences between hospitals and surgical procedures. 84% patients had an appropriate indication for transfusion. 62% of patients were assessed as having been over-transfused. 69% of all units transfused were assessed as appropriate. 15% of all patients had low haemoglobin levels pre-operatively, but only a third of anaemic patients had been investigated with 14% of these treated preoperatively. Use of autologous blood collected in theatre or post-surgery was almost exclusively limited to cardiac surgery. Indication for transfusion was recorded in 46% of transfusions.

Comment

The present audit is the first such audit to look at red cell usage within New Zealand Hospitals on a national basis. This audit identified several areas that could be responsive to education including: a) red cell dosage to avoid over transfusion, b) discourage routine two red cell unit prescribing c) investigate barriers to blood sparing techniques, d) identification and treatment of anaemic patients before surgery, e) documentation and indication for transfusion can be improved.

No conflict of interest to disclose

Tuesday 20 October
ANZSBT Free Communications 1

0830-1000
Hall B

O068

0945

Snapshot of Current Platelet Transfusion Practice in Five South Australian Hospitals

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Aim

To examine the appropriateness of platelet transfusion practice across five metropolitan teaching hospitals in Adelaide, South Australia.

Method

Retrospective case note audits of consecutive platelet transfusion from the last quarter of 2008 were performed by five Transfusion Nurse Consultants. The data was entered into an auditMakerTM database customised to include relevant clinical information (such as patient demographics, clinical diagnosis, and indication for transfusion, consent and documentation of the process). The data was assessed by a haematologist for appropriateness against NHMRC/ABST guidelines.

Results

One hundred and thirteen platelet transfusion episodes (129 units) in 74 patients were audited. The main indications for platelet use were prophylaxis for bone marrow failure, prophylaxis for surgery/invasive procedure, abnormal microvascular bleeding and documented platelet disorders. Fifteen percent of platelet transfusions were found to be outside NHMRC guidelines based on available documentation. Forty percent of the platelet transfusions were used for prophylaxis for bone marrow failure. The reason for transfusion was documented in 90% of cases and consent in 61%.

Conclusion

While overall 15% of platelet transfusion episodes were outside of guidelines based on available documentation, around half of these were prophylactic platelet transfusions in patients with bone marrow failure in the outpatient setting with platelet counts approaching 10. Transfusion in this setting, to avoid a return visit the following day, may have been reasonable. Many of these patients were children. The remainder of transfusions outside guidelines (based on documentation) were for varying reasons including pre-emptive platelet transfusion for critical bleeding, and maybe in line with local protocols.

No conflict of interest to disclose

Tuesday 20 October
ASTH Symposium: Topics in Venous Thrombosis

0830-1000
Hall D
0830

Diagnosis of Venous Thrombosis

Paul A Kyrle

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In patients with suspected deep-vein thrombosis or pulmonary embolism, accurate diagnosis is of utmost importance: an untreated thrombus can result in fatal pulmonary embolism, whereas anticoagulation in the absence of thrombosis is irresponsible. Since only approximately a quarter of patients with suspected venous thromboembolism actually have the disease, the optimal therapeutic strategy is to safely rule out thromboembolism by non-invasive, rapid, and cost-effective methods. To achieve this goal, clinical assessment, laboratory studies, and imaging techniques are combined.

With regard to clinical assessment, several standardized prediction rules for assessing pretest probability of acute deep-vein thrombosis or pulmonary embolism are available and can be accurately applied in inpatients and outpatients by medical staff of various degrees of training and simplified models have been successfully validated in emergency departments.

Regarding laboratory assays, measurement of d-dimer has gained a prominent role as a simple and inexpensive test for ruling out acute venous thromboembolism in patients with a low pretest probability.

Contrast venography is the most sensitive and accurate imaging test for diagnosis of deep-vein thrombosis, but is invasive and has potential contraindication. It should thus be reserved either for patients with negative non-invasive tests and a high clinical probability, or for those in whom non-invasive test are equivocal or non-feasible. Compared with venography, compression ultrasonography has a sensitivity of 97 – 100% and a specificity of around 99%. In one study, the rate of venous thromboembolism in patients with a negative ultrasound was 0.7% during a 6-month follow-up, indicating that few thromboses were missed and that anticoagulation can be safely withheld in these patients. In patients with suspected pulmonary embolism, computed tomographic pulmonary angiography (CTPA) has become the preferred imaging technique, in particular when using a multi-row-detector (MD). Ventilation-perfusion lung scanning is less attractive because of the large number of non-diagnostic readings and compression ultrasound has been shown to be unnecessary in patients with a negative MD-CTPA.

Tuesday 20 October
ASTH Symposium: Topics in Venous Thrombosis

0830-1000
Hall D
0910

Cerebral Venous Sinus Thrombosis: An Update

Andrew Lee

Affiliations: Flinders Comprehensive Stroke Centre, Flinders University and Medical Centre, Bedford Park, South Australia

Cerebral venous sinus thrombosis is caused by a thrombus obstructing the draining veins of the brain and is an uncommon but important cause of stroke. In this presentation, the author will review key areas of the pathophysiology, diagnosis and treatment of this disease.

Tuesday 20 October
ASTH Symposium: Topics in Venous Thrombosis

0830-1000
Hall D
0935

Post-thrombotic Syndrome: Diagnosis, Prevention and Management

Kurosh Parsi

*Sydney Children's Hospital, St. Vincent's Hospital, Sydney & UNSW, Sydney, NSW
& Australasian College of Phlebology*

Post-thrombotic syndrome (PTS) presents with non-specific clinical signs and symptoms of chronic venous hypertension. The diagnostic criteria for PTS are non-specific and the recent ISTH initiative has not been helpful due to its adaptation of Villalta criteria, a collection of clinical signs that are also commonly found with chronic venous insufficiency secondary to varicose veins. Others have defined PTS based on the presence of reflux, a definition that lacks validity because reflux is not a measure of venous insufficiency but venous incompetence. The clinical signs of PTS should be correlated with the site and extent of the previous DVT and the presence of significant post-thrombotic deep vein incompetence (DVI) in a relevant anatomical distribution.

Adequate anticoagulation, as against the duration of anticoagulation, has been associated with a decreased incidence of PTS. This is presumably because adequate anticoagulation would prevent further extension of the thrombus and further damage to the venous wall. Post-thrombotic ultrasound examination should not simply look for evidence of 'residual vein thrombosis', another inaccurate term, but for the presence of sequelae of the previous thrombus which include wall thickening, septae, webbing, and double lumens. Spectral, Colour and Power Doppler examination should be performed to establish the degree and the pathway of DVI. Venous function tests including air or photo plethysmography are helpful in establishing the function of the venous system and should be incorporated into the assessment of PTS. Treatment, apart from conservative measures and compression stockings should include oblitative measures to treat any superficial venous incompetence which can be readily treated. Treatment of the superficial system can help to improve the overall venous hypertension. Modern techniques to achieve this include endovenous laser ablation and ultrasound guided sclerotherapy. In selective cases, the same techniques can be used to target and selectively occlude incompetent deep veins.

Tuesday 20 October

Nurses Symposium: Recovery, Survivorship, and Adolescents Symposium

0830-1000

Hall A

0830

Exercise and Recovery from Cancer: From Research to Clinical Practice

Morgan Atkinson

Centre for Physical Activity in Ageing, Hampstead Rehabilitation Centre, Hampstead, SA, Australia

Treatment for Blood related disorders, including bone marrow transplantation has been associated with treatment related toxicity which can compromise recovery. Functional impairments such as muscular atrophy leading to reduced muscular strength and functional capacity, impaired pulmonary and cardiac function, reduced bone mineral density, impaired glucose tolerance and dyslipidaemia, and cancer related fatigue have been extensively documented in the literature.

Currently cancer rehabilitation is progressing towards standard practice as the evidence base increases. Studies have consistently reported that structured exercise programs for cancer patients increase muscular strength and endurance, cardiorespiratory fitness, flexibility and overall quality of life. Furthermore, studies support the use of exercise participation in reducing feelings of anxiety, depression, pain and nausea, the duration of neutropenia, thrombocytopenia, period of hospitalisation and feelings of fatigue and weakness.

Late and long term side effects and lifestyle diseases such as diabetes, cardiac disease, obesity and osteopenia are commonly reported in cancer survivors. Physical activity is an accepted treatment modality in the management and prevention of such diseases and may have implications in long term health following cancer treatment.

So with the body of evidence growing why isn't exercise an accepted treatment modality, and what should be considered as best practice in exercise rehabilitation?

Tuesday 20 October

Nurses Symposium: Recovery, Survivorship, and Adolescents Symposium

0830-1000

Hall A

0900

Adolescent and Young Adult Issues for Cancer Care

Sharon Bowering

Abstract not received at time of going to print

Tuesday 20 October

Nurses Symposium: Recovery, Survivorship, and Adolescents Symposium

0830-1000

Hall A
0930

A Survivorship Programme in South Australia

Alison Keenan¹, Uwe Hahn¹, Margaret Colbeck²

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Background

Global evidence demonstrates that whilst the cure rate for adult-onset Hodgkin's Lymphoma (HL) is significantly higher in recent years, survivors are also at an increased risk for 'Late Effects' (LE) as a consequence of their curative treatment. Psychosocial and physical LE range from anxiety through to second malignancies and can occur many years later.

Objective

The aim was to develop a Survivorship Programme at the Queen Elizabeth Hospital in Adelaide. Through screening, early detection of risk factors and the initiation of preventative measures, its purpose was to improve the long term outcome for survivors of adult-onset HL.

Methods

Survivors of HL were identified from the South Australian Cancer Registry for the North Western Area Health Service. Survivors diagnosed after 1975, aged over 17 and disease-free for a minimum of five years were eligible for invitation to participate. 74 were invited: 38 accepted, 9 returned to sender and 27 did not reply.

Results

35 survivors have been assessed; 24 females, 11 males. 3 of the female survivors had been diagnosed with breast cancer (a ratio of 1 in 8). All three had received 40Gy of upper mantle radiotherapy at a pre-menopausal age and developed breast cancer 16-21 years post treatment. For the general population the risk is 1 in 12 up to 75 years. Many patients were unaware of the risk of breast cancer and not enrolled in regular screening programs using mammography.

Implications

Whilst the focus in this abstract is on breast cancer, the Programme identified many more Late Effects in this small cohort. It would therefore be reasonable to suggest that further studies of survivors of Hodgkin's Lymphoma are needed to increase our knowledge and consequently provide more efficacious care for this group of individuals.

Tuesday 20 October
HSANZ Free Communications 5

1030-1130
Hall C

O069

1030

Invariant Natural Killer T Cells in Chronic Lymphocytic Leukaemia

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² *Blood and Cancer Centre, Wellington Hospital, Wellington, New Zealand*

Aim

Invariant natural killer T (iNKT) cells release proinflammatory cytokines and induce dendritic cell maturation in response to glycolipid antigens such as α -galactosylceramide (α GalCer) presented on the CD1d molecule. α GalCer can powerfully augment anti-tumour immunity in preclinical models of cancer. We evaluated iNKT cell number, phenotype and function, and expression of CD1d on normal and leukaemic cells in patients with chronic lymphocytic leukaemia (CLL), with a view to using iNKT cells in CLL immunotherapy.

Method

Peripheral blood mononuclear cells were obtained from patients with untreated CLL (n=29) and healthy age-matched controls (n=29). iNKT cell number and phenotype, and CD1d expression of normal and leukaemic cells, was assessed by flow cytometry. Following immunomagnetic B cell depletion, α GalCer-induced cytokine production and in vitro iNKT cell proliferation were evaluated.

Result

iNKT cell number was similar in patients (median 115/mL blood, range 0 – 2273) and controls (median 96/mL, range 0 – 3279). iNKT cell CD4 and CD25 status was unchanged, but CD8+ iNKT cells were significantly reduced in patients. CD1d was expressed on CLL cells in all patients, at a similar level to that of normal B cells. CD1d expression on myeloid dendritic cells and monocytes was normal. α GalCer-induced production of interferon gamma, IL-4, IL-13 and IL-17 was not impaired. Although in vitro iNKT cell proliferation was modestly reduced, patient-derived iNKT cell lines were successfully generated from four of ten patients with CLL.

Conclusion

Patients with untreated CLL have a similar number and phenotype of iNKT cells to healthy controls, and α GalCer-induced cytokine production is not impaired. CLL cells themselves express CD1d, and CD1d expression on antigen presenting cells is normal in patients with CLL. iNKT cell lines can be derived from patients with CLL. Novel immunotherapy strategies exploiting the iNKT/CD1d axis may be feasible in CLL.

No conflict of interest to disclose

Tuesday 20 October
HSANZ Free Communications 5

1030-1130
Hall C

O070

1045

Mutation Screening of *TP53* Exons 2-11 in Chronic Lymphocytic Leukaemia Using High Resolution Melting Analysis

Chelsee Hewitt, Giada Zapparoli, Dennis Carney, John Seymour, David Westerman, Alexander Dobrovic
Peter MacCallum Cancer Centre, East Melbourne, VIC Australia.

Aims

Loss of 17p13 is a prognostic marker for poor survival and chemorefractoriness to alkylating agents and purine analogues in patients with chronic lymphocytic leukaemia (CLL). The target of the 17p13 deletion is thought to be the tumour suppressor gene *TP53*. It has been recently demonstrated that *TP53* mutations are an independent predictor of poor survival and chemorefractoriness in CLL. Consequently *TP53* mutation analysis is likely to become an important tool in the stratification of CLL patients for appropriate treatment. *TP53* mutations are spread throughout the gene but are thought to cluster within the DNA binding region, which stretches from the middle of exon 4 to the beginning of exon 9. However, to some extent the distribution may reflect the fact that the majority of studies limit their mutation detection to exons 5-8.

Methods

We developed high resolution melting (HRM) assays to allow efficient high throughput screening of mutations throughout the entire coding region of *TP53* (exons 2 to 11). In a pilot study we screened 25 CLL patients using these HRM assays followed by sequencing of amplicons with aberrant melting patterns.

Results

We identified *TP53* mutations in 40% (10/25) of the CLL patients. Eight mutations were situated within exons 5-8 (1 nonsense, 6 missense and 1 insertion). A previously reported nonsense mutation was revealed in exon 9 outside the DNA binding domain. One patient harboured 2 alterations in exon 4, a nonsense mutation within the DNA binding domain and a synonymous change outside the DNA binding domain.

Conclusions

1. *TP53* mutations in CLL can occur outside the DNA binding domain, therefore limiting mutation screens to exon 5-8 may underestimate their prevalence.
2. HRM is a cost-effective methodology for the rapid detection of mutations, especially where the probability of a mutation in an individual exon is low.

No conflict of interest to disclose

Tuesday 20 October
HSANZ Free Communications 5

1030-1130
Hall C

O071

1100

CCL2, CXCL2, IL-6 and IL-8 are Expressed in Primary Chronic Lymphocytic Leukaemia Cell Cultures and Enhance CLL Cell Long-Term Survival *in vitro*

Melinda Burgess¹, Karunya Ravindranath¹, Gunjeet Minhas¹, Catherine Cheung², Peter Mollee², Nigel McMillan¹, and Devinder Gill²

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Aim

To investigate the role and clinical relevance of cytokines CCL2, CXCL2, IL-6 and IL-8 in the *in vitro* survival of CLL PBMC cultures.

Methods

PBMCs from CLL patients were purified and grown in culture at high density (10^7 cell/ml) for seven days before supernatants were collected and cytokines detected using the Human Cytokine Antibody Array III (Chemicon). Cultures were undertaken in the presences of these exogenous cytokines and with blocking antibodies and leukaemia cell survival was determined using trypan blue exclusion. The mRNA level of these cytokines was examined by real time PCR and results compared to age-matched normal PBMCs. In addition, expression of these cytokines receptors was also undertaken by FACS.

Results

The cytokines CCL2, CXCL2, IL-6 and IL-8 were found to be produced in CLL PBMC cultures. Addition of these cytokines improved *in vitro* survival of CLL cells; however, no further survival advantage resulted when these cytokines were used in various combinations. Additionally, blocking CCL2 and CXCL2 with specific antibodies resulted in the loss of this effect. Moreover, the expression of CCR2 and CXCR2 was found to decrease on CLL cells and increase on accessory cells over time in culture. Furthermore, the mRNA levels of these cytokines were elevated when compared to age-matched normal PBMCs.

Conclusion

The identification and increased expression of CCL2 and CXCL2 are novel in CLL culture systems and contribute to improved CLL cell survival *in vitro*. Through these chemokines, leukaemic cells and accessory cells may be able to create a supportive microenvironment for CLL.

No conflict of interest to disclose

Tuesday 20 October
HSANZ Free Communications 6

1030-1130
Meeting Rooms 1/ 2

O073

1030

Temporal Differences in Mobilisation of Normal and Malignant Hematopoietic Cells by the CXCR4 Antagonist AMD3100

Robert Welschinger, Florian Liedtke, Kenneth Bradstock and Linda Bendall
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Introduction

The chemokine CXCL12, and its receptor CXCR4, play a major role in the homing and engraftment of B lineage acute lymphoblastic leukemia (ALL) cells in the bone marrow (BM). Inhibition of the CXCL12/CXCR4 interaction results in ALL cell mobilisation into the blood. The separation of ALL cells from the protective BM microenvironment is likely to enhance the effectiveness of chemotherapy. However the CXCR4 antagonist, AMD3100, also mobilises normal hematopoietic stem cells (HSC) potentially increasing chemotherapy-related toxicity.

Aim

To compare the temporal affects of AMD3100 on HSC and ALL cell mobilisation.

Methods

The duration of AMD3100-induced mobilization of ALL cells was examined using a NOD/SCID mouse model of human ALL and compared to the kinetics of normal HSC mobilization in Balb/C mice. In NOD/SCID mice the ALL percentage and absolute cell number was measured, and in Balb/C mice Colony Forming Units (CFU) in the blood was assessed. Adhesion molecule and CXCR4 expression was determined by flow cytometry, and chemotaxis in transwell assays.

Results

HSC and ALL cells showed peak mobilization between 1 and 3 hours post AMD3100 administration. However, while HSC had returned to the BM within 6 hours, in 6 of 7 ALL samples tested significant numbers of leukemic cells remained in the circulation. There was a direct correlation between the expression of CXCR4 and the mobilization of ALL cells. Comparison of the level of mobilisation following AMD3100 administration and the chemotaxis of ALL samples to CXCL12 was indicative of a positive relationship. Similarly, there was a weak negative association between VLA-5 expression and mobilization but no association between VLA-4 or CD44 expression could be detected.

Conclusion

Prolonged mobilization of ALL cells by AMD3100 provides a window in which chemotherapy could be specifically targeted to circulating ALL cells after the HSC have safely returned to the BM.

No conflict of interest to disclose

A144

Tuesday 20 October
HSANZ Free Communications 6

1030-1130
Meeting Rooms 1/ 2

0074

1045

Late Breaking Abstract:

Osteopontin expression levels are a prognostic indicator of overall patient survival in cytogenetically normal acute myeloid leukaemia

Jason A. Powell¹, Daniel Thomas¹, Emma F. Barry¹, Chung H. Kok², Anna Brown², Gregory J. Goodall^{4,5}, Terence P. Speed⁶, Motomi Osato⁷, David N. Haylock⁸, Susan K. Nilsson⁸, Richard J. D'Andrea², Angel F. Lopez³ and Mark A. Guthridge^{1,5}.

Cell Growth and Differentiation Laboratory¹, Cytokine Receptor Laboratory³ and Cytokine Signaling Laboratory⁴, Centre for Cancer Biology, Division of Human Immunology, Frome Rd. Adelaide, SA, Australia. Department of Haematology², Centre for Cancer Biology, Adelaide, SA, Australia. Department of Medicine⁵, University of Adelaide Frome Rd. Adelaide, SA, Australia. Division of Bioinformatics⁶, The Walter & Eliza Hall Institute of Medical Research, Parkville, VIC, Australia. Cancer Science Institute of Singapore, National University of Singapore, Singapore⁷. Australian Stem Cell Centre, Monash University, Clayton, VIC, Australia.⁸.

Acute myeloid leukemia (AML) remains a devastating haematological disease with overall 5 year survival rates in adults of <40%. Cytogenetic analysis remains an important tool for assigning treatment selection and for assessing patient prognosis. However, normal karyotype AML constitutes the largest subset of AML with patients demonstrating diverse responses to therapy and few prognostic indicators. Furthermore, the mechanisms by which normal karyotype patients fail to respond to chemotherapy may not solely reside within blasts themselves but depend on an interaction between the leukaemia stem/progenitor cells and stromal factors in the microenvironment. To discover new functional prognostic markers in AML we performed a global expression screen for cytokine-regulated hemopoietic cell survival genes. This screen examined a specific cell survival pathway emanating from Ser585 of the β c subunit of the IL-3/GM-CSF receptor¹. Importantly, we have shown that while this Ser585 survival pathway is tightly regulated in normal primary myeloid cells, it is constitutively activated in AML². We show that gene targets of this Ser585 survival pathway were over-expressed in AML. Importantly, we validated osteopontin (*OPN*) as a *bone fide* target of the Ser585 survival pathway and siRNA-mediated knockdown of *OPN* expression induces cell death in both primary AML blasts as well as in CD34⁺CD38⁺CD123⁺ leukemic stem/progenitor cells³. We quantified *OPN* mRNA expression in a multicentre cohort of 60 normal karyotype AML samples from patients that received standard induction chemotherapy to test the effect of *OPN* expression on patient outcome. Multivariate analysis indicated that patients with high *OPN* expression had a significantly shorter overall survival (median 384 days) compared to patients with low *OPN* expression (median 1017 days)(n=60, p=0.01, HR=2.22, 95%CI 1.23-4.02). These results identify *OPN* as a new prognostic indicator with therapeutic potential in normal karyotype AML and suggests *OPN* may contribute to leukemic stem/progenitor cell survival and resistance to chemotherapy.

1 Guthridge MA, Stomski FC, Barry EF, et al. Site-specific serine phosphorylation of the IL-3 receptor is required for hemopoietic cell survival. *Mol.Cell.* 2000;6(1):99-108.

2. Guthridge MA, Powell JA, Barry EF, et al. (2006) Growth factor pleiotropy is controlled by a receptor Tyr/Ser motif that acts as a binary switch. *EMBO J.* 2006;25(3):479-85.

3. Powell JA, Thomas D, Barry EF, et al. Expression profiling of a hemopoietic cell survival transcriptome implicates osteopontin as a functional prognostic factor in AML. *Blood.* 2009; In press.

I have no conflicts of interest to declare

A145

Tuesday 20 October
HSANZ Free Communications 6

1030-1130
Meeting Rooms 1/2

0075

1100

The GM-CSF Receptor Utilises β -catenin and TCF4 to Specify Macrophage Lineage Differentiation

Anna Brown^{1,2,3}, Diana Salerno^{1,2}, Chris Wilkinson^{2,3}, Teresa Sadras^{1,3}, Chung Kok^{3,4}, Michelle Perugini^{1,2}, Gregory Goodall¹, Thomas Gonda⁵ and Richard D'Andrea^{1,2,3,4}

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² Women's and Children's Health Research Institute, Adelaide, South Australia, Australia

³ University of Adelaide, Adelaide, South Australia, Australia

⁴ Queen Elizabeth Hospital, Adelaide, South Australia, Australia.

⁵ Diamantina Institute, University of Queensland, Brisbane, Australia.

Background

Over the past several years we have used a factor-dependent murine bi-potential cell line, FDB1, in combination with activated mutants of the GM-CSF receptor common beta subunit (h β c) to dissect signalling and gene expression changes that can contribute to differences in myeloid cell differentiation and growth.

Aim

To determine the mechanisms through which signalling from a single receptor can induce two distinct cell fates (the choice between Granulocyte and Macrophage lineages).

Methods

We have used two derivatives of an activated h β c mutant with an extracellular duplication (F1 Δ) where the presence or absence of a single intracellular tyrosine residue (Y577), can specify bi-lineage Granulocyte-Macrophage differentiation or Macrophage-only differentiation respectively (Brown et al., 2004). Transcriptional profiling of these differentiation states has allowed us to identify genes with altered expression associated with either terminal granulocyte or macrophage differentiation. Signalling pathways correlated with transcriptional changes and differentiation states were also examined.

Results

An examination of the genes displaying expression correlating with macrophage differentiation revealed a potential role for the transcription factor TCF4/TCF7L2 which is a central mediator of the canonical Wnt signalling pathway through its role as the DNA binding co-factor for β -catenin. TCF4/TCF7L2 is also a transcriptional target gene of this pathway. Further examination of signalling in these cells identified that stabilisation of β -catenin was associated with the switch to macrophage-only differentiation and that endogenous GM-CSF signalling also induces β -catenin stabilisation during GM differentiation. Stabilisation of β -catenin and GM differentiation could also be induced using the GSK3 β inhibitor BIO (6-bromoindirubin-3'-oxime), indicating that this pathway for regulation of β -catenin protein stability is intact in FDB1 cells.

Conclusion

Using the FDB1 model of myeloid differentiation we have identified a previously uncharacterised role for β -catenin/TCF signalling downstream of myelopoietic cytokine receptor activation during myeloid differentiation.

No conflict of interest to disclose

Brown, AL, Peters, M, D'Andrea, RJ, and Gonda, TJ (2004). Constitutive mutants of the GM-CSF receptor reveal multiple pathways leading to myeloid cell survival, proliferation, and granulocyte-macrophage differentiation. *Blood* 103, 507-516.

A146

Tuesday 20 October
HSANZ Free Communications 6

1030-1130
Meeting Rooms 1/2

O076

1115

Characteristics of Bone Marrow Derived Mesenchymal Stromal Cells in Myelodysplasia

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⁴ Department of Haematology, Royal Perth Hospital, Perth, Western Australia

Immune dysregulation has been implicated in the pathogenesis of myelodysplasia (MDS). Recent studies indicate that a rare population of bone marrow cells, mesenchymal stromal cells (MSC), may play a role in the modulation of normal immune cells and malignant clones in MDS. In this study, MSC from MDS patients (MDS-MSC) were characterised and compared to those of healthy donors.

MSC were isolated from bone marrow aspirates of MDS patients (n=4) and healthy donors (n=6) using density gradient centrifugation. These cells were culture expanded and characterised for the expression of established MSC phenotype of CD73, CD90 and CD105, and lack of haemopoietic markers CD14, CD34 and CD45 using flow cytometry. Growth kinetics of MSC to passage (P) 4 were recorded and the differentiation capabilities of these cells determined. Immune regulatory actions of MSC on peripheral blood mononuclear cells (MNC) were examined using Cell Titre Aqueous One™ proliferation assay and IL-6 production measured using BD FACSArya bioanalyser™. Cytogenetics and DNA analysis for *FLT3* mutation were performed.

MDS-MSC expressed normal MSC phenotype and underwent multi-lineage differentiation when stimulated. Cytogenetic and DNA analysis of this small cohort of MDS-MSC revealed normal karyotype with no *FLT3* mutation detected. MDS-MSC displayed slower growth kinetics (paired T test; \pm standard error of mean (SEM); $P < 0.05$; 88days vs. 56days to reach P4) and produced higher levels of IL-6 in culture, as compared to MSC from healthy individuals (unpaired T test; \pm SEM; $P < 0.05$; 2576pg/ml vs. 1406pg/ml at 24hrs; 4676pg/ml vs. 1868pg/ml at 96hrs). In addition, as for normal MSC, MDS-MSC inhibited the proliferation of healthy MNC in co-culture.

Preliminary results indicate that MDS-MSC have a role in the inhibition of immune cells. The observation *in vitro* that MDS-MSC produced significantly higher levels of IL-6 may reflect the *in vivo* MDS bone marrow microenvironment and may contribute to the disease

No conflict of interest to disclose

A147

Tuesday 20 October
HSANZ Free Communications 7

1030-1130
Meeting Rooms 10/11

O077

1030

EBV-specific T Cells As Therapy for Relapsed / Refractory EBV-Positive Lymphomas

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² Peter MacCallum Cancer Centre, East Melbourne, Vic

We and others have shown a critical pathogenetic link between Epstein-Barr Virus (EBV) and the development of a range of malignant lymphomas, including Hodgkin's, DLBCL, PTLD and ENKTL. These occur in the immunosuppressed and the 'overtly' immunocompetent who demonstrate a selective impairment of EBV immunity. The presence of EBV within the lymphoma cell is an adverse prognosticator but is also a potential target. Restoration of EBV-specific T cell immunity is an attractive therapeutic option. We hypothesize that adoptive immunotherapy of clinical grade EBV EBNA1-LMP1/2 specific T cells for relapsed / refractory EBV-positive lymphomas is safe, results in reconstitution of anti-viral immunity, and induces tumour lysis. We have recently commenced an NHMRC phase I clinical trial of adoptive immunotherapy of *in-vitro* expanded, EBV-specific cytotoxic T lymphocytes (CTL) in EBV-positive lymphomas. This utilizes a novel replication-deficient adenoviral construct ("AdE1-LMPpoly") that encodes specific EBV proteins expressed by all latency II and III EBV-positive lymphomas. CTL are generated in QIMR's cGMP licensed facility. Previous methodology was slow, technically demanding and resulted in CTL with minimal EBNA1 specificity. Our approach is a new technology that circumvents these limitations and utilizes a highly efficient, but relatively brief, autologous EBV-specific CTL expansion protocol. To date 4 patients have been enrolled (2 Lymphomatoid Granulomatosis [LYG], 1 Hodgkin's, 1 DLBCL). In all cases clinical grade EBV-specific CTL were generated. CTL were re-infused in both LYG patients. In the first a short-lived remission was induced including complete eradication of skin lesions with demonstrable anti-viral efficacy but subsequent relapse of disease, but interestingly no return of skin disease. In the second patient remission was induced and is ongoing. Our data although preliminary, indicates that this approach is potentially feasible, safe and efficacious. The trial is ongoing and aims to recruit a further 16 patients.

No conflict of interest to disclose

Tuesday 20 October
HSANZ Free Communications 7

1030-1130
Meeting Rooms 10/11

O078

1045

Epstein-Barr Virus Specific Cytotoxic T Cells for Clinical Use in Immune Reconstitution Post Haemopoietic Stem Cell Transplant

Emily Blyth¹, Leighton Clancy², Upinder Sandher³, Rajiv Khanna⁴, David Gottlieb³

¹ Westmead Millennium Institute, Westmead, NSW, Australia. ² Sydney Cellular Therapies laboratory, Westmead, NSW, Australia. ³ Blood and Marrow Transplant Service, Westmead Hospital, Westmead, NSW, Australia. ⁴ Queensland Institute for Medical Research, Herston, QLD, Australia

Introduction

Uncontrolled EBV replication can lead to life threatening post-transplant lymphoproliferative disorder (PTLD). The incidence of EBV reactivation and PTLD is proportional to the degree of cellular immunosuppression. Treatment with EBV specific cytotoxic T lymphocytes (CTL) effectively controls EBV replication but the generation of CTL using autologous EBV-transformed B cells as stimulators is cumbersome.

Aim

To develop a rapid and reliable method for production of a clinical grade EBV specific T cell product using an adenoviral vector encoding genes for EBNA-1 and immunogenic LMP peptides.

Method

Monocyte derived dendritic cells (DC) were generated from peripheral blood by adherence to plastic and exposure to IL-4 and GM-CSF. DC were transfected with a clinical grade adenoviral vector encoding EBNA-1 and HLA Class I epitopes of LMP-1 and LMP-2a and matured with TNF. Mature DC were used to stimulate non-adherent cells. Cultures continued for 21 days with a second stimulation on day 7 in the presence of increasing doses of IL-2. The cellular product was analysed on day 21 for phenotype, antigen specificity and functional capacity by tetramer staining, cytokine production in response to antigen stimulation and cytotoxicity.

Results

Cellular proliferation was seen in all of 10 EBV seropositive normal donors with a mean fold increase in total cell number of 5.25 (SEM 1.094). In all donors, the cellular product was mainly T cells (mean CD3+ 94.7%, range 81 to 99.4) with both CD4 (mean 65.7% of CD3, range 17.0 to 93.2) and CD8 cells present (mean 29.6%, range 4.4 to 74.4). EBV specificity was demonstrated by tetramer staining or intracellular cytokine detection in 70% of donors. Tetramer analysis of HLA-A2 positive donors showed up to 429-fold expansion of LMP-2a tetramer specific CD8 cells. Cytokine responses to stimulation with EBNA, LMP and adenoviral peptides were present in both CD4 and CD8 cells and showed significant individual variation. Cytotoxic activity was seen with up to 75% specific lysis of EBV antigen coated targets.

Conclusion

The use of an adenoviral vector containing genes encoding EBNA-1 and LMP antigens allows the rapid generation of a clinical grade EBV-specific T cell product from the majority of EBV seropositive normal donors. This technique will permit the incorporation of adoptive immunotherapy for EBV into routine haemopoietic stem cell transplantation.

There are no conflicts of interest to disclose

Tuesday 20 October
HSANZ Free Communications 7

1030-1130
Meeting Rooms 10/11

0079

1100

Comparison of Cytomegalovirus (CMV) pp65 Specific T Cell Generation from Mobilised Peripheral Blood Stem Cell (PBSC) Collections and Whole Blood

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Introduction

CMV reactivation post allogeneic haemopoietic stem cell transplant (HSCT) can cause significant morbidity. We have recently conducted two clinical trials of *ex vivo* expanded donor derived CMV specific T cells given prophylactically to HSCT recipients. Our ongoing investigations suggest this strategy is safe and controls reactivation without the need for antiviral therapy. Our approach requires 100mls of donor blood to be collected before stem cell mobilisation. This can result in significant logistical and regulatory obstacles which could be alleviated if a proportion of the mobilised PBSC product in excess of that required for HSCT could be allocated to generate CMV specific T cells.

Aims

The aim of this study was to compare the generation of CMV specific T cells from mobilised PBSC harvests to whole blood obtained prior to stem cell mobilisation.

Methods

Samples consisted of 1.6-1.8ml (<1%) of PBSC products or 100mls of blood from transplant donors. Mononuclear cells were isolated and dendritic cells (DC) generated. DC transfected with a clinical grade vector encoding CMVpp65 were co-cultured with PBMC to stimulate expansion of T cells. Cultures were re-stimulated after 7 days and continued for 14 days.

Results and Discussion

There was a 14.5-17 fold increase in cell number in cultures from PBSC harvests consisting primarily of T cells (range 92.6-98.3%). In one donor, there was a 100 fold increase in T cells recognizing the HLA-A2 restricted epitope NLVPMVATV (49% of CD8 T cells). 15% of CD8 T cells recognised a HLA-A24 restricted epitope in a second donor. In cultures from whole blood we observed a 7.9-10.1 fold increase in cell number. Cultures were mainly T cells (75.2-80%) but contained more NK cells (>20%). Differences were observed in CD4:CD8 ratio and percentage of CMV tetramer⁺ cells. This study shows CMV specific T cells can be generated from PBSC harvests, however further work is required to compare T cell function in cells generated from mobilised PBSC harvests.

No conflict of interest to disclose

A150

Tuesday 20 October
HSANZ Free Communications 7

1030-1130
Meeting Rooms 10/11

O080

1115

Developing a Therapeutic Anti - Dendritic Cell Antibody to Prevent GVHD

Derek Hart², Therese Seldon¹, Martina Jones², Yonghua Sheng¹, Anna Palkova¹, Hannah Cullup¹, Trent Munro², Alison Rice¹, John Wilson¹, Ross Barnard², Stephen Mahler², David Munster.

¹ Mater Medical Research Institute. ² University of Queensland.

Graft versus host disease (GVHD) following allogeneic haematopoietic stem cell transplantation (alloHSCT) is the major contributor to transplant related mortality. The risk of GVHD limits the application of alloHSCT and current immunosuppressive strategies, which focus on controlling donor T cell responses using nonspecific agents, increase the risk of leukaemia recurrence and post transplant infections, including the reactivation of pre-existing cytomegalovirus (CMV) infections. Host and donor dendritic cells (DC) stimulate alloreactive donor T lymphocytes, and initiate GVHD. We have shown that polyclonal antibody to the DC surface activation marker human CD83 (anti hCD83), which depletes activated DC, can prevent human DC and T cell induced lethal xenogeneic GVHD without impairing T cell mediated anti-leukaemic and anti-viral (CMV and influenza) immunity (J Exp Med 2009; 206: 387). Therefore, we investigated the effect of polyclonal anti mCD83 antibody in both autologous and allogeneic murine HSCT models. The anti mCD83 had no effect on autologous HSC engraftment and we confirmed that it delayed acute GVHD, at least as effectively as cyclosporin A in a fully MHC mismatched combination. We are currently developing humanized mouse models that will, in conjunction with our established mouse alloHSCT models, enable us to test whether the anti leukaemic CTL capacity in anti CD83 treated recipients will control leukaemia recurrence.

Based on these preclinical studies, we are developing a humanized monoclonal antibody against hCD83, to test as a new therapeutic immunosuppressive agent. We have panned single chain variable fragment (scFv) phage libraries on recombinant human CD83 extracellular domain and isolated six phage clones that bind to CD83. After screening for specificity and affinity, the clones are reformatted to human IgG1 and expressed as whole immunoglobulin by transfected CHO cells. The purified antibodies are tested further, including in a functional assay (mixed lymphocyte reaction (MLR)). To date, we have reformatted and tested two of the six clones. One has lost binding affinity for antigen, probably as a result of immunoglobulin variable region glycosylation, which does not occur with prokaryote expressed scFv. The other reformatted anti-CD83 clone binds and is functionally active as it blocks a mixed leucocyte reaction (MLR), by an antibody dependent cellular cytotoxicity mechanism.

This clone and others in the pipeline will be subjected to preclinical testing in the humanized NOD-SCID mouse of human cell induced lethal GvHD. The most effective antibody, which prevents GVHD without impairing the desired graft versus leukaemia effect of alloHSCT will then be developed for phase 1 clinical trials in clinical alloHSCT.

No conflict of interest to declare

Tuesday 20 October
HSANZ Free Communications 8

1030-1130
Meeting Room 3

O081

1030

Determinants of Survival in Australian Patients With AL Amyloidosis

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⁵*Cancer Centre, Coffs Harbour, Australia*

Aim

As there are few reported cohorts of patients with AL amyloidosis in Australia, we aimed to assess the characteristics and outcomes of such patients referred to a single centre.

Methods

A retrospective analysis of consecutive patients with symptomatic AL amyloidosis was performed, identifying prognostic variables and the impact of haematologic and organ responses on survival. AL amyloidosis associated with overt myeloma was excluded. Organ involvement, and haematologic and organ responses were defined as per the International Society of Amyloidoses consensus criteria.

Results

52 pts with AL were referred between Jan'99 and Jun'09: median age 61 yrs; 33% female. AL was lambda light chain restricted in 67% and the FLC ratio was abnormal in 94%. 47% had an ECOG performance status of ≥ 2 . Organ involvement: renal 62%; cardiac 69%; liver 22%; and neurologic 41%. Initial therapy was autologous stem cell transplantation (n=12), melphalan and dexamethasone (n=29), other chemotherapy regimens (n=2), and 9 received no treatment. On an intent-to-treat basis 30% achieved haematologic CR, 28% PR and 42% had no response. 29% achieved an organ response at a median of 5 months post-therapy (range 1 to 16 months). With a median follow-up of 19 months, median overall survival was 25 months. In univariate analysis, inferior survival was predicted by worse ECOG performance status ($p<0.0001$), weight loss ($p=0.0005$), syncope ($p=0.04$), absence of renal involvement ($p=0.02$), cardiac involvement ($p=0.005$), liver involvement ($p=0.004$), a postural drop in blood pressure ≥ 20 mmHg, and number of organs involved ($p=0.05$). Amongst treated patients, haematologic response ($p<0.0001$) and organ response ($p<0.0001$) strongly predicted survival, although CR did not provide additional benefit over PR.

Conclusions

Patients with AL amyloidosis continue to have a poor prognosis which is predominantly influenced by performance status and extent of organ, particularly cardiac, involvement. Achievement of a haematologic PR is a powerful predictor of survival and is the critical initial goal of therapy.

No conflict of interest to disclose

A152

Tuesday 20 October
HSANZ Free Communications 8

1030-1130
Meeting Room 3

O082

1045

Bone Marrow Plasma Cells at Diagnosis of Waldenstrom's Macroglobulinemia

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2. Peter MacCallum Cancer Centre (PeterMac), East Melbourne, Victoria, Australia.
3. University of Melbourne, Parkville, Victoria, Australia.

Aim

The diagnostic and prognostic significance of the bone marrow plasma cell (PC) component in Waldenstrom's Macroglobulinaemia (WM) is unknown. Because WM lacks a hallmark immunophenotype or molecular-cytogenetic abnormality, we investigated whether the PC compartment offers useful additional information at diagnosis.

Method

Percentage and topography of B-lymphocytes and PCs in the marrow were defined in 5% increments by consensus between two observers interpreting immunohistochemistry in patients presenting with WM between 1999-2009 at RMH and PeterMac. B-lymphocytic infiltration was classified as: Scattered-nonaggregated, interstitial, peri-sinusoidal, nodular, and diffuse. PC infiltration was classified as: Scattered-interstitial, peri-vascular, microaggregates (≥ 10 non-peri-vascular PCs), permeative (occupying at least one inter-fat space), or nodular (effacement of fat-space/s). Associations between percentage and pattern of marrow B-lymphocytes, PCs, and monoclonal serum IgM were also investigated.

Result

Thirty-nine patients (10F:29M) presented with monoclonal serum IgM between 4-64g/L (median 21g/L). The most common pattern of B-lymphocytic infiltration was interstitial (33/39, 84.2%); 12/39(28.9%) demonstrated a nodular component; 8/39(21.1%) paratrabecular; and three (7.9%) peri-sinusoidal. PC burden ranged between 1-30% (median 5%) and was $>5\%$ in the majority (33/39, 85%). Distribution was peri-vascular in 37/38(97.4%); scattered-interstitial, 22/38(57.9%); micro-aggregates, 7/38(18.4%); and permeative, 4/38(10.5%). Plasma cells outnumbered B-lymphocytes in 4/39(10.3%). No nodules were identified. Notably, bone marrow PC percentage was positively associated with serum IgM ($p < 0.01$, Spearman's rank), as was the character of the infiltrate (microaggregates $p < 0.05$ and permeative $p < 0.05$, Wilcoxon rank sum; interstitial and perivascular, no association). B-lymphocyte percentage was not associated with paraprotein level.

Conclusion

Marrow plasmacytosis at time of diagnosis of WM is usually modest and often peri-vascular. However, higher PC burden, presence of microaggregates, and permeative changes all appear related to higher IgM. Changes to PC compartment following treatment and their correlation with outcome in WM require evaluation.

No conflict of interest to disclose

Tuesday 20 October
HSANZ Free Communications 8

1030-1130
Meeting Room 3

O083

1100

Borderline High Serum Free Light Chain (FLC) Kappa:Lambda Ratios Are Seen Not Only in Dialysis Patients, But Also in Non-Dialysis Dependent Renal Impairment and Inflammatory States

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²*Haematology Department, Pathology Queensland, Princess Alexandra Hospital, Brisbane, Australia*

Aim

An abnormal FLC ratio (normal range 0.26-1.65) is "diagnostic" of clonal plasma cell or lymphoproliferative disorders, although renal reference intervals (extending ULN from 1.65 to 3.1) are required when interpreting the FLC ratio in patients on dialysis. As we have noted other patients with false positive borderline FLC ratios we further characterised the clinical features of these patients.

Methods

We performed a retrospective audit of FLC assays performed between 1/1/2006 and 13/9/2008 at three hospitals and correlated cases with a borderline abnormal ratio (arbitrarily defined as 0.13-0.25 and 1.66-3.2) with their renal function, laboratory measurements of inflammatory parameters, serum and urine protein electrophoresis, clinical features and their final clinical diagnosis.

Results

2524 FLC assays were requested in 955 patients. Of these, 374 tests in 214 patients had a borderline FLC ratio of which 324 tests were from 167 patients known to have a plasma cell dyscrasia. Thus, 47 patients (4.9%) had a borderline abnormal ratio and no known plasma cell disorder: median age 71yrs; median eGFR 34mls/min/1.73m² (range 5-90); median clinical follow-up post FLC assay 264 days. All 47 patients had a borderline high kappa:lambda FLC ratio ranging from 1.67 to 3.2, median kappa FLC 110mg/L (range 12 to 580) and median lambda FLC 49mg/L (range, 7 to 300). No patient without clonal disease had a borderline low ratio. 33 patients (72%) had eGFR < 50ml/min/1.73 m², six being dialysis dependent. Other diagnoses besides renal impairment included infection or gangrene (n=9), chronic liver disease (n=7), malignancy (n=3), colitis (n=2), vasculitis (n=1) and rheumatoid arthritis (n=1). 56% of patients had features on serum protein electrophoresis suggestive of an inflammatory process.

Conclusions

Patients without plasma cell dyscrasias may have borderline high FLC ratios in the setting of non-dialysis dependent renal failure as well as in patients with a polyclonal inflammatory response even in the absence of renal impairment.

No conflict of interest to disclose

A154

Tuesday 20 October
HSANZ Free Communications 8

1030-1130
Meeting Room 3

O084

1115

The Potential role of Curcumin (diferuloylmethane) in Patients with MGUS[Monoclonal Gammopathy of Undetermined Significance]

Terry Golombick and Terry Diamond
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Aims

To determine whether curcumin can decrease paraprotein load and reduce bone resorption in MGUS patients.

Methods

26 patients randomised into 2 groups on a 2:1 randomisation: Group A patients administered curcumin at the start, then crossed over to placebo. Group B patients administered placebo, then crossed over to curcumin. Full blood count, B2 microglobulin, serum paraprotein and immunoglobulin electrophoresis determined. Urine was collected for uNTx measurements.

Group values expressed as the mean \pm standard error of the mean. Data from different time intervals within groups compared using paired student t-test. Statistical significance assigned as $P < 0.05$.

Results

Serum paraprotein concentration ranged from 8-36g/L, with a median value of 20g/L. Of the 17 patients who were commenced on curcumin, 10 had a baseline serum paraprotein level \geq to 20g/L and 7 $<$ 20g/L. In patients with a serum paraprotein \geq 20g/L, fifty percent of these had a 12-30% decrease in serum paraprotein levels in response to curcumin. The most significant decrease was seen at V2 ($p < 0.05$). This decrease remained stable in most patients until they were crossed over to placebo. Patients with a baseline serum paraprotein $<$ 20g/L did not show a response to curcumin. In contrast to a decrease in serum paraprotein seen in patients initiating curcumin therapy, patients receiving placebo demonstrated stable or increased serum paraprotein levels. Twenty seven percent of patients showed a decrease in their uNTx levels when taking curcumin, but the change in uNTx did not reach statistical significance.

Conclusions

Currently no treatment is recommended for MGUS. Given the uncertainty of disease progression to multiple myeloma, early intervention with the aim of reducing the paraprotein load and the potential negative effects on the skeleton would provide an innovative therapeutic tool. Our pilot study suggests that curcumin may decrease both serum paraprotein and uNTX in a select group of patients with MGUS (paraprotein levels \geq 20 g/L). These findings warrant further investigation.

No conflict of interest to disclose

Tuesday 20 October
ASTH Free Communications 2

1030-1130
Hall D

O085

1030

Lepirudin Use and Laboratory Monitoring in Patients on Chronic Haemodialysis with a history of Heparin-induced Thrombocytopenia(HIT)

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⁴Department at Renal Medicine, Royal North Shore Hospital, St. Leonards, NSW, Australia

Aim

The use of anticoagulants to maintain circuit patency in chronic hemodialysis patients with a previous history of HIT remains challenging. A worldwide shortage of danaparoid has led to increasing use of the direct thrombin inhibitor, lepirudin, which accumulates in renal failure. We report the use of lepirudin, and subsequent monitoring via the activated partial thromboplastin time(APTT) and ecarin clotting time(ECT) in 2 patients. Actin FS was the APTT reagent used.

Method

Both patients were on long-term hemodialysis via polysulfone based high flux haemodialysers (studies have shown these membranes to be permeable to lepirudin). Lepirudin was commenced at a dose of 0.1 mg/kg pre dialysis (2-3 times/week). Pre and post-dialysis blood samples were obtained from the arterial needle of the arterio-venous fistula/graft. Pooled normal samples were used to obtain a normal range for APTT and ECT. Lepirudin concentrations of patient samples were obtained via interpolation of a standard curve obtained by spiking pooled normal samples with increasing lepirudin concentrations.

Results

Elevations in APTT and ECT occurred in both patients, suggesting systemic absorption of lepirudin. Initially, there was increased bleeding (epistaxis) in one patient, accompanied by raised APTT. There was persistent clotting of the dialysis circuit in the other patient. Dose adjustments occurred in both patients. At sub-therapeutic to near therapeutic concentrations of lepirudin (0.1-0.6ug/mL), APTT appeared to be more sensitive to dose changes than ECT. With increasing lepirudin concentrations, the linear dose response was maintained with ECT, whereas a plateau response was observed with APTT.

Conclusion

The use of lepirudin in chronic haemodialysis is safe in this setting. It requires frequent laboratory monitoring, with lepirudin retention in the systemic circulation post dialysis and unpredictable lepirudin loss during dialysis. At sub-therapeutic to near therapeutic lepirudin concentrations, APTT is more sensitive to dose adjustments than ECT.

No conflict of interest to disclose

A156

Tuesday 20 October
ASTH Free Communications 2

1030-1130
Hall D

O086

1045

Heparin-induced Thrombocytopenia (HIT): Evaluation of Soluble Platelet Glycoprotein VI as a Biomarker for HIT

Huy Tran,¹ Mohammad Al-Tamimi,² George Grigoriadis,¹ Hatem Salem,^{1,2} Ross I Baker,³ Erica Malan,⁴ Michael C. Berndt,⁵ Robert K. Andrews,² and **Elizabeth E Gardiner**²

¹Haematology Department, Alfred Hospital, and ²Australian Centre for Blood Diseases, Monash University, Alfred Medical Research & Education Precinct (AMREP), Melbourne, Victoria, Australia; ³Department of Haematology, and Centre for Thrombosis and Haemophilia, Murdoch University, Royal Perth Hospital, Perth, Australia; ⁴Monash Medical Centre Haematology Laboratory Clayton Campus, Melbourne Victoria; ⁵College of Medicine and Health, University College Cork, Cork, Ireland

Heparin-induced thrombocytopenia and thrombosis (HITT) is a serious adverse event associated with the widespread use of heparin as an inexpensive, fast-acting and reversible anticoagulant. The final diagnosis of HIT is still based on clinical suspicion as not all heparin/PF4 antibodies cause HIT, and the results from currently available assays for HIT-related Ig or its functional effects on platelets may be negative despite a convincing clinical picture. Consequently, it is difficult to distinguish HIT patients at risk of thrombosis from non-thrombotic HIT. Our previous studies showed that the platelet-specific receptor, glycoprotein (GP)VI, is stable on normal circulating platelets, but undergoes metalloproteinase-mediated ectodomain shedding in response to Fc γ RIIa-mediated platelet activation to HIT antibodies *in vitro*.

Aim

To determine whether soluble GPVI (sGPVI) is an early biomarker of HIT pathology.

Methods

We used an enzyme-linked immunosorbent assay (ELISA) to measure shed sGPVI in plasma from patients at risk or confirmed to have HIT.

Results

Initial studies showed sGPVI levels in plasma from 192 healthy individuals (19.5 \pm 15.4 ng/mL) were independent of age, gender and common GPVI polymorphisms (associated with Gln317/Leu). In contrast, sGPVI levels in HIT patients ranged from normal to >180 ng/mL (mean 81 \pm 37.9 ng/mL) in plasma samples collected at the onset of thrombocytopenia. Levels of sGPVI tended to be higher in confirmed HIT with high anti-heparin/PF4 antibody compared to low antibody levels.

Conclusions

These findings suggest a potential correlation between HIT-related Ig and plasma levels of sGPVI *in vivo*, consistent with experimental data. Current studies will assess whether sGPVI levels are predictive of thrombosis.

No conflict of interest to disclose

Tuesday 20 October
ASTH Free Communications 2

1030-1130
Hall D

O087

1100

Rapid Diagnosis of Heparin-induced Thrombocytopenia by Whole Blood Impedance Aggregometry

Marie-Christine Morel-Kopp^{1,3}, Margaret Aboud^{1,2}, Chandima Kulathilake³, Chee Wee Tan^{1,3} and Christopher Ward^{1,3}

¹ Northern Blood Research Centre, University of Sydney. ² Pacific Laboratory Medical Services (PaLMS), ³ Department of Haematology and Transfusion Medicine, Royal North Shore Hospital, Sydney, NSW Australia.

Aim

Heparin-induced thrombocytopenia (HIT) is a serious complication of heparin use. IgG antibodies to complexes of platelet factor 4 (PF4) and heparin trigger the clinical manifestations of HIT. Only a subset of these antibodies will activate platelets, and these can only be identified with platelet functional assays; most of those being time-consuming and complex to perform. We have developed a whole blood impedance (WBI) test using the new Multiplate[®] analyser to simplify HIT diagnosis confirmation step.

Methods

All samples (N=107) referred to our laboratory over a 10 month period were screened for heparin-PF4 antibodies by an ELISA method (Zymutest HIA IgG). The 4T's score was used to assess HIT pretest probability. Antibody positive samples were further tested by all three functional assays: LTA, SRA and WBI.

Results

Twenty out of 107 samples were Zymutest positive. Thirteen out of twenty samples were positive by LTA (10 patients) and 15 by WBI (11 patients). SRA, considered to be the gold standard, was used as a confirmatory test and 12 were found to be positive (10 patients); the three discrepant samples were weakly positive by LTA or WBI. The prevalence of a positive functional test was strongly correlated with the 4T's clinical risk score, but a small number of low-risk patients had positive functional assays.

Conclusion

In this study, the WBI assay detected all SRA and LTA-positive samples, and was positive for three others, suggesting greater sensitivity. The WBI is easy to perform with rapid turnaround time, and should be considered as an alternative confirmatory assay for platelet-activating HIT antibodies.

No conflict of interest to disclose

Tuesday 20 October
ASTH Free Communications 2

1030-1130
Hall D

O088

1115

Heparin-Induced Thrombocytopenia: evaluation of ELISA assays

Marie-Christine Morel-Kopp^{1,3}, Margaret Aboud^{1,2}, Chandima Kulathilake³, Chee Wee Tan^{1,3} and Christopher Ward^{1,3}

¹ Northern Blood Research Centre, University of Sydney. ² Pacific Laboratory Medical Services (PaLMS), ³ Department of Haematology and Transfusion Medicine, Royal North Shore Hospital, Sydney, NSW Australia

Heparin-induced thrombocytopenia (HIT) is a rare immuno-allergic complication of anticoagulant treatment by heparin and is triggered by the production of antibodies to complexes of heparin (H) and platelet factor 4 (PF4). Enzyme linked immunosorbent assays (ELISA) are designed to detect these antibodies. A critical subset of these antibodies actually mediate HIT *in vivo*, and laboratory evidence for this subset relies on platelet functional assays: ¹⁴C-serotonin release assay (SRA) and light transmission aggregometry (LTA).

Methods

In evaluating four ELISA assays (Stago Asserchrom IgGAM, GTi PF4 IgG and Hyphen HIA IgGAM and IgG) to detect antibodies against H-PF4 complexes, we used patient sera from 111 consecutive requests for laboratory screening for HIT. ELISA assays have high sensitivity for the detection of heparin-dependent antibodies. Specificity for the antibody that confers high risk of triggering HIT was assessed by further testing all ELISA-positive samples by SRA and LTA.

Results

10 samples were positive by SRA (the positivity of one sample was not suppressed by 100U/mL of heparin rendering it potentially non-specific). Of these 10 SRA positive samples, 8 were also positive by LTA.

All four ELISA assays clearly identified these 10 potentially clinically significant positive results.

Non-specific positivity was similar in the four assays (Stago IgGAM 14, GTi PF4 IgG 8, Hyphen HIA IgGAM 8 + 4 equivocal, Hyphen IgG 7 + 3 equivocal).

Conclusion

All assays had well-defined cut-off ODs, with the Stago and GTi assays yielding no equivocal results. One patient with a non-specific positivity in the Stago assay had a clearly demonstrated lupus anticoagulant. As all assays satisfactorily identified all clinically significant antibodies, our decision in favour of the Hyphen IgG ELISA assay was based on cost-competitiveness. The ELISA assays are suitable for screening patient plasma for heparin-dependent antibodies, but a more specific functional assay is recommended to confirm the laboratory diagnosis of HIT.

No conflict of interest to disclose

Tuesday 20 October
Nurses Symposium: Consent in Blood Transfusion

1030-1130
Hall A
1030

Consent in Blood Transfusion Symposium- ANZSBT Survey

K Robinson^{1,2} & R Hunt¹ on behalf of ANZSBT Clinical Practice Improvement Committee

1. *BloodSafe Program, Adelaide, South Australia*

2. *Australian Red Cross Blood Service, Adelaide, South Australia*

Introduction

The NH&MRC/RCNA blood component administration guidelines recommend that patient consent be gained for transfusion and this has been incorporated into ACHS EQulP 4 standards.

Aim: To determine current transfusion consent policy and practice across Australian and New Zealand hospitals. To pilot a standardised audit tool usable by hospital staff without specific expertise in transfusion.

Method

An online survey tool was developed to assess hospital transfusion policy and practice and included a retrospective audit of up to 25 medical records of patients transfused between July 2008 & 2009. An invitation to participate was placed in the ACHS newsletter and sent out to ANZSBT members and through local networks.

Results: Table 1 summarises the results to date from 63 Australian and 11 New Zealand hospitals. 73% of respondents reported having a hospital transfusion consent policy. 57% of transfused patients audited had a signed consent form and 10% had other medical record documentation. 30% had no documented consent for transfusion.

Table 1

Results to 22/6/2009	Overall
Metropolitan Location	54%
Public Hospital	66%
Private Hospital	22%
Public/private combination	12%
Hospitals with a transfusion practitioner	40%
Hospitals with a specific transfusion committee	58%
Hospital transfusion consent policy	73%
Requirement for a signed consent form	58%
Medical Record Transfusion Consent Audits:	
Documented on consent form	57%
Documented in medical record	10%
Documented consent unable to be gained	1%
No documented consent	30%

Conclusion

The ANZSBT consent survey has proved a meaningful and practical tool to assess and benchmark current transfusion consent policy and practice.

No conflict of interest to disclose

A160

Tuesday 20 October
Nurses Symposium: Consent in Blood Transfusion

1030-1130
Hall A
1100

A Patient's Perspective - The Patient from Hell

Shannon Farmer

WA Department of Health Patient Blood Management Program; Medical Society for Blood Management; School of Surgery, Faculty of Medicine, Dentistry and Health Sciences, University of Western Australia; Centre for Population Health Research, Curtin Health Innovation Research Institute (CHIRI), Curtin University, Perth, Western Australia.

Complex issues surround contemporary transfusion medicine. The precautionary principle and vigilant efforts to protect the blood supply from infectious agents have resulted in supply challenges and burgeoning costs. New and re-emerging infectious agents present an ongoing challenge to blood safety efforts. Transfusion-related circulatory overload (TACO), transfusion-related acute lung injury (TRALI), wrong blood component transfused, acute transfusion reactions and bacterial contamination of blood remain the leading causes of transfusion-related death and major morbidity. Greater safety issues may relate to the growing literature implicating transfusion in short- mid- and long-term adverse patient outcomes. Patient informed consent relating to transfusion risks, benefits and alternatives is also becoming an important issue in health care. With greater access to medical information, patients are now demanding more say in health care decisions and requesting explanations on options and alternatives. This talk will dramatise the modern informed patient who has done his/her research and is seeking information on transfusion risks, benefits and alternatives. It highlights challenges and opportunities presented by the modern information age where medical information is the most retrieved on the internet and where surveys suggest consumers outweigh clinicians in accessing sites intended primarily for clinicians. An article in the *British Medical Journal* states, "The fact that patients have access to the same databases as clinicians leads to increased consumer knowledge, which is pushing clinicians to higher quality standards and evidence based-medicine."

Tuesday 20 October
ANZSBT: Ruth Sanger Oration

1130-1230
Hall C

Falsifiability of Component Therapy – Karl Popper, Thomas Kuhn and the Common Sense of Transfusion Medicine

Albert Farrugia

Plasma Protein Therapeutics Association, Annapolis MD, USA

Falsifiability is an important concept in the philosophy of science. Its main developer and proponent was Karl Popper, who asserted that a proposition is scientific only if it is falsifiable, that is, that it can be shown false by observation or experiment. That something is "falsifiable" does not mean it is false; rather, that if it is false, then this can be shown by observation or experiment. Conceptually, Thomas Kuhn's development of paradigm shifts as a way of explaining scientific developments complements and furthers the concept of falsifiability, in that scientists work within a conceptual paradigm that strongly influences the way in which they see data, and will go to great length to defend their paradigm against falsification, by the addition of ad hoc hypotheses to existing theories. Changing a 'paradigm' is difficult, as it requires an individual scientist to break with his or her peers and defend a heterodox theory.

This presentation will assess key concepts in transfusion medicine, including component therapy, haemovigilance and the safety-supply balance in the light of falsifiability and the evolution of the current transfusion paradigm. The overall framework of blood policy and decision making in the author's experience over the past thirty years will be reviewed. It is suggested that there is a need to examine current dogma, asserted as common sense by many, in relation to many of these key concepts as a necessary prelude to the development of a new, more relevant and productive paradigm, which, in its turn, should be falsifiable and conducive to further development....when the time comes.

Tuesday 20 October
HSANZ Symposium: Risk Adapted Therapy

1330-1500
Hall C
1330

Myeloma

Thierry Facon
CHU Lille, France

The concept of risk-adapted therapy has largely emerged with the availability of new prognostic factors and the use of novel agents (specifically thalidomide, bortezomib and lenalidomide). Theoretically, treatment choice could be adapted to individual patient characteristics (for example eligibility for autologous stem cell transplantation (ASCT), preference for one of the novel agents based on comorbidities) or to the risk of the disease (different treatment approaches for low-risk or high-risk MM). In many countries, cost will also effect treatment choice.

Patient- based risk-adapted therapy

Eligibility for ASCT is somewhat variable across countries and is still under debate in patient subgroups, e.g., in patients >65 years of age and in those with renal failure. The use of novel agents, such as MP with or without thalidomide or bortezomib, in frail elderly patients is also a matter of debate, especially in community hospitals. In limited cases, the patient's history strongly suggests the use of a specific agent. For instance, a history or risk of DVT may indicate treatment with bortezomib, while a history of peripheral neuropathy may point to the use of lenalidomide. Other treatment considerations warrant careful consideration, such as the use of bortezomib in patients with renal failure or the use of lenalidomide or thalidomide in patients living far from a hospital.

Recently assays have been developed, using GEP to identify patients likely to respond in the early courses of therapy (bortezomib study conducted at the UAMS)

MM risk-adapted therapy

Despite major advances in identifying risk factors, using cytogenetics, FISH and more recently GEP and CGH, selecting definitive treatment based on cytogenetic abnormalities (IMW 2009 consensus panel 2) is premature. Targeted therapies that can reverse primary cytogenetic changes do not yet exist for MM. Risk-directed trials have been rare. This concept was probably pioneered by the IFM group 10 years ago in the IFM 99/02 and IFM 99/03-04 trials, for low/intermediate and high-risk patients, respectively (based on beta-2-microglobulin and del13). At the present time, the only example of trials researching GEP-defined, risk-directed therapies appears to be the total therapy programs TT4 and TT5 from UAMS.

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HSANZ Symposium: Risk Adapted Therapy

1330-1500
Hall C
1400

Lymphoma

Martin Dreyling

Abstract not received at time of going to print

Tuesday 20 October
HSANZ Symposium: Risk Adapted Therapy

1330-1500
Hall C
1430

Monitoring Response in Chronic Myeloid Leukaemia

Tim Hughes

In CML, close monitoring of response is needed to enable early recognition of drug resistance and disease progression. The risk of imatinib resistance is strongly related to the phase of disease with almost universal resistance in blast crisis and a very low risk of resistance in de-novo chronic phase CML. Resistance can be either primary or secondary. Primary resistance, seen in less than 10% of newly diagnosed CML patients is poorly understood and rarely attributable to kinase domain mutations. Poor intracellular uptake of imatinib and suboptimal plasma levels may be important contributors to primary failure. Secondary resistance emerges in about 10-15% of CML patients treated with imatinib first-line. Around 60% of cases have kinase domain mutations. To address the problem of imatinib resistance two second generation ABL kinase inhibitors, dasatinib (BMS) and nilotinib (Novartis) have been developed and assessed in CML patients. These drugs have several potential advantages over imatinib that may allow them to overcome drug resistance. They are both much more potent than imatinib so that if imatinib resistance is mediated by additional copies of the Ph-chromosome or overexpression of BCR-ABL, enhanced potency may lead to greater efficacy. Nilotinib and dasatinib are also less vulnerable to resistance mediated by kinase domain mutations. Whereas imatinib is partially or totally resistant to over 90 amino acid substitutions in the kinase domain of BCR-ABL, dasatinib and nilotinib are vulnerable to a limited spectrum of mutations. The emerging evidence from clinical trials in imatinib-resistant patients is that in-vitro sensitivity studies using mutant constructs of BCR-ABL are predictive of clinical resistance to second-line therapy. It is also evident that treatment of imatinib resistance when it is still only cytogenetic is more effective than treatment of hematologic resistance. This strengthens the argument for close molecular and/or cytogenetic monitoring. The indications for regular mutation screening include imatinib failure, advanced phase disease and in any patient being considered for a second line tyrosine kinase inhibitor

Tuesday 20 October
ANZSBT Nurses Combined Symposium: Blood Management

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1330

Do Transfusion Guidelines Reduce Transfusion Requirements

Dafydd Thomas

Abertawe Bro Morgannwg University NHS Trust, Swansea, Wales, UK

There are numerous examples of transfusion guidelines available in many languages and many countries. As a doctor working in the UK I have been involved and am therefore aware of guidelines that have been produced to help guide my clinical practice. More recently I have been involved as a representative of the Royal College of Anaesthetists with a number of guidelines aimed at promoting appropriate use of red cells, fresh frozen plasma and platelets in addition to the management of massive haemorrhage.

The surprising result of this involvement is my increased awareness of how few of my colleagues back at my base hospital are unaware or not interested in these guidelines. They most certainly do not therefore incorporate the recommendations suggested in these guidelines into their clinical practice.

Why then is there such poor awareness about transfusion guidelines amongst the multidisciplinary groups within our clinical environment. There is no doubt that many healthcare workers are overwhelmed by information and guidelines need to be succinct, up to date and evidenced base to have any impact. They also have to be made easily available to busy clinicians and incorporated into local guidelines and standard operating protocols.

Of course even when this is achieved change is never easy or quick. Many authors have described a lag time of implementation of even the best evidenced base practice which can take 15 to 20 years to become embedded in practice. In my presentation I will give examples of how practice and patient care has changed in response to guidelines - albeit over a predictable 15 year period.

Tuesday 20 October
ANZSBT Nurses Combined Symposium: Blood Management

1330-1500
Hall B
1400

Where Does All Our Blood Go?

Rachel Whitford

Department of Health, Government of South Australia

Background

Blood transfusion is a common clinical procedure essential for treatment of specific patient groups. Ageing populations and advancing medical care are driving an increasing demand for red cells against a decreasing donor pool. Electronically linked pathology, clinical and patient epidemiological databases can be useful in developing data systems to monitor, compare and model transfusion practices.

Methods

A linked electronic database was developed using clinical, epidemiological and red cell transfusion data from twenty five public hospitals across South Australia.

Data were electronically extracted from four pre-existing databases on all hospital admissions during the 2006 calendar year. Data analysis included aggregation of blood usage by surgical and medical procedures (ICD-10-AM codes), specialty related groups (SRGs), patient demographics and type of admission.

Results

Of the 327,995 admissions, 12,803 (3.9%) received a total of 40,124 red cells (average: 3.1, range: 1 to 64). Patients >65 years accounted for 56.9% used. Red cells were transfused in 6.4% of surgical admissions, accounting for 46.1% of total use and in 3.3% of medical admissions, 47.6% of total use. The remaining 6.3% use was associated with various types of endoscopic procedures. Red cells were transfused in 5.5% of emergency admissions, 61.9% of total use and in 2.8% of non-emergency admissions, 38.1% of total use. SRGs related to the treatment of haematological malignancies, orthopaedics, GIT endodscopy, cardiothoracic surgery, medical oncology, colorectal and vascular surgery formed the largest clinical entities, accounting for 55.0% of all red cells transfused, but only represented 19.1% of all admissions.

Conclusion

The electronic linkage of laboratory, epidemiological and clinical data into a single database has provided baseline information on red cell use in relation to patient diagnoses, clinical procedures and demographics. The study highlights patterns of blood use and will provide useful input into blood contingency planning based on known disease trends, patient profiling and transfusion audits.

No conflict of interest to disclose

Tuesday 20 October
ANZSBT Nurses Combined Symposium: Blood Management

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1430

Developing a Blood Management Program

Sherri Ozawa

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The pressing issues surrounding blood transfusion, including ever increasing costs, emerging pathogens, inadequate supply, and the association of poor clinical outcomes has prompted numerous institutions across the world to implement organized patient blood management programs. Such programs approach appropriate transfusion practice from a number of different angles. Current blood use patterns by physicians and specific clinical areas must be analyzed, blood ordering practices must be scrutinized, and measures must be taken to ensure that patients for whom blood is not an option have their wishes and needs sufficiently addressed. Ethical and legal aspects of patient blood management must be taken into consideration and appropriate documentation and procedures must be created to address the specific needs of this population.

The implementation of such programs requires the acquisition of knowledge, manpower, equipment, and ongoing education to be successful. An entirely new mindset is necessary in the culture of the institution, a mindset which views transfusion as an invasive procedure that should be carefully undertaken only when evidence of benefit outweighs risk, it is acceptable to the patient, and no alternative is available. This requires a dramatic change in practice for many physicians who have been trained to use allogeneic transfusion as a default treatment to manage anemia, even when safer options exist. Hospitals that implement such programs often see dramatic reduction in financial expenditures for blood products, improved provider and patient satisfaction, and improved clinical outcomes.

Tuesday 20 October
ASTH Symposium: Rare Bleeding Disorders

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Hall D
1330

Platelet Function Disorders – Mechanism and Diagnosis

Marco Cattaneo

Clinica Medica, Ospedale San Paolo, Università degli Studi di Milano. Milano, Italy

Inherited platelet disorders can alter circulating platelet numbers, function or both. These conditions are typically manifested by symptoms of excessive mucocutaneous bleeding and rapid onset, excessive bleeding following invasive surgical and dental procedures or trauma. Disorders of platelet function include defects of: 1) platelet receptors for adhesive proteins (e.g., Bernard-Soulier Syndrome, Glanzmann Thrombasthenia), 2) platelet receptors for soluble agonists (e.g., defects of P2Y₁₂), 3) platelet granules (e.g., storage pool deficiency); 4) signal transduction pathways (abnormalities of the arachidonate/thromboxane A₂ pathway, of the stimulatory G-protein alpha-subunit), 4) procoagulant phospholipids (Scott syndrome). The diagnostic laboratory assessment for evaluation of a suspected platelet function defect should include an assessment of blood counts, a careful evaluation of the blood smear, and an evaluation of platelet size (mean platelet volume). Assessments of platelet function, by assays of aggregation and secretion, are commonly used for the diagnostic evaluation of platelet disorders. More specialized tests are helpful to confirm conditions that have been suspected based on the results of the initial screening tests. Therapy is not warranted for bruising. Platelet transfusions should be reserved for individuals with serious bleeding unresponsive to medical therapies. Recombinant Factor VIIa is useful in the treatment of bleeding episodes of patients with alloimmunization from platelet transfusions. Desmopressin and fibrinolytic inhibitors are useful for treatment of less severe bleedings. Treatment of menorrhagia needs to be individualized, and should take into consideration the individual's wish for pregnancies or contraception.

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ASTH Symposium: Rare Bleeding Disorders

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Factor XIII Deficiency

Elizabeth M Duncan

Haematology Division, Institute of Medical and Veterinary Science, Adelaide, SA, Australia

Factor XIII (FXIII) is a transglutaminase that circulates in plasma as a tetramer, comprising two catalytic A sub-units (FXIII-A) and two carrier B-subunits (FXIII-B). In fibrin, activated FXIII-A catalyses the covalent cross-linking of adjacent α and β chains of fibrinogen to stabilise the clot. It also incorporates antifibrinolytic factors into fibrin, to improve resistance to fibrinolysis. Inherited, severe FXIII deficiency is a rare, homozygous recessive condition (1 per 2 million) with patients characteristically showing delayed bleeding and poor wound healing. Commonly reported clinical symptoms include umbilical stump bleeding, intra-cranial haemorrhage and spontaneous abortion. Both cryoprecipitate and a highly purified formulation of FXIII-A provide effective treatment, and a new recombinant product is currently undergoing clinical trials. Prophylaxis prevents bleeding, with levels as low as 5% sufficient to prevent spontaneous bleeding and maintain pregnancy. In the non-bleeding patient FXIII has a half-life of ten days, but this may be shorter in the bleeding patient and the levels required to support haemostasis much higher. The prothrombin time and APTT will not detect a deficiency of FXIII, and test requests should be guided by clinical symptoms. Quantitative measurement of FXIII-A is preferred for laboratory diagnosis, using methods to measure FXIII activity or antigen levels. These methods differ in degree of automation, levels of accuracy at low levels of FXIII and cost. Clot lysis tests are still commonly used to screen for FXIII deficiency but they will only detect a severe deficiency, may give a false positive result and cannot be used to monitor treatment. With more than 60 mutations of the A-subunit gene and 4 mutations of the B-subunit gene described, genetic analysis can be of value to confirm a diagnosis. Acquired deficiency of FXIII, e.g. secondary to auto-antibodies or liver disease, is also a clinically significant disorder requiring diagnosis and suitable treatment.

No conflict of interest to disclose

Tuesday 20 October
ASTH Symposium: Rare Bleeding Disorders

1330-1500
Hall D
1435

Paraproteinemia and Coagulation Disorders

Simon McRae

SA Pathology Royal Adelaide Hospital, Adelaide, SA, Australia

Abnormal screening coagulation tests are frequently observed in patients with underlying plasma cell disorders, with the majority of such patients having no bleeding history. The mechanism of the prolongation of clotting time remains unclear in the majority of these patients. However, a small percentage of such patients will have rarer conditions that are associated with a clinically significant coagulopathy, such as acquired von Willebrand Syndrome, amyloid associated acquired factor Xa deficiency, and inhibitors directed against other coagulation factors. The incidence, diagnosis, and management of the paraprotein related acquired coagulopathy will be discussed, including examples of recent single centre experience with managing such patients around the time of major surgery.

Tuesday 20 October
HSANZ Symposium: Drug Approval

1530-1630
Hall C
1530

Access to New and High Cost Drugs in Australia - Some Insights

Andrew Roberts^{1,2,3}

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In Australia, the TGA licences pharmaceuticals for sale and use after a formal evaluation of efficacy and safety. However for most new drugs, access by the general population relies on the Federal Government including the drug on the Pharmaceutical Benefits Scheme. For the past three decades, inclusion on the PBS has required a formal evaluation by an expert committee, the PBAC. By legislation, no item can be listed on the PBS without a positive recommendation by the PBAC. Successive Australian governments have tasked the PBAC to consider the cost-effectiveness of medications as a key element within the decision-making process. I will outline the current processes and highlight how cost-effectiveness is evaluated, with particular reference to how this applies to haematology-related drugs.

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New Zealand Experience

Peter Browett

Abstract not received at time of going to print

Tuesday 20 October
HSANZ Symposium: Drug Approval

1530-1630
Hall C
1610

Challenges to New Drug Development and Approval: Experience and Opinion of an Industry Medical Director

Kevin P Lynch

Celgene Pty Ltd, Melbourne, Australia

In an environment characterised by an ageing population, technological advances enabling new target identification, rapidly emerging economies of third world countries and a lifestyle contributing to an epidemic of chronic disease, one would speculate that the Pharmaceutical Industry would be very buoyant indeed. However, an understandable and increased level of scrutiny by regulators and payors, enormously high development costs, and fierce competition for patients and resources provides substantial constraints to this enthusiasm. Indeed, despite large increases in investments, the number of new medical entities approved by authorities such as the FDA and TGA has roughly halved in the last decade. Identification of lead compounds for clinical development is less of a bottleneck, especially in the field of cancer medicine where hundreds of new compounds targeting recently understood oncogenic pathways are in development. The challenge is more focused on efficient completion of Phase I and II studies utilising better biomarkers, codevelopment of molecular diagnostics, and rigorous attention to translational questions that can help design the pivotal studies as well as feeding clinical information all the way back to the discovery programme. Adoption and acceptance of flexible designs for registration studies, often using less conventional statistical techniques, may be crucial to more efficient late stage development programmes. Demonstrating improvement in survival outcomes at this stage is made more complex by the confounding features of additional lines of therapy and the sometimes contentious inclusion of a cross-over design. In this respect, the understanding and support from academic investigators and regulatory authorities is key. Fundamental to the process of drug development is a recognition that regulatory approval of a drug does not equate with access. Australian and New Zealand have in some ways lead the way as countries that demand convincing health economic data before approving funding for new medicines. It is only reasonable that the Industry in seeking funding for high cost medicines, should bring to the use of a drug a high level of confidence that it will work in the selected population. This will only be possible through close attention to disease biology, drug mechanism of action and appropriate patient selection. Early and consistent engagement of the academic research community is critical to this effort.

The opinions expressed in this abstract are those of the author alone and do not necessarily reflect those of Celgene nor of the Pharmaceutical Industry more broadly

Tuesday 20 October
ANZSBT Symposium: IVIg – Have We Got It Right?

1530-1630
Hall B
1530

A National Overview

Alison Turner

Abstract not received at time of going to print

Tuesday 20 October
ANZSBT Symposium: IVIg – Have We Got It Right?

1530-1630
Hall B
1550

A View from the Australian Red Cross Blood Service

Marija Borosak

Australian Red Cross Blood Service (ARCBS), Melbourne, Victoria, Australia

Intravenous immunoglobulin (IVIg) is a unique and precious plasma derived product used in immune replacement and immune modulation for a number of clinical indications. In Australia ARCBS collects plasma from volunteer, non-remunerated donors, the plasma is pooled and fractionated by CSL Bioplasma. Currently Australia is not self-sufficient in IVIg, and imported IVIg supplements domestic supply.

IVIg has been managed intensively for over a decade as IVIg demand has been increasing, in recent years at about 14% per annum. ARCBS has been an integral participant in this management and actively manages IVIg clinical review and supply.

In December 2007 new Criteria for the Clinical Use of Intravenous Immunoglobulin in Australia (the Criteria) were approved by Australian Health Ministers with input from clinical experts, professional colleges, societies and other organisations including substantial input from the ARCBS Transfusion Medicine Team (TMS). The Criteria were implemented from March 2008 with a six month transition phase and have ensured more equitable access to IVIg supplied across Australia under the National Blood Agreement, providing a consistent framework for jurisdictions by which all IVIg requests are assessed. The Criteria identifies clinical conditions where IVIg can be used provided qualifying criteria are met. The introduction of the Criteria has lead to a period of change with some reclassification of patients according to Criteria labels, as well as increased use of IVIg due to changes to disease indications included for access to IVIg under the National Blood Agreement. The ARCBS TMS team, review requests and outcomes as required according to the Criteria. Data on requests, issues, outcomes and complications of therapy are recorded in the national STARS database.

Overall there has been successful implementation of the Criteria with opportunity to build further on a good foundation. The review of the Criteria is anticipated to commence within the next 12 months.

No conflict of interest to disclose

Tuesday 20 October
ANZSBT Symposium: IVIg – Have We Got It Right?

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1610

A Clinician's Perspective

Robert Heddle

SA Pathology, IMVS Campus, Adelaide, Australia

Clinical Immunology Unit, Royal Adelaide Hospital, Adelaide, Australia

Aim: Descriptive analysis of difficulties experienced in managing IVIg as (1) head of a Clinical Immunology Unit in a major teaching hospital (2) Chairperson of SA IVIg Users' Group

Methods: Review of emails, correspondence considered in role as chairperson of IVIg Users' Committee. Discussions with clinical and ARCBS colleagues

Results

Generic problems

- 1) Difficulties in promoting to clinicians the December 2007 Criteria
- 2) "Grandfathered" patients; IVIg started on criteria no longer current
- 3) Reluctance of many clinicians to communicate details of prospective IVIg recipients
- 4) Diversity of gate-keepers (in SA)
- 5) Major difficulties in meeting review criteria- diversity of criteria, poor understanding, logistic difficulties of follow up/communication and poor definition of pre- and on-treatment clinical criteria.

Condition specific problems

- 1) Specific antibody deficiency- deals with subjects with recurrent sino-pulmonary infection/bronchiectasis with normal total IgG but suspected inadequate antibody responses. Little consensus on "what" antibody responses to measure, let alone "how" or normal ranges; propose more emphasis on rigorous clinical criteria
- 2) Acquired hypogammaglobulinaemia secondary to haematological malignancies. Low IgG inherent to both criteria but frequently not determined. Review criteria suggest trial off IVIg- this has been performed rarely; is it a reasonable review criterion?
- 3) Adult ITP. Criteria for ongoing IVIg require adequate trials of other therapies and need to maintain platelet count >30,000. These details are often missing from requests.

Conclusions

The December 2007 Criteria represent a necessary attempt to advance evidence based use of a limited resource. The criteria are necessarily developmental and their application difficult. Authorities need to continue to review the utility of criteria. Clinicians cannot be helped unless they communicate relevant clinical details.

No conflict of interest to declare

Tuesday 20 October
ASTH Symposium: Acquired Platelet Disorders

1530-1630
Hall D
1530

The Good and the Bad - Plasma Lipoproteins in Coagulation and Venous Thrombosis

Natalie Pecheniuk

School of Pharmacy and Molecular Sciences, James Cook University, Townsville, QLD, Australia

It is widely accepted that high density lipoproteins (HDL), the good cholesterol, shows inverse correlation with atherothrombosis. The role of HDL in venous disease however has only recently become evident. The molecular mechanisms of HDL's beneficial antithrombotic qualities observed in both venous and arterial thrombosis remain to be elucidated but evidence suggests that HDL and not LDL shows activated protein C (APC) cofactor activity. HDL particles appear to enhance the protein S-dependent APC pathway down-regulating thrombin generation via enhanced proteolysis of factor Va. Strong correlations are observed in both biochemical and translational clinical studies between HDL particles, in particular the larger HDL particles, with decreased thrombotic potential and venous thromboembolism (VTE). These findings suggest molecular components of the HDL particle may contribute to these beneficial observations. In particular, elevated levels of the major apolipoprotein in HDL, apolipoprotein AI, appear to correlate with reduced VTE recurrence. Recent studies have also suggested that apolipoprotein AI incorporation into lipid vesicles ablates the procoagulant effect of anionic lipids. Further, many enzymes such as lipases, CETP and PLTP are involved in lipoprotein metabolism and contribute to the flux of cholesterol, lipid and protein components between lipoprotein particles. Genetic associations with polymorphisms of CETP have been observed in VTE and these specific genotypes known to associate with an unfavourable lipoprotein profile and elevated plasma CETP were observed to be more prevalent in VTE subjects. Interestingly, elevated plasma CETP antigen has recently been shown to contribute to blood coagulability. The potential benefits of HDL particles stem further than its well described role in reverse cholesterol transport and includes anti-inflammatory, anti-oxidant, anti-apoptotic and anti-thrombotic properties, all of which have a role in the aetiology of venous thrombus formation.

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Stroke, Thrombotic Thrombocytopenic Purpura and ADAMTS13 Activity

Ross Baker, Grace Gilmore and Jim Thom

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ADAMTS13 plays an important role in preventing arterial thrombosis and thrombotic thrombocytopenic purpura (TTP) by cleaving the thrombogenic ultralarge high molecular weight von Willebrand factor (ULvWF) multimers to less active forms. When the enzyme is deficient, circulating ULvWf causes thrombosis by the formation of intravascular aggregation of platelets in the microcirculation and at sites of high shear flow associated with blood vessel damage. Diagnostic assays for ADAMTS13 antigen, function and autoantibodies are now becoming more widely available but their utility for clinical practice is uncertain.

Generally patients with idiopathic TTP have low ADAMTS13 activity when compared to those with other causes of secondary TTP such as metastatic tumours, organ transplantation or the use of drugs such as mitomycin C or cyclosporine. The secondary causes of TTP do not tend to respond to plasma exchange probably because the TTP is caused by the massive endothelial stimulation and release of ULvWf rather than ADAMTS13 deficiency.

Around 75% of patients with idiopathic TTP have severe deficiency of ADAMTS13 (less than 5%) at diagnosis. Recent data suggests that patients with idiopathic TTP with normal or deficient ADAMTS13 activity had a similar response rate to plasma exchange and short term survival. However, those who have severe ADAMTS13 deficiency had a significant increased risk of relapse. In smaller studies the detection of auto-antibodies to ADAMTS13 was also associated with refractory disease, higher mortality and an increased risk of relapse. Rituximab has been reported to benefit some patients with primary TTP particularly those with detectable auto-antibodies.

During plasma exchange around a third of patients will have persisting ADAMTS13 deficiency despite a good clinical response. Around 60% of these patients will relapse. In those who had severe ADAMTS13 whose levels are corrected with treatment, relapse almost is always associated with a falling ADAMTS13 level.

Low of ADAMTS13 and high vWf are strongly associated with risk of cardiovascular disease and in experimental models infusing recombinant ADAMTS13 reduces mortality without increasing haemorrhage.

Measuring ADAMTS13 activity may provide clinicians with useful biomarker for prognostic information in patients with TTP and a rationale for targeted therapy with recombinant ADAMTS13 in those severely deficient or with a vascular event.

Tuesday 20 October
Nurses Free Communications 6

1530-1630
Hall A
1530

O089

A Changing Model of Care – The Role of the Blood Transfusion Clinical Nurse Consultant

Emily Allen

Prince of Wales Hospital, Randwick, NSW, Australia

Aim

As a changing model of care the CNC for transfusion was introduced to Prince of Wales Hospital in September '07. The aim was to develop, implement and establish a sustainable role that can initiate and maintain an ongoing process of improvement in quality and safety of transfusion practices at Prince of Wales Hospital. Since this role commenced a number of initiatives have been introduced to the hospital and Area Health Service.

Methods

3 monthly red cell appropriateness audits of 20 consecutive transfusion episodes in specific high use clinical areas, and yearly appropriateness audits of 1 week red cell transfusion episodes hospital wide are carried out, as well as yearly audits of documentation and knowledge surveys.

An education and assessment process is provided by Bloodsafe e-learning and regular education sessions on blood and blood products, transfusion safety and up to date practices in transfusion medicine.

Communication improvement initiatives include the publication of a monthly transfusion newsletter, 'Bloodflash', providing timely transfusion news via Northern Network email and participation in local and Area Health Service transfusion committees.

Results

Hospital wide audits identify a significant improvement in inappropriate transfusion rates – 32% in March '08 compared to 21% in March '09. 3 monthly audits in one specific high use clinical area show a marked improvement in inappropriate transfusions from 25% in February '07 to 5% in September '08.

Documentation audits in September '07 compared to June '09 show considerable improvements in the consenting process from 28% to 64%.

Conclusion

There have been some convincing developments in transfusion practices at Prince of Wales Hospital and by continuing to support the initiatives that have been implemented by the CNC it is hoped that there will be further reductions of inappropriate transfusions and an increase in quality and safety in transfusion practices around the hospital.

No conflict of interest to disclose

A180

Tuesday 20 October
Nurses Free Communications 6

1530-1630
Hall A
1545

O090

Collaborative Nurse Led Transfusion Clinic – 18 months On!

Charlene McLaren

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Introduction

In 2006 there were increasing numbers of elderly frail patients causing increased stress and frustration for medical staff in the Haematology Outpatient service at Christchurch Hospital. This highlighted the need for change to occur within the service and the introduction of a Collaborative Nurse Led Transfusion Clinic was proposed and implemented in November 2007.

Aim

The aim of this clinic was to allow a nurse who had completed appropriate post graduate qualifications to carry out comprehensive assessments for transfusion dependant patients who had stable haematological conditions or were palliative.

There was a need to improve patient assessment pre transfusion and improve patient management plans. The nurse was able to do a full holistic assessment addressing a range of problems relating to the needs of frail elderly patients. The establishment of this clinic decreased medical loads and department fiscal costs. This also created increased self autonomy and opportunities for the nurse who had advanced haematology clinical knowledge.

Conclusion

Now 18 months on this has been extremely exciting for the Haematology Service. The clinic is continuing to improve and expand with positive patient experiences. It also will create further opportunities for other senior nurses to expand their roles while providing a more holistic service for our patients thus continuing to improve Outcomes for the out patients in the Haematology Outpatient service.

No conflict of interest to disclose

Tuesday 20 October
Nurses Free Communications 6

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Hall A
1600

O091

Influencing Factors on Chelation Compliance in Transfusion Dependant Haemoglobinopathy Patients

Emily Allen

Prince of Wales Hospital, Randwick, NSW, Australia

Aim

Complications of iron overload are thoroughly documented in the literature and methods of chelating excess iron are effective when administered as prescribed. Regularly transfused patients are required to take full responsibility in complying with their prescribed chelation regime at home although there are a number of physical and psychological issues that impact on the patients' ability to comply with their treatment. By understanding these issues, providing relevant education and support health professionals can work with the patient to develop strategies that may help to improve their compliance. Improving the patients understanding of haemoglobinopathy and related complications can lead to an increased autonomy in the management of their condition and demonstrate a positive influence on patient compliance.

Patient case studies will be utilised to illustrate some of the issues, methods implemented, and their latest outcomes.

Methods

One to one patient support sessions are provided to identify issues, provide education and improve the patients' knowledge. Presentation of graphs showing individual patient ferritin levels and T2* measurements are utilised as a visual aid and educational tool during these sessions and facilitated patient focus groups are held quarterly to provide patients with the opportunity to voice issues and concerns.

Results

Influencing factors include site problems with subcutaneous infusions, patients feel too busy to prepare or forget to take tablets among others.

Preliminary results of mean ferritin levels indicate an overall reduction from 2680ug/L in Jan '07 to 1551ug/L in May/June '09. Recent T2* results show a decrease in iron stores following appropriate changes to chelation therapy regimes combined with improved compliance.

Conclusion

This work in progress has provided the foundation to explore the clinician's understanding of the issues affecting patient compliance to chelation therapy and in turn encourage the patients' active participation in the management of their haemoglobinopathy to prevent or reduce iron overload.

No conflict of interest to disclose

A182

Tuesday 20 October
Nurses Free Communications 6

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0092

So You Think Giving a Blood Transfusion is Easy!

Linley Bielby^{1,2}, Erica Wood^{1,2}, Russell Hunt³, David Roxby³, David Westerman² and Axel Hofmann⁴

¹*Australian Red Cross Blood Service, Melbourne, Victoria, Australia,* ²*Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia,* ³*Flinders Medical Centre, Adelaide, South Australia, Australia,* ⁴*Medical Society for Blood Management, Vienna, Austria*

Introduction

Administration of red cells is a common activity, especially for Haematology/Oncology Nurses, however many would not consciously consider the risks, time and complexity-associated costs related to transfusion.

Methods

To determine the real cost of red cell transfusion in Australia, each step of the transfusion process was mapped including phlebotomy, specimen delivery, laboratory procedures, collecting units for transfusion and bedside administration. Nurses play key roles in many of these steps.

Results

The process maps demonstrated the complexity (number of steps involved in each aspect of the transfusion process) and points where critical failures can occur. In clinical areas these include: phlebotomy, requiring 35 process steps; collection of the unit from the laboratory, 20 steps or more; and actual administration of the unit; >120 process steps. Critical points where failure can occur include checking correct patient identification (ID) before pre-transfusion sample collection and administration. Incorrect patient/sample ID and cross-match sample documentation required an average 4% of patients to be re-bled at one centre. Sample collection time ranged from 3.5 – 11 mins. The average time to collect a red cell unit from the blood bank for transfusion was 12 mins and the average associated nursing time for administration of this red cell was 28 mins, with extra time required for additional precautions (~2-3 min each additional intervention). Preliminary financial data attributed to each of process step indicates that the cost is AUD\$650.00-\$690.00 for administration of a single red cell unit.

Conclusions

The transfusion process is complex, time-consuming and involves many individuals and resources. Nurses have key roles in the transfusion process and by understanding the real risks and costs related they can encourage better transfusion practice, to optimise red cell utilisation, to improve patient outcomes, and to reduce risks and costs of unnecessary transfusions.

This study was funded by an unrestricted grant from Amgen, Australia.

Tuesday 20 October
HSANZ Masterclass 7

1630-1730
Meeting Rooms 1/2

Myeloma - from Genetic Abnormalities to Targeted Therapy

Thierry Facon
CHU Lille, France

Our understanding of multiple myeloma (MM) disease biology has increased substantially over the recent years and has led to the identification of a number of factors that are associated with a poor prognosis. The 2009 International Myeloma Workshop consensus panel 2 summarized that the specific cytogenetic abnormalities considered as poor risk comprise cytogenetically detected deletion of chromosome 13 (del13), translocation(4;14) and deletion of chromosome 17 (del17p), as well as detection by FISH of t(4;14); t(14;16) and del17p.

A number of studies have investigated the novel agents in patients with poor-risk cytogenetic factors to establish if these agents may offer the possibility of improving outcomes in these patients over traditional treatments. These studies have many limitations: 1) they are usually post-hoc subgroup analyses of phase 3 studies or expanded access programs, 2) limited number of patients, 3) in most studies response was evaluated but TTP, PFS and OS were not reported. Bortezomib in MM patients with cytogenetic abnormalities was investigated in several studies. In a retrospective analysis of the SUMMIT and APEX trials, response and survival appeared to be similar in bortezomib-treated patients with and without del(13). In the front-line setting, similar results have been obtained. Bortezomib induction regimens achieved similar response rates in patients with or without cytogenetic abnormalities. In the IFM bortezomib/dexamethasone vs VAD study, the combination of bortezomib and dexamethasone resulted in a significantly higher VGPR rate than VAD in patients with del(13) and t(4;14) and/or del(17p). In addition, in patients with newly diagnosed MM not eligible for transplantation, who were treated with bortezomib plus melphalan and prednisone (MPV), response, TTP and OS were not negatively affected by the presence of t(4;14), t(14;16) and del(17p). Collectively, these data suggest that bortezomib may overcome, at least partly, the poor prognostic impact of del13 and t(4;14).

Data from several lenalidomide studies suggest that del(13) and t(4;14) do not influence response rate or EFS. However, a recent report by the IFM group indicated that in patients treated with lenalidomide plus dexamethasone, the presence of del(13) and t(4;14) resulted in a significant reduction in response rate, PFS and OS compared with patients without cytogenetic abnormalities.

Overall, further studies with larger patient numbers and longer follow-up are needed to confirm these encouraging results and to assess the effect on PFS and OS.

Tuesday 20 October
HSANZ Masterclass 8

1630-1730
Meeting Room 3

Waldrenstrom Macroglobulinaemia – What's New?

Martin Dreyling

Notes:

Tuesday 20 October
ANZSBT Masterclass 10

1630-1730
Meeting Room 4

Haemodilution – The Role in Transfusion Practice

Paul M Ness

Transfusion Medicine Division, Johns Hopkins Medical Institutions, Baltimore, Maryland, USA

Acute normovolemic haemodilution (ANH) has been advocated to reduce or eliminate the need for volunteer blood during surgery. The principle of ANH is to reduce the patient's haematocrit by phlebotomy prior to surgical blood loss so that, for a given volume of surgical bleeding, a smaller mass of red cells will be lost. Since the amount of blood saved can be small, another important mechanism by which ANH might reduce transfusion requirements is by providing fresh autologous platelets and plasma that can correct the acquired perioperative haemostatic deficiencies.

Despite its introduction over three decades ago, ANH has not emerged as a standard intervention, nor has it been discredited. The use of ANH began in cardiac surgery and was driven by a desire to reduce blood required to prime the extracorporeal pump. The AIDS epidemic shifted the focus to minimizing exposure to homologous blood and its adverse effects. In this context, ANH has been advocated as a more cost effective means of avoiding transfusion than preoperative autologous donation (PAD) or intraoperative salvage.

The benefits of ANH may be quite modest, depending on the magnitude of surgical blood loss and patient characteristics. The benefit increases with the extent of haemodilution. Moderate ANH has generally been carried to a haematocrit of 25-35%, based on the concept that oxygen delivery is maximal at a haematocrit of about 30% and a reluctance to accept the risks of more extreme ANH. Even with optimal implementation, blood conservation is modest in the absence of extreme haemodilution. On the other hand, we have demonstrated that aggressive ANH can be performed with a blood substitute, so-called augmented ANH, in aortic aneurysm patients. Since ANH has been proposed as an effective alternative to transfusion. Ness attempted to assess its value compared in a prospective, randomized, controlled trial of ANH versus PAD in radical prostatectomy; no differences in transfusion utilization or perioperative morbidity were found but there was no untreated control group.

Often discussed but little explored is the impact of ANH on the hemostatic mechanism. One of the touted benefits of ANH has been the preservation of coagulation factors and platelets, since blood removed in the operating room is not cooled. We provided limited evidence that blood collected by ANH may reduce postoperative bleeding in cardiac surgery compared to control patients.

A final concern about ANH is whether it is cost effective. In addition, the resources to perform ANH in the operating room require training and reliable protocols and it is not clear that many hospitals have set up the appropriate systems so that ANH can be offered as a reliable alternative to blood transfusion in elective surgery. If a safe blood substitute becomes available, augmented ANH may become a feasible option for high risk cases or religious objectors in the future.

A186

Tuesday 20 October
ANZSBT Masterclass 11

1630-1730
Meeting Room 5

Preparation and Implementation of a Practical Hospital Based Blood Shortage Contingency Plan

Taher Rad

Notes:

Tuesday 20 October
ASTH Masterclass 12

1630-1730
Meeting Room 11

Managing Patients with Venous Thrombosis

Paul Kyrle

Notes:

Wednesday 21 October
HSANZ Symposium: Targeted Therapy

0830-1000
Hall C
0830

Acute Leukaemia

Daniel Tenen

Abstract not received at time of going to print

Wednesday 21 October
HSANZ Symposium: Targeted Therapy

0830-1000
Hall C
0900

JAK2 Mutation Positive Myeloproliferative Diseases

Radek Skoda

University Hospital Basel, Experimental Hematology, Department of Biomedicine, Basel, Switzerland

The majority of JAK2 mutations in patients with myeloproliferative disorders (MPD) are located in the “pseudo-kinase” domain and they activate the kinase domain through a poorly understood allosteric mechanism. Therefore, attempts to derive inhibitors have focused on targeting the ATP binding site and so far only a few attempts have been made to search for drugs that would specifically bind the mutated JAK2. There appear to be some differences in the way the different JAK2 mutations activate signaling. The most frequent mutation, JAK2-V617F located in exon 14, is found in all 3 MPD entities polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF), whereas mutations in exon 12 are associated solely with PV and mutations in exon 16 are found in patients with B cell ALL and Down syndrome. However, it can be expected that the JAK2 inhibitors that act as ATP analogues will be effective in all three classes of JAK2 mutations and will also be effective in mutations activating the thrombopoietin receptor, MPL. The experience with JAK2 inhibitors in clinical phase I and II studies with PMF patients so far showed beneficial effects on spleen size and constitutional symptoms, but little impact on the mutant allele burden. Some of these JAK2 inhibitors are now entering phase III trials. An update on the current state of these studies will be given and some results from pre-clinical animal models will be presented.

Wednesday 21 October
HSANZ Symposium: Targeted Therapy

0830-1000
Hall C
0930

Molecular Targeted Therapy of CML

Junia V Melo

Dept. of Haematology, Centre for Cancer Biology, IMVS, Adelaide, SA, Australia & Imperial College London, UK

The clinical features of chronic myeloid leukemia (CML) are caused by a functionally overactive tyrosine kinase, Bcr-Abl, which is the product of the *BCR-ABL* fusion gene, consequent to a reciprocal t(9;22)(q43;q11) chromosomal translocation. The tyrosine kinase inhibitor (TKI) imatinib has become the first-line treatment for CML, and has improved survival and may alter the natural course of the disease in many patients. Despite these remarkable results, the emergence of resistance to this TKI has become a significant problem. Much progress has been recently made in elucidating the mechanisms which underlie imatinib resistance. The most common cause of such drug resistance is the selection of leukaemic clones with point mutations in the Abl kinase domain leading to amino acid substitutions which prevent the appropriate binding of the drug. Genomic amplification of *BCR-ABL*, modulation of drug efflux or influx transporters, and Bcr-Abl independent mechanisms also play important roles in the development of resistance. Persistent disease is another therapeutic challenge and may, in part, be due to the inability of imatinib to eliminate primitive stem cell progenitors. There is a pressing need, therefore, to develop and test novel drugs and strategies for the effective treatment of CML. A multitude of agents targeting signal transduction pathways downstream of Bcr-Abl have been developed, and have shown *in vitro* and *in vivo* efficacy in overcoming imatinib resistance. However, the Bcr-Abl tyrosine kinase activity of the oncoprotein still remains the most promising therapeutic target, and the focus has been directed in more recent years to developing TKIs with greater potency and improved 'fitting' in the kinase pocket. There are currently three such 'second generation' TKIs being explored in clinical trials: nilotinib, dasatinib and bosutinib. Furthermore, the emerging role of immunotherapy and exploitation of tyrosine kinase-independent pathways are promising aspects of translational research.

Wednesday 21 October
ANZSBT Free Communications 2

0830-1000
Hall B

0093

0830

An Age-dependent ABO Discrepancy Between Mother And Baby Reveals A Novel A^{weak} Allele.

Jennifer Condon¹, Annika K. Hult^{2,3}, Lanny Ramadi¹, Kate Green¹, Andrew Harrison⁴, Åsa Hellberg², Jill R. Storry^{2,3}, Martin L. Olsson^{2,3}.

1. Australian Red Cross Blood Services, Melbourne, Victoria, Australia; 2. Nordic Reference Laboratory for Genomic Blood Group Typing, Clinical and Regional Laboratories, Lund, Sweden; 3. Division of Hematology and Transfusion Medicine, Department of Laboratory Medicine, Lund University, Lund, Sweden; 4. St John of God Pathology, Geelong, Victoria, Australia.

Background: A and B antigens are synthesized on glycoproteins and glycolipids of erythrocytes (RBCs) and other tissues by glycosyltransferases encoded by the ABO gene. A¹ alleles differ from B alleles by 7 nucleotides of which 4 encode amino acid differences. The O¹ allele differs from A¹ by a nucleotide deletion that creates a frameshift. However, 48 different A subgroup alleles are known to encode A^{weak} expression. Cord blood from an infant born to an A₂B mother typed group O, H+. Results were confirmed on a heel prick sample. All samples were investigated to determine the reason for the discrepancy.

Methods: Genomic DNA was analysed by ABO genotyping using in-house PCR-RFLP/PCR-ASP assays and by sequence analysis. RBCs were characterised by flow cytometry using monoclonal anti-A.

Results: Surprisingly, routine analysis showed the mother's genotype as A¹B and the infant's as A¹O¹. PCR-ASP screening for known mutations causing weakened A/B expression and rare O alleles were negative. DNA sequencing revealed a novel mutation, 311T>A in the A¹-like allele of both subjects, which predicts an amino acid change, Ile104Asn. Flow cytometry demonstrated A antigen on the mother's RBCs equivalent to the A₂ phenotype. A antigen was barely detectable on the cord RBCs, however, a sample drawn at 11 months demonstrated increased A expression.

Discussion: Amino acid 104 resides in the α₂-helix of the stem domain but is not involved directly with catalysis. Possibly, Asn104 destabilises the helix and/or changes the subcellular localization. Based on structural data and analogous glycosyltransferases, we hypothesised that Ile104 is involved in dimer formation and that the mother's normal B-transferase stabilises the altered A-transferase (so-called allelic enhancement). However, the altered A-transferase alone may be too unstable to function effectively. Speculation that the baby's A antigen might strengthen as more complex/branched carbohydrate chains are produced was confirmed by the later sample.

No conflict of interest to disclose

A192

Wednesday 21 October
ANZSBT Free Communications 2

0830-1000
Hall B

O094

0845

PUPPY Underpins Platelet Planning: Results from the Prospective Utilisation of Platelets and Plasma (Puppy) Study

Neil Waters, Mary Comande, Mark Polizzotto, George Grigoriadis, Marija Borosak, Damien Jolley, Erica Wood

Australian Red Cross Blood Service, ²Monash Institute of Health Services Research

Background

In order to plan for platelet demand and meet clinical need in an emergency an understanding of platelet use is required. Restricting platelet use to clinically urgent cases and/or deferring elective surgery may be required, however few data exist to inform planning.

Aims

To determine indications for, and urgency of, platelet transfusions in Victoria.

Methods

Random sample survey, adapted from Bloodhound study of red cell utilisation. 1252 platelet units (752 pooled, 500 apheresis, including 7x4 Paediatric) were randomly tagged with case report form (CRF). CRFs were completed by issuing hospital scientist.

Results

1158 units were issued to hospitals. Analysis of 1149 (99.2% response) returned CRFs shows 830 (72.2%) issued for transfusion, 300 (26.1%) expired in hospitals, 19 (1.6%) recalled or other disposal.

Clinical conditions requiring transfusion included malignant haematology 413 (49.8%), benign haematology 49 (5.9%), Oncology 68 (8.2%), other medical 52 (6.2%), cardiothoracic surgery 91 (11.0%), urological surgery 30 (3.6%), other surgery 87(10.4%) and other/unknown 40 (4.8%)

Clinical urgency of transfusion was acute (<1 hour) in 126 (15.2%); urgent (<24 hours) in 527 (63.5%); semi-urgent (<1 week) in 130 (15.7%) and unknown in 45 (5.4%) cases; 2 (0.2%) transfusions were deferrable >1 week. Support for elective procedures used 66 (7.9%).

Where sufficient information was supplied (540 [65.1%]), appropriateness was assessed against NHMRC guidelines, with 454 (84.1%) were assessed as appropriate.

Median platelet count prior to transfusion was $19 \times 10^9/L$ (IQR 11 - 55); for bleeding patients $25 \times 10^9/L$, for prophylaxis (no risks) $11 \times 10^9/L$, and prophylaxis (with risks): $15 \times 10^9/L$.

Conclusions

Platelet usage is concentrated in treating haematological and malignant disorders, and supporting major surgery. High levels of urgent transfusion and low numbers of transfusions supporting elective surgery demonstrate that in a shortage conventional triage strategies would have little impact on requirements and additional strategies are required to ensure continued adequacy of supply.

No conflict of interest to disclose

Wednesday 21 October
ANZSBT Free Communications 2

0830-1000
Hall B

O095

0900

Bacterial Screening of Platelets in Australia: First 12 month experience

Marija Borosak¹, Janet Wong², Peta Dennington², Phillip Mondy², Lynn Aston⁴, Ben Saxon⁵, Shane Winzar¹, Erica Wood¹, Joanne Pink³
Australian Red Cross Blood Service, ¹Melbourne, Victoria, ²Sydney, NSW, ³Brisbane, QLD, ⁴Perth, WA, ⁵Adelaide, SA, Australia.

Aims

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

Methods

Seven ARCBS testing laboratories (5 new facilities) use the BacT/ALERT® 3D automated microbial detection system. Closed system sampling (15-20mL) from each platelet component occurs at 24h; samples are inoculated for aerobic and anaerobic culture. Platelets are released 'negative to date' while culture continues over component shelf-life. Initial machine positive (IMP) and all follow up results are notified immediately to transfusing laboratories. Communication and education was undertaken to inform clinicians and laboratory personnel about introduction of bacterial screening. In the 12 months following implementation (late April 2008 – end April 2009) ARCBS transfusion medicine staff provided clinical follow up of cases where transfusion had occurred prior to IMP notification.

Results

Feedback from clinicians showed understanding of the need to undertake bacterial screening and follow up. Initial concerns related to management of different clinical scenarios and potential workload for laboratories and clinical staff. Of 116 594 platelet components screened there were 1364 (1.17%) IMP notifications, of which 372 (27.3%) platelets or their associated components (red cells, plasma) were transfused. Follow up of these cases found three possible reactions related to transfusion. Many patients (39%) were already on antibiotic and no confirmed or high probability bacterial cases were reported. Of all screened platelets, 205 were confirmed positive/indeterminate (0.18%). In 59 (29%) of these the organisms were deemed clinically significant. Transfusion was prevented in 59% (35/59) of these cases due to early notification; no transfusion reactions were reported.

Conclusion

ARCBS has successfully implemented bacterial contamination screening of platelets in Australia, contributing to a small but important further improvement in transfusion safety. ARCBS transfusion medicine staff work with hospital and laboratory personnel to manage the clinical follow up of bacterial screening.

No conflict of interest to disclose

Wednesday 21 October
ANZSBT Free Communications 2

0830-1000
Hall B

O096

0915

Combined Prion and Leukocyte (PR*LR) Filters Vary with respect to Vesicle Formation During Storage

Kenneth Nollet¹, Akiko Sugawara¹, Shunichi Saito¹, Kentaro Yajima², Morikazu Miura², Tomo Yokomizo², Hitoshi Ohto¹
¹ Fukushima Medical University, Division of Blood Transfusion and Transplantation Immunology, Fukushima City, Fukushima, Japan. ² Asahi Kasei Medical Co., Ltd., Sepacell Division, Tokyo, Japan

Aim

Prior work (in press) showed that platelet-derived microparticle (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

Following 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 minutes. Supernatant aliquots were incubated with PE-conjugated anti-CD42b or mouse IgG1 (control), and fixed with paraformaldehyde. PE fluorescence, forward scatter, and side scatter 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.

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).

This research was supported by Asahi Kasei Medical Co., Ltd., which provided materials and financial support for an independent investigation of filtration technology.

Wednesday 21 October
ANZSBT Free Communications 2

0830-1000
Hall B

O097

0930

FFP Puppy Digs Up Some Surprising Results - Prospective Utilisation of Platelets and Plasma (Puppy) Study

Mary Comande¹, Neil Waters¹, Mark Polizzotto¹, George Grigoriadis¹, Marija Borosak¹, Damien Jolley², Erica Wood¹

¹ Australian Red Cross Blood Service, ² Monash Institute of Health Services Research

Aim

Determine clinical indications for, and urgency of, Fresh Frozen Plasma (FFP) use to inform supply and contingency planning.

Methods

Random sample survey adapted from Bloodhound red cell utilisation study, performed August 2008 to February 2009. FFP units (n=1993) were randomly tagged with case report form (CRF) at production and distributed to Victorian transfusion laboratories. At time of issue for transfusion, CRFs were completed by laboratory scientists.

Results

Interim analysis of the first 1343 (67.4%) returned CRFs shows 1243 FFP units (92.6%) issued for transfusion, 96 (7.1%) discarded and 4(0.3%) recalled.

Major clinical requirements for FFP were: cardiothoracic surgery 215 cases (17.3%); gastroenterology 172 (13.8%); solid organ transplant, including plasma exchange for renal transplant, 161 (13.0%); haematology/oncology 147 (11.8%) and trauma 78 (6.3%). In 636 cases (51.2%) FFP was used to support an interventional procedure, of these, 112 (19.2%) were elective, 316 (49.7%) non-elective and 198 (31.1%) urgency was unknown to the laboratory. Use for warfarin reversal was often in conjunction with other clinical indications.

Clinical urgency of transfusion was acute (required <1 hour) in 471 cases (35.1%); urgent (1-24 hours) in 661 (49.2%); semi-urgent (24 hours -1 week) in 58 (4.3%) and non-urgent (>1 week) in 9 (0.7%). In 44 cases (3.3%) urgency was not known. AB plasma is in demand for trauma, other emergencies and ABO-incompatible renal transplantation.

Conclusions

Analysis of interim data shows 84% FFP transfusions required within 24 hours with significant use in cardiothoracic surgery and organ transplantation. Surprisingly, solid organ transplantation, including use of AB FFP for ABO-mismatched renal transplants, was third largest use. High use in critical illness and complicated coagulopathy has implications for contingency planning, as alternatives to FFP in those contexts are typically unavailable (unlike warfarin reversal). Information about changes in clinical use of FFP will inform future blood supply planning.

No conflict of interest to disclose

Wednesday 21 October
ANZSBT Free Communications 2

0830-1000
Hall B

0098

0945

Routine Autologous Blood Collection and Subsequent Transfusion for Donors of Bone Marrow (BM) is Unnecessary

Zane Kaplan, Christopher Hogan, Jeff Szer, Andrew Grigg.

Departments of Laboratory Haematology and Bone Marrow Transplant Service, Royal Melbourne Hospital, Parkville, VIC

Background

Australian Bone Marrow Donor Registry and World Marrow Donor Association guidelines stipulate harvest centres should collect ≥ 1 autologous red cell units from allogeneic donors for transfusion during or after a marrow harvest. However, the necessity for routine autologous collection has not been established. This practice increases cost and inconvenience and does not eliminate the most significant transfusion associated risks, including bacterial contamination and administrative error.

Aim

To perform a single centre retrospective analysis of the utility of autologous blood collection pre-allogeneic BM donation and review transfusion practices of stored autologous units.

Methods

Fifty-nine consecutive BM donors presenting between January 2004 and December 2009 were identified. Sibling and volunteer unrelated donors were included. Haemoglobin (Hb) measurements pre-autologous blood collection, pre-harvest and post-harvest (prior to transfusion), where available, were retrospectively analysed. Transfusion of autologous units was audited.

Results

The donors comprised 34 males (mean age 40yrs–range:16-52) and 25 females (mean age 39yrs–range:24-66). Forty-seven donors had autologous blood collected (33:1unit and 14:2units). The mean Hb pre-autologous donation for males ($n=27$) was 156g/L (range:139-179g/L) and for females ($n=20$) was 137g/L (range:116-157g/L). The mean reduction in Hb post-autologous collection was 12g/L (-31g/L to -1g/L) and 7g/L(-30g/L to +4g/L) for males ($n=23$) and females ($n=17$) respectively, despite routine iron supplementation. The mean BM harvest volume was 1068ml (range:200-1725ml). The mean post-harvest Hb pre-transfusion in males ($n=19$) was 122g/L (range:92-151g/L) with a mean drop of 23g/L (range:5-43g/L), for females ($n=12$) the respective results were 108g/L (range:84-145g/L) and 26g/L (range:10-49g/L). No donor who had a post-harvest Hb measured met NHMRC minimum criteria for transfusion ($Hb < 70g/L$). Twenty-seven of the 47 donors who had autologous blood collected, were transfused, although in 56% their Hb pre-transfusion was not checked. There was no significant difference in post-harvest Hbs of transfused (pre-transfusion 115g/L range:84-151g/L) versus non-transfused donors (118g/L range:88-145g/L).

Conclusion

Routine autologous blood collection prior to BM harvest leads to a drop in Hb pre-harvest, wasting of blood and unnecessary transfusions. Post-harvest Hb did not decrease to levels considered detrimental to healthy persons in any donor. We conclude routine autologous blood collection from healthy BM donors is unnecessary.

No conflict of interest to disclose

Wednesday 21 October
ANZSBT Free Communications 3

0830-1000
Hall A

0099

0830

Haemovigilance In New Zealand – Four Years and Counting ...

Krishna Badami¹, Dorothy Dinesh², **Susanta Ghosh**³, John Dagger², Peter Flanagan⁴
New Zealand Blood Service, ¹Christchurch, ²Wellington, ³Hamilton, ⁴Auckland, New Zealand

Aim

To collect data on transfusion- and blood donation-related hazards.

Methods

For transfusion-related events reporting is voluntary. European Haemovigilance Network definitions are used. Specialists review reports to determine if the stated category and 'imputability' to transfusion are appropriate. Data for 2005 – 2008 is presented. Donor incidents are collected using an incident reporting system. A central office collates data and prepares an annual report.

Results

Adverse events in recipients and blood donors are summarised below.

Table 1. Adverse events in recipients receiving blood components, 2005 - 2008 – number (frequency/ 10,000 components transfused):

	Febrile non-haemolytic transfusion reactions	Allergic reactions (all grades)	Transfusion-associated circulatory overload	Transfusion-related acute lung injury	Acute haemolytic transfusion reaction	Delayed haemolytic transfusion reaction	Incorrect blood component transfused	Other [@]	Total
2005*	131 (12)	89 (8)	8 (1)	10 (1)	0	6 (1)	10 (1)	17 (2)	271 (26)
2006	190 (12)	148 (10)	7 (<1)	10 (1)	2 (<1)	10 (1)	22 (1)	31 (2)	420 (28)
2007	193 (13)	155 (10)	17 (1)	9 (1)	4 (<1)	12 (1)	21 (1)	44 (3)	455 (30)
2008	207 (12)	160 (9)	20 (1)	4 (<1)	1 (<1)	19 (1)	39 (2)	70 (4)	520 (30)

* data for 8 months only.

[@] events not fitting clearly-defined categories and previously-unrecognised complications of transfusion.

In 2008 there were 185,738 whole blood & apheresis donations and 1416 adverse events involving 1306 donors (762 events / 100,000 donations).

Conclusion

Greater awareness and ease and standardization of reporting are the keys to a successful HV programme. This can promote rational transfusion use and the introduction of measures to improve blood safety.

No conflict of interest to disclose

Wednesday 21 October
ANZSBT Free Communications 3

0830-1000
Hall A

O100

0845

Hospital Influences Risk of Transfusion in Cardiac Surgery: An Analysis of a Large Patient Cohort

Zoe McQuilten¹, Erica Wood¹, Merrole Cole-Sinclair², Chris Reid³, Louise Phillips³.

¹*Transfusion Medicine Services, Australian Red Cross Blood Service*

²*Department of Haematology, St Vincent's Hospital, Melbourne, Victoria, Australia*

³*Department of Epidemiology and Preventive Medicine, Monash University, Melbourne, Victoria, Australia*

Background

The international literature reports significant variation in transfusion practice in cardiac surgery; however there are very few Australian data on transfusion in this setting. We aim to investigate the variation in transfusion practice between the major cardiac surgery units in Victoria.

Methods

Data were prospectively collected on 9363 cardiac surgery patients at six major Victorian hospitals between January 2005 and December 2008 through the Australasian Society of Cardiac and Thoracic Surgeons Cardiac Surgery Database. There included patient demographics, co-morbidities and medication use, surgery type, peri-operative complications, clinical outcome and peri-operative transfusion.

Results

Procedure types were: coronary artery bypass graft (CABG) surgery in 60%, valve surgery 14%, CABG + valve 10% and other procedure 16%. There was significant variation in transfusion of all blood components between the six hospitals and this variation was not accounted for by patient or surgery related factors. The adjusted odds ratio for risk of transfusion for red cells varies at each hospital from 0.22 to 1.98, for platelets from 0.39 to 3.3 and for plasma from 0.27 to 2.0.

Conclusion

There was significant variation in transfusion practice across the major hospitals in Victoria performing cardiac surgery which was not accounted for by patient or surgery related factors. Patient laboratory results and pre-operative transfusion details were not available for inclusion in this study, however a project is currently underway to address this through the linkage of hospital laboratory data with this and other clinical outcome registries. With increasing concern about potential adverse outcomes associated with peri-operative transfusion in cardiac surgery, further studies are required to determine factors contributing to this variability in transfusion practice and how it may influence patient outcomes.

No conflict of interest to disclose

Wednesday 21 October
ANZSBT Free Communications 3

0830-1000
Hall A

O101

0900

An Audit of the Safety and Efficacy of Prothrombinex®-VF for Acute Reversal of a Prolonged INR, at Two Large City Hospitals

Louise Bobbitt¹, Jacqueline Raynes², Sue Dainty¹, Hilary Blacklock², Ross Henderson¹ and Sanjeev Chunilal¹

1. North Shore Hospital, Waitemata District Health Board, Auckland, New Zealand

2. Middlemore Hospital, Counties Manukau District Health Board, Auckland, New Zealand.

Aim

To determine the 30 day risk of thromboembolism (arterial and venous) following administration of Prothrombinex-VF (PTX) to acutely reverse a prolonged INR.

Method

A prospective audit of patients who received PTX between August 2008 and May 2009 at two Auckland hospitals. Patients receiving PTX for reasons other than a prolonged INR were excluded. Eligible patients were followed for 30 days to determine the rate of thromboembolism and mortality. All issues of PTX were authorised by a Clinical Haematologist with the decision to use vitamin K and/or Fresh Frozen Plasma (FFP) made on a case by case basis.

Results

A total of 122 doses of PTX administered to 120 patients for acute reversal of a prolonged INR. PTX (+/- vitamin K and FFP) reversed the INR to <1.5 in 80% of cases. Patients were elderly (71y +/-12), predominantly male (58%) and unwell (45% haemodynamically compromised). Indications for warfarin administration included atrial fibrillation (AF) (70%), mechanical heart valve (MHV) (12%) and venous thromboembolism (VTE) (8.5%). PTX was administered (+/- vitamin K and FFP) to rapidly reverse the INR due to life threatening bleeding (55%), for an acute procedure/surgery (33%) and for medically unwell patients with a raised INR (9%). By 30 days, 21 (18%) patients were dead. There were 16 thrombotic events (1 venous; 15 arterial) of which 9 were fatal. These thromboses occurred in high risk patients (13 with AF or MHV). Only 2 unexpected nonfatal events (1 major cerebrovascular accident; 1 myocardial infarction) occurred in patients treated for VTE. There was no observed difference in the rate of thrombosis if patients did or did not receive FFP with PTX (12% versus 14% p=NS).

Conclusion

There is a high rate of thrombosis and death in our elderly patients receiving PTX used to rapidly reverse an elevated INR. Most events occurred predictably in patients with pre-existing AF or MHV and in those patients treated for an acute haemorrhage. Our data suggest that PTX is effective in reversing a prolonged INR, and the frequent thrombotic events associated with its use reflect the patient population and co-morbidities. The high rates of thrombosis were not attenuated by concomitant administration of FFP.

Conflict of Interest Statement

This research was supported by CSL. The company had no role in the design, data analysis or preparing the abstract.

A200

Wednesday 21 October
ANZSBT Free Communications 3

0830-1000
Hall A

O102

0915

How is a Tool Developed for the Aerospace Industry Being Used to Prevent a Leading Cause of ABO Incompatible Transfusions?

Jo Main¹, Justine Mizen¹, Linda Nolte¹, David Westerman^{1,2}

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² University of Melbourne, Parkville, Victoria, Australia

Introduction

Errors in sample collection and labelling represent a leading cause of transfusion related patient morbidity and mortality. Accurate specimen labelling is a critical step in pre-transfusion compatibility testing.

A shift in the thinking about how errors occur has provided new ways to approach possible solutions in specimen labelling using Failure Mode and Effect Analysis (FMEA). FMEA is a systematic method for identifying potential process failures before they occur, with the intent to eliminate or minimise the risks associated with them.

Aim

To reduce the number of mislabelled blood grouping specimens in the Outpatient Pathology Department (OPD) at the Peter MacCallum Cancer Centre.

Method

We employed FMEA on the labelling of blood grouping specimens in the OPD to:

- Map the *process* of labelling of a specimen tube
- Identify the *potential* failure modes
- Identify the *causes and effects* of the failure modes
- Evaluate the *risks* associated with the failure modes
- Identify the current controls

Results

The following failure modes were identified:

- Specimen tubes – poorly designed tube labelling
- Physical environment – privacy and workflow issues
- Human factors – errors in positive patient identification

These were prioritised according to their detectability, frequency and the seriousness of their consequences. Proposed corrective actions were assigned to the high risk failures and the process was redesigned to address these failures. The FMEA tool was then used to determine the effectiveness of the redesigned process before the implementation of the proposed corrective actions.

Conclusion

The next phase is to fully implement the proposed corrective actions in the OPD and then to evaluate the effectiveness of these actions in reducing the number of mislabelled blood grouping specimens.

Our experience has demonstrated that FMEA is a simple and effective method to proactively identify failure modes and prioritise risk reduction strategies.

No conflict of interest to disclose

Wednesday 21 October
ANZSBT Free Communications 3

0830-1000
Hall A

O103

0930

Bedside Transfusion Practice Audit in eight New Zealand Hospitals

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New Zealand Blood Service Auckland New Zealand

Aim

To determine the level of adherence to the ANZSBT guidelines with the administration of resuspended red cell transfusions at the patients bedside at eight large public hospitals in New Zealand, and to check the hospital blood policies against the ANZSBT component administration guidelines

Method

Episodes were collected prospectively by the Transfusion Nurse Specialists at each site. Data were recorded by direct observation. Further data were recorded from the patients clinical notes after the Transfusion had been completed.

Results

420 transfusions were audited from a spread of specialities. Identity checks were generally well performed. Notable exceptions were asking patients to state their identity (45% compliance overall), and wearing wristbands in neonates (33%) and daycases (57%). The two-person check of unit against patient and prescription was performed variably with one hospital failing to do this at the bedside in almost a quarter of transfusions audited. Checking patient vital signs revealed confusion over the role of pulse oximetry vs observed respiratory rate. A nurse stayed with the patient for the first 15 minutes of the transfusion in only 86% of cases. Only 60% of adverse reactions were reported to blood bank. Post-transfusion documentation was well performed. Transfusion duration was over 4 hours in up to 10% of transfusions. To identify the extent of overlapping omissions in safety checks, a composite safety check list was compiled. Only 67% of transfusions satisfied this list, with up to five omissions per transfusion

Comment

Direct observation by the auditors may have an effect on compliance. However the audit demonstrated areas of non compliance for recommend best practice for patient safety with the administration. of red blood cells transfusions

No conflict of interest

Wednesday 21 October
ANZSBT Free Communications 3

0830-1000
Hall A

O104

0945

Procedural Adverse Events in Transfusion – Lessons from STIR 2006-09

Lisa Stevenson, David Beilby, Karen Botting, Slavica Curcic, James Daly, Bridget Glazebrook, Geoff Magrin, Ellen Maxwell, Richard Rogers, Merrole Cole-Sinclair, Carole Smith, Neil Waters, Deane Wilks, Larry McNicol, Erica Wood.

STIR expert group and advisory committee, Blood Matters-better safer transfusion program, Department of Human Services and Australian Red Cross Blood Service, Melbourne, Victoria, Australia

Aim

STIR (Serious Transfusion Incident Reporting) is the voluntary haemovigilance framework developed by the Blood Matters-better safer transfusion program. Hospitals and laboratories in Victoria, Tasmania, ACT and Northern Territory participate. The program aims to;

- measure and monitor serious transfusion incidents, including near misses
- derive recommendations for better, safer transfusion practice and disseminate these to health services and the Australian Red Cross Blood Service.

Method

STIR provides a central system for reporting events related to administration and handling of fresh blood components and pre-transfusion samples. Health services electronically submit initial reports to STIR. Detailed case report forms relevant to the event type (e.g. incorrect blood component transfused, IBCT) are provided to the reporting institution to complete. Information (de-identified for institution) regarding the case is returned to STIR for data entry and review by an expert group. STIR links with the Victorian sentinel event program.

Result

To date STIR has been notified of **439** adverse events in **432 patients**. Combined procedural adverse events, incorporating incorrect blood component, wrong blood in tube and near miss events, accounted for **40%** of all reports during this period.

Common themes include:

- failure to comply with established patient identification procedures when collecting pre-transfusion samples
- failure to identify patients at the bedside before transfusion administration

STIR has received **38** IBCT events, including **22** where the component did not meet specific patient requirements, **9** incorrect but ABO-compatible events, and **3** ABO incompatible events. Four reports are still to be investigated. No deaths related to transfusion were reported.

Conclusion

Procedural adverse events are preventable events. The common theme through all the procedural reports is failure to identify the patient correctly, either due to incorrect identification procedures or failure to follow local hospital procedures. STIR recommends that **all** staff administering transfusions should be trained in the correct procedure, particularly the importance of patient identification.

No conflict of interest to disclose

Wednesday 21 October
ASTH Free Communications 3

0830-1000
Hall D

O105

0830

Patients with Enhanced Clot Solubility in Acid Conditions Require Differentiation between Raised Plasma Levels of the Aspartic Protease Pepsinogen I and Inhibitory Antibodies to Factor XIII

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² Sansom Institute, School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, SA Australia

Background and Aims

The *in vitro* solubility of plasma clots in 2% glacial acetic acid (GAA) indicates either Factor XIII (FXIII) deficiency, antibodies to FXIII or the presence of an unknown acid-activated protease (AP). The AP was previously postulated to be "pepsin-like activity" or cathepsin D (CD). We describe four subjects with clot solubility in GAA (but not urea) and normal FXIII levels. This study aims to assess their plasma levels of common APs and to explore the possibility of inhibitory antibodies.

Methods and Results

We mixed subject plasma with normal plasma or cryoprecipitate and added calcium to form a clot. The clots lysed in GAA, consistent with presence of an inhibitor. However IgG purified from subjects' plasma did not cause clot lysis and IgG-depleted plasma clots retained the ability to lyse in GAA. Pepstatin, an AP inhibitor, prevented clot lysis in the subject group, confirming lysis was due to an AP. Clots only dissolved at pH <3.2 (acetate/acetic acid) consistent with pepsin activity and lower than expected for CD. In subjects 1- 4 CD levels (Calbiochem ELISA) were 41, 46, 38 and 44 ug/L respectively and within the normal range (n=20, 36-70 ug/L). However pepsinogen I levels (Biohit ELISA) were 570, 780, 490 and 540 ug/L and were markedly elevated compared to 16 normal donors (65-200 ug/L). Pepsinogen II levels (Biohit ELISA) were normal.

Conclusions

An AP, and not a FXIII inhibitor, caused clot lysis in plasma from subjects with acid-soluble clots and normal FXIII. These subjects had high plasma levels of pepsinogen I, the acid-activated precursor to pepsin, and we postulate that pepsin digested the clot. Abnormal clot lysis tests require follow up with a FXIII assay and possibly also a pepsinogen assay, to confirm or exclude a true FXIII deficiency or inhibitor.

No conflict of interest to disclose

Wednesday 21 October
ASTH Free Communications 3

0830-1000
Hall D

O106

0845

Exon 28 Mutation Testing in von Willebrand Disorder

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Aim

To determine the usefulness of exon 28 mutation testing in patients with von Willebrand disorder (VWD).

Method

The size and complexity of the von Willebrand factor (VWF) gene makes full gene mutation testing impractical for most laboratories. Of the 52 exons of this gene, exon 28 is by far the largest, coding for 14% of the mature protein. This exon contains all the mutations for type 2B VWD, approximately 70% of known mutations for type 2A and type 2M, and 20% of type 1 mutations.

We have performed sequencing of exon 28 in 15 families with type 1 and type 2 VWD with ristocetin cofactor levels <40IU/dl.

Results

Mutations in exon 28 of the VWF gene were identified in 9 of the 15 families tested. Type 2B mutations were found in two families thought to have this sub-type, as well as in another family with previously unclassified type 2. A type 2A mutation was found in another patient with unclassified type 2.

In four families with presumed type 2M, mutations were found in all of them, with two having the same mutation and similar phenotype. In one of these families, two of the six affected family members did not have the identified mutation, casting doubt on its causative nature. These three mutations have been previously reported as type 1, 2A or 2M, but bioassay results of the reported index cases seem to be consistent with type 2M.

A type 1 mutation was found in one of four presumed type 1 patients, with no mutations found in the other three. No mutation was found in a further three patients with possible acquired type 2 VWD.

Conclusion

Mutations were detected in exon 28 in all families with type 2. Exon 28 analysis is particularly useful for identification of type 2B.

No conflict of interest to disclose

Wednesday 21 October
ASTH Free Communications 3

0830-1000
Hall D

O107

0900

Gestational Thrombocytopenia in Three Sisters with Type 2B von Willebrand's Disorder

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Aim

To describe three sisters with von Willebrand's disorder (VWD) who developed thrombocytopenia during their first pregnancies.

Method

All three sisters had been previously diagnosed with mild unclassified VWD. BC developed mild thrombocytopenia early in her first pregnancy with normal von Willebrand's studies on presentation. The provisional diagnosis of immune thrombocytopenia (ITP) was made. Her platelet count was $32 \times 10^9/L$ when she was treated with immunoglobulin and steroid, but with no response to either. Her thrombocytopenia resolved after delivery. Her second sister, NT had a similar experience during her first pregnancy. Her eldest sister received desmopressin prior to delivery and was later diagnosed with thrombocytopenic thrombotic purpura (TTP). She was managed with steroids and plasma exchange. Post partum platelet aggregometry did not demonstrate increased aggregation to low concentrations of ristocetin.

Results

Type 2B was suspected on the basis of thrombocytopenia during pregnancy. NT was tested following an injury - FVIII 27 IU/dl; VWF Antigen 39 IU/dl; RiCof 27 IU/dl ; RiCof/Ag 69%; CBA 14 U/dl, CBA/Ag 36%, platelet $145 \times 10^9/L$. Ristocetin-induced platelet aggregation (RIPA) at low concentration is required to diagnose Type 2B VWD. As the sisters were unavailable for platelet aggregometry; exon 28 of VWD gene was sequenced, since all type 2B mutations are detected in this exon. The mutation 3916C>T; Arg1306Trp was detected. This common type 2B mutation is well recognised on the VWD mutation database, confirming the family as type 2B VWD.

Conclusion

All three women were misdiagnosed during their first pregnancies. They demonstrated the increase in VWF during times of stress such as pregnancy, trauma or surgery, leads to increased platelet binding to platelet Glycoprotein 1b in type 2B VWD and hence thrombocytopenia, which resolved after delivery. Type 2B also needs to be differentiated from pseudo (platelet-type) VWD and mutation studies provide a definitive diagnosis for type 2B.

No conflict of interest declared

Wednesday 21 October
ASTH Free Communications 3

0830-1000
Hall D

O108

0915

Vancomycin Induced Thrombocytopenia in Hospitalised Patients

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Aim

Vancomycin is an antibiotic used widely in Australian hospitals to treat Methicillin-resistant *Staphylococcus aureus* (MRSA). Only recently has vancomycin been implicated in causing drug-induced thrombocytopenia (DIT) and as such, there are very limited studies dedicated to establishing the characteristics of this disease. This will be the first prospective study on vancomycin induced thrombocytopenia (VIT). It aims to establish vancomycin as a causative agent in thrombocytopenia through the detection of vancomycin dependant antibodies and describe the clinical presentation of these patients.

Methods

Blood samples were obtained with informed consent from inpatients at St George Hospital and Sutherland Hospital at a mean of 13 days after vancomycin initiation. Vancomycin dependent antibodies were detected using flow cytometry on patient sera and, for any positive results, monoclonal antibody immobilisation of platelet antigens (MAIPA) was used. Patient medical records were reviewed for duration and dose of vancomycin treatment, other concomitant medications and for clinical signs and symptoms of DIT. Platelet counts for the duration of their stay in hospital were also monitored.

Results

Out of 33 enrolled patients (11 females and 22 males; aged between 30 and 89), 6 patients tested positive for vancomycin dependent antibodies (3 females and 3 males; aged between 30 and 80). Of these 6 patients, 4 patients showed an average of 45.5% drop in platelet count within two days of vancomycin commencement. The other 2 patients showed no significant platelet drop within the time of vancomycin administration.

Conclusions

In this preliminary study, the incidence of vancomycin dependent antibodies detected was 18.2% and 66.7% of these patients developed thrombocytopenia due to vancomycin. This also suggests that, like other DITs (e.g. heparin induced thrombocytopenia), pathogenic and non pathogenic antibodies may be associated with vancomycin induced thrombocytopenia.

No conflict of interest to disclose

Wednesday 21 October
ASTH Free Communications 3

0830-1000
Hall D

O109

0930

Is Bone Marrow Examination Necessary To Safely Manage Idiopathic Thrombocytopenic Purpura In Adults?

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Aim

Guidelines for the management of adults below 60 years presenting for the first time with apparent clinical Idiopathic Thrombocytopenic Purpura (ITP) suggest that bone marrow aspirate and trephine (BMAT) is not routinely required. However, in certain settings, it is often clinical practice to perform BMAT for this indication. We retrospectively determined the prevalence of alternative pathologies in patients with a clinical diagnosis of ITP.

Method

This study was conducted at St John's Medical College Hospital, Bangalore, a tertiary centre in southern India. The clinical notes accompanying BMAT request forms between January 2004 and July 2008 (excluding July to December 2008) were reviewed. Cases were excluded if subjects were outside the age 18-60 years, had bi- or pancytopenia (excluding mild-moderate, micro/normocytic anaemia), fever, HIV infection, previous or suspected malignancy, organomegaly, lymphadenopathy, or were on medication known to cause thrombocytopenia. All patients whom had a marrow and did not meet these exclusion criteria had their charts selected for review. Where the clinical presentation was consistent with acute ITP, the patient's BMAT report was selected for review.

Results

Of 4483 BMAT slips reviewed, 374 cases were selected for chart review, and 312 charts obtained. 117 cases had a clinical presentation suggestive of ITP and had BMAT results available. The mean age was 33 years. Females comprised 81/117 (69%). Anaemia was seen in 72/121 (59%). In 116/117 cases there was no alternative pathology on BMAT. In one case, myelofibrosis was identified. Megakaryopoiesis was described as "normal" in 108 and "reduced" in 8 cases.

Conclusion

In this large study investigating BMAT in adults with apparent ITP, it appears that alternative pathology is rare, suggesting that routine BMAT is unnecessary. These finding suggest that in countries with a high burden of anaemia, BMAT may be safely omitted in patients with a clinical diagnosis of ITP.

No conflict of interest to disclose

Wednesday 21 October
ASTH Free Communications 3

0830-1000
Hall D

O110

0945

Antiphospholipid Testing:- Is More Necessarily Better?

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². *Australian Centre for Blood Diseases, Prahran, Victoria, Australia*

Aim

International Society on Thrombosis and Haemostasis (ISTH) recommendations for lupus anticoagulant testing currently propose that at least two tests utilising different assay principles be used for the diagnosis of the antiphospholipid syndrome. Recent North American and European questionnaires have demonstrated that of the laboratories surveyed, the majority use the dilute Russell's viper venom time (dRVVT) and the activated partial thromboplastin time (APTT) using a lupus anticoagulant sensitive reagent. It has been the practice of our laboratory to perform four different screening tests including the dRVVT and APTT, as well as the kaolin clotting time (KCT) and dilute thromboplastin time (DTT). We aim to determine whether the use of four tests as opposed to the recommended two is more sensitive or specific for the detection of a lupus anticoagulant.

Methods

We undertook a retrospective analysis of the last 18 months of lupus anticoagulant testing undertaken in our laboratory (n=1283). After the application of exclusion criteria, we analysed 1003 results. Sensitivity and specificity of positive mixing studies (i.e failure to correct) for APTT, KCT, dRVVT and dTT and combinations of these assays were calculated.

Results

Sensitivity for APTT, DTT, DRVVT and KCT was 67%, 86%, 86% and 76% respectively. Specificity of all individual tests was similar at ~ 96-97%. Standard combination as used by most laboratories (i.e APTT + DRVVT) revealed 86% sensitivity (confidence interval 83-89%); no better than DTT or DRVVT analysis alone. The most sensitive combination of tests was APTT + DRVVT + DTT (97% sensitivity – 94-100% confidence interval).

Conclusion

Although current ISTH recommendations stipulate a requirement for two screening tests, our data suggests that for maximal sensitivity, analysis of APTT, DRVVT and DDT in combination yields the best results.

No conflict of interest to disclose

Wednesday 21 October
HSANZ Free Communications 9

1100-1230
Hall C

O111

1100

Prognostic Impact of Elevated Pre-Transplant Serum Ferritin in Patients Undergoing Allogeneic Haemopoietic Stem-Cell Transplantation For AML

Elango Subramonia Pillai, Glen Kennedy, Simon Durrant and Jason Butler
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Aim

To identify the prognostic impact of pre-transplant iron load in patients with acute myeloid leukaemia (AML) undertaking allogeneic stem cell transplantation (SCT).

Methods

Retrospective audit of consecutive patients with AML undertaking allogeneic SCT at RBWH between January 2000 and May 2007. Patients were identified from an institutional data base. Iron load was measured by serum ferritin concentration prior to commencing conditioning for transplantation.

Results

Data was censored after May 2008 to ensure a minimum post-SCT follow-up of ≥ 12 mths. For the entire cohort, median overall survival (OS) was 7.2 yrs, with 5yr OS 55%. Transplant related mortality (TRM) and relapse probability at 5yrs were 25% and 31% respectively. Pre-SCT ferritin values were available in all patients. Of the entire cohort, only 2 patients (1%) had normal ferritin levels pre-SCT; median pre-SCT ferritin was 2220 $\mu\text{g/L}$ (range 151-9080 $\mu\text{g/L}$; normal range 10-200 $\mu\text{g/L}$). On univariate analysis, increased ferritin $>$ median was significantly associated with reduced OS; 3yr OS 48% vs 68% for pre-SCT ferritin $>$ vs $<$ median respectively ($p=0.014$). When pre-SCT ferritin was divided into quartiles, the reduced OS post-transplantation appeared to be restricted to that subgroup of patients with the highest pre-SCT ferritin values (i.e 4th quartile); 3yr OS 40% vs 65% for 4th quartile vs quartiles 1-3 respectively ($p<0.005$). Patients with the highest pre-SCT ferritin values also experienced significantly greater TRM (3yr TRM 33% vs 16% for 4th quartile vs quartiles 1-3 respectively; $p=0.02$). On multivariate analysis, raised pre-SCT serum was significantly associated with reduced OS ($p<0.01$), whether analyzed by $>$ vs $<$ median values, or 4th quartile vs quartiles 1-3 respectively.

Conclusion

Iron overload, as measured by raised ferritin, is extremely common in AML patients undertaking allogeneic SCT. Pre-SCT serum ferritin levels appear to represent a new prognostic marker for OS post allogeneic SCT in these patients. Prospective studies to limit iron-overload prior to allogeneic SCT are needed.

No conflict of interest to disclose

Wednesday 21 October
HSANZ Free Communications 9

1100-1230
Hall C

O112

1115

Increased Expression of the Chemokine Receptor CCR5 on Human Blood Dendritic Cells is Associated with Acute Graft vs Host Disease post Allogeneic Stem Cell Transplantation

Mary Sartor, Jenny Lau and Ken Bradstock

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Dendritic cells(DC) are potent antigen presenting cells, that are central to the development of acute graft-versus-host disease (aGVHD) following allogeneic hematopoietic cell transplantation (alloHCT). DC migration is tightly regulated by the expression of chemokine receptors.

In this study we investigated the patterns of expression of the chemokine receptors CCR5 and CCR7 on circulating blood CD11c⁺myeloid and CD11c⁺plasmacytoid dendritic cells by multiparameter flow cytometry from the peripheral blood of 32 patients post alloHCT, and assessed their correlation with aGVHD.

Peripheral blood was collected twice weekly up to day 120 post transplant. CCR7 receptor expression on both CD11c⁺DC was detected in 60% of patients the mean percentage of CCR7 receptor expression was 12%(range 0.3-39%). In contrast, CCR5 receptor expression on both CD11c⁺DC was detected in all but one patient, the mean percentage of CCR5 receptor expression was 30%(range 0.4-90%). The maximum percentage expression of the two chemokine receptors on DC was correlated with the development of aGVHD and its severity. No correlation was found between CCR7 receptor expression and the incidence or severity of aGVHD($p=1.0$). However, there was a correlation between CCR5 expression on DC and the development of aGVHD. 19 patients who developed aGVHD, all had detectable levels of CCR5 expression on both CD11c⁺DC median 42%(range 5.2-89%) prior to the onset of aGVHD, while patients with no aGVHD CCR5 was expressed at a significantly lower level ($p<0.0001$). Patients with grade 2-4 aGVHD had higher CCR5 expression on both CD11c⁺DC post transplant (median 56%, 73% respectively), compared to patients with grade 0-1 on both CD11c⁺(median 4.3%, 2.7% respectively)($p<0.0001$).

CCR5 receptor expression is higher on DC of patients who develop severe aGVHD. This raises the possibility of monitoring CCR5 expression on DC as a means of predicting the development of aGVHD and of using targeted therapies to block CCR5 interactions to reduce or prevent the severity of aGVHD.

No conflict of interest to disclose

A211

Wednesday 21 October
HSANZ Free Communications 9

1100-1230
Hall C

O113

1130

Fludarabine/Melphalan Allografting in Australia and New Zealand, 1998-2008: Excellent Overall Survival in a High-risk Population

Adam Bryant¹, Anna Kalff², David Ritchie², Biju George³, Mark Hertzberg³, Keith Fay^{1,4}, Paul Cannell⁵, Leanne Berkahn⁶, Leonie Wilcox⁷, John Moore¹.

¹St Vincent's Hospital, NSW., ²Royal Melbourne Hospital, VIC., ³Westmead Hospital, NSW., ⁴Royal North Shore Hospital, NSW., ⁵Royal Perth Hospital, WA., ⁶Auckland City Hospital, NZ., ⁷Australasian Bone Marrow Transplant Recipient Registry.

Background

Over the last ten years, Reduced Intensity Conditioning (RIC) has increasingly been used in allogeneic Haematopoietic Stem Cell Transplantation (HSCT). RIC has generally been reserved for patients who have been heavily pre-treated, are of advanced age or have significant co-morbidities. The Fludarabine Melphalan (Flu/Mel) combination is the most commonly used regimen in Australia and New Zealand, however its efficacy and relative benefit in myeloid and lymphoid malignancies has not been previously assessed.

Method

This was a retrospective analysis of Flu/Mel allografts performed in nine allogeneic HSCT centres in Australia and New Zealand between January 1998 and December 2008. All centres were sent an electronic Case Report Form which was returned to the coordinating centre at St. Vincent's Hospital, Sydney. This current data set analyses the outcome from six centres that have responded to date. Statistical analysis was performed using Prism 5 software.

Results

There were n=214 patients with a slight male preponderance (M=54%). The median age of recipients was 54 years (18-67) with 88 patients transplanted for lymphoid malignancies (excluding acute lymphoblastic leukaemia) and 126 patients for myeloid malignancies. Transplant related mortality at D100 was 12.6% and similar for both myeloid and lymphoid malignancies (12.5% vs 12.6%, p=0.97). The median overall survival was 51.4 months with no significant difference in overall survival between the myeloid and lymphoid cohorts (p=0.20). Further analysis will be presented with a particular emphasis on variables associated with outcome.

Conclusion

This study is, to our knowledge, the largest analysis of Flu/Mel conditioning ever published. The data demonstrates that Flu/Mel can be delivered to heavily pre-treated patients of advanced age with excellent long term overall survival. In this analysis there did not appear to be an advantage of the Flu/Mel regimen for lymphoid or myeloid malignancies.

No conflicts of interest to disclose

A212

Wednesday 21 October
HSANZ Free Communications 9

1100-1230
Hall C

O114

1145

Factors Predicting the Outcome of the Blood and Marrow Transplant Patients Admitted to Intensive Care Unit.

Nalini Pati¹, Biju George¹, Ian Kerridge¹, Nicole Gilroy², Vineet Nayyar³, Eddie Stachowski³, Mary McGurgan¹, Gillian Huang¹, Ken Bradstock¹, David Gottlieb¹, Mark Hertzberg¹

¹ Blood and Marrow Transplant Unit, ² Department of Infectious Diseases,

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Aim

To identify factors predicting outcome of patients admitted to intensive care (ICU) following allogeneic haematopoietic stem cell transplantation (allo-HSCT).

Methods

Retrospective audit of all allo-HSCT patients requiring ICU admission.

Results

Between 2000 and 2009, 392 patients underwent an allo-HSCT. 106 (27%) required ICU admission (n = 129). The median age was 47 (range 16-65) with myeloablative transplant in 89 and reduced intensity in 40 patients. Respiratory failure was the main reason for admission (54.6%) followed by sepsis (41.5%). Improvement in organ failures were seen in 29.2% of the patients, 39.2% remained stable while 28.4% deteriorated following admission. Most patients (n = 67, 51.9%) were discharged from ICU but only 48 (37%) were discharged from the hospital (ICU). A higher proportion of patients were admitted after day + 30 post-transplant (40% Vs 29%), with those admitted prior to day +30 having a lower likelihood of survival (17% vs 23%). Univariate analysis identified; number of organ failures at admission, progression of organ failure during ICU admission, APACHE II score at admission, steroid refractory GVHD, and requirement for inotropic support or dialysis as significant predictors for survival in ICU. Patients requiring intubation and mechanical ventilation had a poor outcome than the group did not (p=0.001). While prior ICU bacterial infection did not alter the outcome (p=0.221) but the onset of a new infection in ICU did influence the outcome (p=0.0001). In contrast factors which did not alter the outcome are type of transplant, graft source, presence of neutropenia and mucositis.

Conclusion

While ICU support is justified for HSCT patients leading to improved survival, high APACHE II score, multiorgan failure, progression of organ failure during ICU stay, and the need for ventilation or dialysis, carries a dismal prognosis and is unlikely to be influenced by lengthy ICU admissions. There remains the importance of a good scoring system in regard to prognosticate and decide upon the continuity of the treatment on these critically ill patients.

No conflicts of interest to declare

A213

Wednesday 21 October
HSANZ Free Communications 9

1100-1230
Hall C

O115

1200

The Impact Of An Outpatient Based Autologous Stem Cell Transplant Service (ASCT) On Hospital Bed Stay And Clinical Resources. The Sir Charles Gairdner Hospital Experience

Dejan Radeski, Susan Hyde, David Joske, Bradley Augustson, Steven Ward, Patrick Crawford and Gavin Cull

Haematology Care Centre, Sir Charles Gairdner Hospital, Nedlands, Western Australia

Aim

To examine the impact of outpatient based ASCT (OP-ASCT) on hospital bed stay and clinical resources.

Method

A retrospective analysis of 107 consecutive ASCT at SCGH between January 2007 and June 2009. Data was collected from patient notes and computerized hospital information systems.

Results

Patients for ASCT were referred from their consultant to the nurse practitioner/transplant coordinator. All patients were considered candidates for OP-ASCT. High dose melphalan was administered in the day care ward. BEAM was administered using infusional chemotherapy in the day care ward and via CADD pump at home. Review was daily during chemotherapy and every 1-2 days after chemotherapy. The nurse practitioner was responsible for OP management of problems including nausea, mucositis, dehydration and blood products. Febrile neutropenia was not managed as an OP and mandated admission.

Indications for ASCT included myeloma (42%), NHL (30%), Hodgkin disease (17%) and other (11%). Following pre-transplant assessment, 85/107 (79%) were planned for OP-ASCT and 22/107 (21%) for IP-ASCT. Reasons for IP-ASCT included co-morbid medical problems, psychosocial problems and geographical issues. For IP-ASCT who remained until haematological recovery, median length of stay in hospital (LOS) was 18 days. For OP-ASCT, 17/85 (20%) did not require admission at any point. For those requiring admission, the median was day +6 post-transplant and the main indication febrile neutropenia. Median LOS for OP-ASCT was 6 days. For the entire cohort, median time to first post-transplant transfusion with red cells was 6 days and platelets 7 days. Median time to neutrophil count >1.0 was 11 days post-transplant. Transplant related mortality was 2%.

Conclusion

Planned outpatient based ASCT substantially reduces the median length of stay in hospital. Benefits include reduced pressure on in-patient beds and timely administration of conditioning chemotherapy. A suitably resourced day care ward and dedicated nurse transplant coordinator are required to run this service.

No conflict of interest to disclose

A214

Wednesday 21 October
HSANZ Free Communications 9

1100-1230
Hall C

O116

1215

Haplo-identical Transplant Without T Cell Depletion Using a Reduced Intensity Conditioning Protocol is Feasible in Older Patients with Haematological Malignancies

Biju George, Ian Kerridge, Mary McGurgan, Kenneth Bradstock, David Gottlieb, Mark Hertzberg
Blood and Marrow Transplant Unit, Westmead Hospital, Sydney

Aim

To explore the feasibility of haplo-identical transplants in older patients with haematological malignancies lacking a suitable histocompatible donor.

Methods

Between April 2008 and May 2009, 6 patients underwent haploidentical transplant using Fludarabine 30 mg/m²/day (Days - 6 to -2), Cyclophosphamide 14.5 mg/kg daily (Day - 6 and -5) and TBI 200 cGy on day -1. Graft source was unmanipulated bone marrow. Cyclophosphamide (50 mg/kg on Day +3) with tacrolimus and mycophenolate (started on day +4) was used as GVHD prophylaxis.

Results

Four males and 2 females with a median age of 55 years underwent haploidentical transplant for AML (n = 3), high grade MDS (n = 1) and non-Hodgkin's lymphoma (n = 2). Median CD34 cell dose infused was 2.1 x 10⁶ CD34/Kg (range: 1.3 – 2.9). All patients engrafted with a median neutrophil and platelet engraftment of 15 (range: 13 – 55) and 19 days (range: 0 – 66) respectively. Acute GVHD occurred in 2 (33%) [both grade II] with limited chronic GVHD in 3 (60%). Toxicity was minimal with grade 1-2 mucositis in all. None had bacterial or fungal infections but CMV reactivation occurred in 2. One patient with AML had early relapse at day 21 and expired Day 89 post transplant. Four patients achieved complete chimerism by day 30 while 1 patient who had mixed chimerism by day 30 (donor < 90%) rapidly lost donor chimerism and is presently well with autologous reconstitution. Non- relapse mortality at 3 and 6 months is 0%. At median follow up of 7 months (range: 2-12), 5 (83.3%) are alive with a DFS of 66%.

Conclusions

Reduced intensity haplo-identical transplants without T cell depletion is a feasible option with low toxicity in elderly patients who lack a suitable histocompatible donor. Further studies will determine the place of haploidentical transplantation in patients without suitable donor options.

No conflict of interest to disclose

Wednesday 21 October
HSANZ Free Communications 10

1100-1230
Meeting Rooms 1/2

O117

1100

Long-Term Outcomes of 90 Patients with Primary Cutaneous B-Cell Lymphoma: Analysis of the Peter MacCallum Cancer Centre/St Vincent's Hospital Melbourne Cutaneous Lymphoma Database

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Background

Primary cutaneous B-cell lymphomas (PCBCL) are a rare group of neoplasms. There have been few long-term follow-up analyses worldwide of large patient cohorts. The WHO/EORTC classification system identifies three main subtypes of PCBCL: primary cutaneous marginal zone lymphoma (PCMZL), primary cutaneous follicle centre lymphoma (PCFCL), and primary cutaneous diffuse large B-cell lymphoma, leg type (PCLBCL, LT). The former two subtypes are typically considered to have an indolent behaviour.

Aims

We aimed to identify long-term outcomes and prognostic indicators in all patients with PCBCL treated at our institutions. Patients whose diseases behaved more aggressively than expected (compared with previous reports of these subtypes) were analysed in particular detail.

Methods

The Victorian Cutaneous Lymphoma Database was interrogated to identify all patients with PCBCL since 1991 (n = 90). All patients had a biopsy-proven diagnosis of B-cell lymphoma of skin with no systemic disease identified on staging investigations at time of diagnosis. Survival data were analysed using Kaplan-Meier survival analysis. Univariate analyses were performed for the following factors: subtype, site/extent of cutaneous involvement, age at diagnosis, initial treatment and time-to-relapse.

Results

The 90 patients with PCBCL had a biopsy-proven diagnosis of PCMZL (n=31, 34.4%), PCFCL (n=39, 43.3%), PCLBCL, LT (n=20, 22.2%) and subtype unknown (n=4). Interestingly, 5 patients (5.5%) had concurrent diagnoses of two different cutaneous B-cell lymphoma subtypes. First-line treatments included: radiotherapy (63.6% of patients), chemotherapy (18.2%), surgical excision (18.2%) and rituximab (12.1%). Relapse rate post-initial treatment was 51.5%. Fifteen patients presented with unusual disease courses: either progression of indolent disease (PCMZL or PCFCL) to systemic involvement, concurrent diagnoses of two B-cell lymphoma subtypes, or development of PCLBCL after initial diagnosis of an indolent subtype.

Conclusions

Progression to more aggressive disease in PCBCL indicates a relatively poor prognosis and our analysis suggests that this may be more common than previously recognised. We describe a subgroup of patients with unusual and most interesting disease courses.

No conflict of interest to disclose

Wednesday 21 October
HSANZ Free Communications 10

1100-1230
Meeting Rooms 1/2

O118

1115

Hyper-CVAD + Rituximab Followed by High-dose Busulfan, Melphalan and Autologous Stem Cell Transplantation in First Response is Well-tolerated and Produces Prolonged Event Free Survival in Patients with Mantle Cell Lymphoma (MCL)

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Introduction

Hyper-CVAD+Rituximab (R) alternating with high dose methotrexate + cytarabine (HDMTX/ARA-C +R) results in a >85% CR rate (cf CHOP (30%) or CHOP+ R (50%)). The role of consolidating the excellent initial Hyper-CVAD+R responses with AuSCT remains controversial. We report on our experience of using Hyper-CVAD+R followed by a consolidative BuMel ASCT as initial therapy for patients (pt) <65 years old with MCL.

Patient characteristics

n=25, female=8, median age=53 (range 29-62), Stage IV n=19, \uparrow \square 2microglobulin n= 9, \uparrow LDH n= 7. Median calculated MIPI score = 5.4 (range 4.4 – 6.1). Twenty pt have completed therapy, one declined AuSCT after achieving a CR. Four pt are yet to undertake planned autografts; of these 2 have completed R-HyperCVAD and are in CR, 1 has completed 3 cycles of R-HyperCVAD and 1 has completed 2 cycles of R-HyperCVAD and has been de-escalated to R-CHOP due to recurrent sepsis.

Treatment outcome

Median follow is 65 Mo (range 2-96 Mo). Hyper-CVAD+R induced CR in 21 of 22 evaluable patients (95%). Median time to AuSCT = 8 Mo (range 4-12 Mo). One patient (age=58) died at day +21 post-AuSCT. Of 20 patients completing therapy, 14 (82%) remain in CR (median follow-up 60m). Five have relapsed (40 Mo, 42 Mo, 49 Mo, 54 Mo and 55 Mo respectively) and 2 have died of progressive disease. On an intention to treat, the 5y EFS and OS for all 25 pt are 58% and 87% respectively, and for those completing AuSCT 63% and 87% respectively. We have observed no cases of MDS or secondary AML.

Conclusions

These data confirm the tolerability and efficacy of Hyper-CVAD+R regimen in MCL. Further, consolidation with BuMel AuSCT may delay the time to disease progression compared to Hyper-CVAD+R alone and improve the OS of 60% at 5 years predicted by the MIPI score.

No conflict of interest to disclose

Wednesday 21 October
HSANZ Free Communications 10

1100-1230
Meeting Rooms 1/2

O119

1130

Protein Kinase C β II Expression in Diffuse Large B Cell Lymphoma Predicts for Inferior Outcome of Anthracycline Based Chemotherapy with and Without Rituximab

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Protein Kinase C β II (PKC β II) expression has been reported to indicate inferior prognosis in diffuse large B-cell lymphoma (DLBCL) treated with anthracycline-based chemotherapy.

Aim

To compare the prognostic significance of immunohistochemically determined PKC β II expression in *de novo* DLBCL treated with CHOP chemotherapy with and without rituximab.

Method

80 consecutive patients treated at St. Vincent's Hospital with *denovo* DLBCL, 48 treated with CHOP, and 32 with rituximab plus CHOP (R-CHOP), were studied using immunohistochemistry for PKC β II on diagnostic tissue samples. Staining results were correlated with patient characteristics and clinical outcome. Overall Survival (OS) and Progression-Free Survival (PFS) were determined by the Kaplan-Meier method, and comparisons were determined by the log-rank test.

Result

PKC β II expression correlated with inferior OS and PFS in CHOP treated patients with low-risk International Prognostic Index (IPI) disease (0-2 adverse factors), but not in the overall patient group unstratified by IPI. PKC β II expression significantly correlated with inferior OS and PFS in R-CHOP treated patients unstratified by IPI status.

Conclusion

PKC β II expression has prognostic significance not only for CHOP therapy in low-risk IPI disease, but also for all patients receiving CHOP plus rituximab. Immunohistochemically-demonstrated PKC β II expression thus identified patient subgroups where alternative treatment strategies may confer superior outcome.

This research was supported by Novartis Pharmaceutical Australia. The company had no role in analysing the data or preparing the abstract.

Wednesday 21 October
HSANZ Free Communications 10

1100-1230
Meeting Rooms 1/2

O120

1145

Retrospective Analysis of Patients with Primary CNS Lymphoma Treated with Methotrexate and Reduced Dose Whole-brain Radiotherapy

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Aim

Radiotherapy dose is a major risk factor in development of neurotoxicity in patients with primary CNS lymphoma. This study assessed the effect on outcome and toxicity of reducing whole brain radiotherapy to 30Gy when combined with methotrexate chemotherapy.

Methods

Retrospective observational study of patients with histologically proven PCNSL in one tertiary referral centre in Western Australia. The primary endpoint was event-free survival of the patient group. Response rate and therapy toxicity were secondary end points. Results were compared to TROG 92.01 study, where a whole-brain dose of 45.4Gy plus 5.4Gy boost of radiotherapy was combined with methotrexate. A subset of patients receiving rituximab were analysed separately to determine any significant differences in survival or toxicity.

Results

23 patients between 2001 and 2008 with primary CNS B cell lymphoma were included. They were all immunocompetent with no extra-cerebral disease. Median age of the patient group was 62 years (range 42 to 76 years) and ECOG varied from 1-3. All patients received methotrexate (1g/m² day 1 and day 8) and whole-brain radiotherapy of 30G starting day 15. In addition ten patients received rituximab.

Treatment was generally well tolerated with only four patients requiring dose reduction. 14 (61%) patients had complete radiological remission. As of May 2009 16 patients remain alive with a median follow-up of 18 months and overall survival was 70%. Seven patients had relapsed lymphoma with five occurring in the CNS. Four deaths occurred as result of PCNSL relapse. Median event-free survival was 37 months. Six patients had symptoms consistent with neurotoxicity and it contributed to one death. There were no significant differences in the rituximab group.

Conclusion

For PCNSL patients treated with methotrexate and radiotherapy, reducing the dose of radiotherapy to 30Gy does not compromise survival. Severe neurotoxicity may be reduced. Longer-term follow-up is required to confirm these findings.

No conflicts of interests to disclose

Wednesday 21 October
HSANZ Free Communications 10

1100-1230
Meeting Rooms 1/2

O121

1200

Cardiovascular Disease Is A Frequent Late Complication In Survivors Of Hodgkin Lymphoma

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Aims

The primary aim of this study was to define the frequency of and risk factors for late complications of the therapy for Hodgkin's lymphoma (HL). Here, we focus on cardiovascular disease (CVD) coronary and carotid artery disease, and valvular dysfunction.

Methods

The South Australian Cancer Registry was used to identify HL patients within the North Western Area Health Service of Adelaide. Patients older than 18 yrs, free of disease for at least 5 years and diagnosed after 1975 were invited to participate.

Results

At the time of writing 58 patients were fully evaluable. 43 patients received chemotherapy and 36, radiotherapy (RT), (16 RT only and 20 combined), 28/36 patients received mantle field RT. 35/58 (60 %) evaluable patients were alive at a median 18 years post treatment. 12/58 (20%) had a confirmed diagnosis of symptomatic CVD (3 cerebro-, 10 cardiovascular) . Three patients died from complications of CVD. There was an association between the incidence of CVD and radiotherapy with 10/12 CVD patients having received radiotherapy compared to 2/12 having had chemotherapy alone.

Conclusions

The incidence of CVD was similar to that observed in the literature (17%). Not surprisingly a high number of survivors were unaware of this risk and also the interference of life-style choices and personal risk factors. Hence, early detection, counseling and preventative measures are of paramount importance.

This research was supported by Roche. The company had no role in data analysis or abstract preparation.

Wednesday 21 October
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Meeting Room 3

O123

1100

The Tyrosine Kinase Inhibitor Dasatinib Dysregulates Bone Remodelling Through Inhibition of Osteoclasts *In Vivo*

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Aims

Dasatinib is a tyrosine kinase inhibitor that is used to treat chronic myeloid leukaemia in patients resistant or intolerant to imatinib mesylate. We have previously demonstrated that therapeutic concentrations of dasatinib potentially inhibit osteoclast formation and activity *in vitro* due, in part, to its specificity for the macrophage colony stimulating factor (M-CSF) receptor, c-fms. In the present study, we examined whether dasatinib could significantly alter bone remodelling *in vivo*.

Methods

Nine-month-old Sprague Dawley rats were administered dasatinib (5 mg/kg/day) or vehicle control (10% DMSO/90% polyethylene glycol 300 [v/v]) by gavage or zoledronic acid (ZOL; 100 µg/kg/6 weeks) sub-cutaneously. Following 4, 8 and 12 weeks of treatment, animals were sacrificed and serum biochemical, bone morphometric and histological analyses were carried out.

Results

Micro-computed tomographic (µ-CT) analysis of cancellous bone at the proximal tibia showed that trabecular bone volume (BV/TV) and trabecular thickness (Tb.Th) were increased in dasatinib-treated animals, at levels comparable to the ZOL-treated group. These changes were associated with a greater than 50% decrease in osteoclast numbers (Oc.N/BS) and osteoclast surface (Oc.S/BS). While no significant changes in serum calcium levels were observed, hypophosphataemia was induced in the dasatinib-treated animals. Following 8 weeks of treatment, serum levels of the bone resorption marker C-terminal collagen crosslinks (CTX) were decreased in the dasatinib-treated group, relative to the vehicle control, while the levels of the bone formation marker osteocalcin remained unchanged.

Conclusions

This study demonstrates that dasatinib increases trabecular bone volume in a rat model of normal bone remodelling, at least in part, by inhibiting osteoclast activity. While these data suggest that dysregulated bone remodelling may be a possible side-effect of dasatinib therapy, they also suggest that suggest dasatinib may be useful in the treatment of diseases characterised by bone loss.

No conflict of interest to disclose

Wednesday 21 October
HSANZ Free Communications 11

1100-1230
Meeting Room 3

O124

1115

Enhancing the Functional Activity of the OCT-1 Influx Pump in De-novo CML Patients May Greatly Improve Response to Imatinib

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Aim

The primary active influx protein for imatinib (IM) is the human organic cation transporter 1 (OCT-1), and the functional activity of the OCT-1 protein (OA) is predictive of response to IM in CP-CML patients. Patients with a low OA have a poorer response to imatinib compared to those with high OA (MMR by 24 months: 45% vs 85% respectively $p = 0.004$). Increasing dose may overcome the effect of a low OA but is not tolerated by most patients. As an alternative here we investigate strategies to increase the active uptake of IM.

Method

Potential OA enhancers (OA-E) were chosen using the connectivity map (CMAP) which allows the observation of functional interactions that are present between various drugs and genes of interest. In this study, the OA was measured in a cohort of 56 de-novo CML patients with and without the presence of diclofenac; fasudil; and LM1685.

Results

Overall, a significant increase in OA was observed when enhancers were introduced ($p = 0.001$). The mean OA without enhancers was 3.7ng/200000 cells, which was increased to 4.9 with fasudil and 5.3 with diclofenac ($p = 0.026$ and 0.003 respectively). An increase was observed in 64% of all samples tested with fasudil and 80% of samples tested with diclofenac. LM1685 also increased OA in 66% of patients; however, there was no significant change in the mean OA. In patients with an initial OA of >4ng/200000 cells OA-E were less effective, suggesting enhancing OA in patients with a higher OA is of little benefit.

Conclusion

In patients having a low OA and hence increased risk of suboptimal response to imatinib, enhancing the OA may be of great benefit to these patients. The potential to improve response to imatinib by enhancing drug uptake warrants further study and clinical investigation.

No conflict of interest to disclose

A222

Wednesday 21 October
HSANZ Free Communications 11

1100-1230
Meeting Room 3

O125

1130

Short-Term Intense Bcr-Abl Kinase Inhibition is Adequate to Trigger Cell Death in CML-CD34⁺ Cells But Only if They are Simultaneously Cytokine Deprived

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Aim

Preclinical studies of imatinib set the paradigm of continuous Bcr-Abl kinase inhibition for optimal response in CML. However, clinical studies with dasatinib suggest that intermittent kinase inhibition also leads to good clinical responses. We assessed the impact of the intensity and duration of Bcr-Abl kinase inhibition on proliferation and apoptosis of CML cells.

Method

Cell death triggered by short-term intense (cells were cultured with dasatinib or imatinib for 30min, and following thorough drug washout, cells were recultured in drug free media for remainder of 72hr) and long-term partial (cells were cultured with dasatinib or imatinib for 72hr) Bcr-Abl kinase inhibition was studied in *BCR-ABL*⁺ cell lines and CML-CD34⁺ colony forming cells (CFC). Effect of combined Janus tyrosine kinase (Jak) and Bcr-Abl kinase inhibition on p-STAT5 expression and CD34⁺-CFC was studied in the presence or absence of cytokines.

Results

Despite reactivation of Bcr-Abl kinase activity within 4hr of drug washout, short-term intense kinase inhibition with 100 nM dasatinib induced apoptosis in 70-80% of cells from *BCR-ABL*⁺ cell lines. By contrast, in the presence of cytokines, short-term intense Bcr-Abl kinase inhibition did not trigger apoptosis in BaF3 BCR-ABL cells or in CD34⁺ primary CML cells. However, without cytokines, short-term 100 nM dasatinib reduced CFCs by 75-80%. Cytokines rescued CML-CD34⁺ cells and BaF-3 BCR-ABL cells by activating Jak-STAT5 pathway. In the presence of cytokines, a combination of Jak inhibitor and short-term dasatinib inhibited STAT5 and triggered cell death in BaF3 Bcr-Abl and CD34⁺ CFC.

Conclusion

Cytokines added during or immediately after short-term exposure to dasatinib prevented apoptosis of CML-CD34⁺ cells suggesting that oncogene dependence of these cells can be overcome by exposure to cytokines. Therapeutic strategies combining short-term intense Bcr-Abl kinase inhibition and blockade of cytokine pathways warrant further assessment as a novel strategy for eradication of CML progenitors.

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Wednesday 21 October
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Meeting Room 3

O126

1145

OCT-1 Activity in CML CD34+ Cells is Not Predictive of Molecular Response to Imatinib Treatment in CP-CML Patients

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Aim

The functional activity of the OCT-1 protein (OCT-1 Activity, OA) in mononuclear cells (MNC) is variable between patients and is predictive of response to imatinib¹. The OA in CML CD34+ cells is significantly lower than that found in mature CML cells. The aim of this study was (1). To assess whether a relationship exists between the OA in MNC and the OA in CD34+ cells. And (2). To identify if CD34+ OA predicts for the achievement of major molecular response (MMR).

Methods

MNC and CD34+ were isolated from imatinib naïve, newly diagnosed chronic phase CML patients. OA was determined using [14C]-labelled imatinib and the OCT-1 inhibitor prazosin [1].

Results

No correlation was found between the OA in MNC and the OA in CD34+ cells in 35 CML patients ($R=0.306$, $p=0.0739$). When patients were divided as having high ($n=23$) or low ($n=12$) OA in their MNC¹, no difference was seen between the OA in their corresponding CD34+ cells (mean low OA: 3.18, high OA: 4.32 ng/200,000 cells, $p=0.236$). Lastly, in 21 patients where 12 month response data was available patients were grouped according to the achievement or not of MMR by 12 months. MNC OA was found to be significantly associated with the achievement of MMR (mean OA, MMR: 13.75, no MMR: 5.31 ng/200,000 cells, $p=0.042$). However, assessment of CD34+ cells failed to demonstrate a relationship between OA and achievement of MMR (mean OA, MMR: 4.11, no MMR: 4.07 ng/200,000 cells, $p=0.409$).

Conclusion

We were unable to demonstrate a relationship between OA measured in a patient's MNC and that in their CD34+ cells. Furthermore, unlike our observations in MNC, CD34+ OA is not predictive of patient's response to imatinib treatment. This suggests, the predictive value of the MNC OA primarily reflects the effective targeting and subsequent eradication of mature CML cells.

References.

1. White DL, Saunders VA, Dang P, et al. Most CML patients who have a suboptimal response to imatinib have low OCT-1 activity: higher doses of imatinib may overcome the negative impact of low OCT-1 activity. *Blood*. 2007;110:4064-4072.

This research was supported by Novartis Pharmaceuticals. The company had no role in analysing the data or preparing the abstract

Wednesday 21 October
HSANZ Free Communications 11

1100-1230
Meeting Room 3

O127

1200

A Population-Based Study of Responses to Imatinib in the Treatment of Newly Diagnosed Patients with Chronic Myeloid Leukaemia in New Zealand

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Aim

New Zealand was one of the first countries to approve and fund imatinib as first-line treatment for chronic phase chronic myeloid leukaemia (CML). To date, most published data has arisen from clinical trials. This study aimed to characterise responses to imatinib in patients with newly diagnosed CML in the New Zealand population.

Method

Laboratory and clinical records of sixty consecutive patients with chronic phase CML in the wider Auckland region receiving imatinib as first-line therapy between March 2003 and February 2008 were analysed for haematological, cytogenetic and molecular responses, event-free, progression-free and overall survival. Toxicities and resistance were also recorded. In addition, clinical practice patterns were evaluated against published guidelines. Statistical analyses were performed by Kaplan Meier and log-rank methods.

Result

Patients in the study had a median follow-up of 30.5 months (range: 3-63 months). Best responses were: complete haematological response 97%, complete cytogenetic response (CCyR) 78% and major molecular response, 62%. Event-free survival (EFS) at 30 months was 77%. Freedom from progression to accelerated phase or blast crisis was 95%, overall survival, 100%. Patients achieving a CCyR by one year had significantly better EFS than those who did not (83% vs 57%, $P=0.024$). Seventeen percent of patients discontinued imatinib for resistance and 7% with intolerance. The rate of primary resistance was 2%, secondary resistance 15% and incidence of kinase domain mutations in resistant patients, 70%. Imatinib toxicities were similar to other studies.

Conclusion

These outcomes mirror the key IRIS study, and are similar to the recently published Hammersmith population-based study. Treatment approaches met European LeukaemiaNet Guidelines. This study provides a pilot for national evaluations of CML treatment outcomes and clinical management in New Zealand.

No conflict of interest to disclose

Wednesday 21 October
HSANZ Free Communications 11

1100-1230
Meeting Room 3

O128

1215

OCT-1 SNPs Known to Reduce Function Do Not Account for the Observed Interpatient Variability in OCT-1 Activity or Response to Imatinib in the Australian TIDEL Trial

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Aim

Intrinsic sensitivity of CP-CML patients to imatinib-induced kinase inhibition is related to the functional activity of OCT-1, but not directly related to the level of OCT-1 mRNA. Single nucleotide polymorphisms (SNPs) can influence protein function or substrate recognition. OCT-1 is known to be highly polymorphic, with several reduced-function (R61C, C88R, P341L) or loss-of-function (G401S, G465R) SNPs reported. We hypothesised that the presence of non-synonymous polymorphisms in OCT-1 may explain interpatient variation in OCT-1 Activity (OA) and therefore predict patient response to imatinib.

Methods

Blood was collected from 91 newly diagnosed CP-CML patients, enrolled to the TIDEL trial. Three fragments of the OCT-1 coding region, corresponding to the SNPs above, were amplified by PCR and sequenced. Sequences were aligned in Mutation Surveyor against the GenBank OCT-1 reference sequence. The presence or absence of polymorphisms was assessed against the achievement of a major molecular response (MMR : $\leq 0.1\%$ BCR-ABL, IS) by 24 months, and OA.

Results

Table 1: Achievement of MMR by 24 months based on presence or absence of a reduced-function or loss-of-function SNP in OCT-1. (* denotes homozygosity)

Achievement of MMR by 24 months	R61C (n=12)	P341L (n=1)	G401S (n=2)	G465R (n=2)	No reduced-function or loss-of-function SNP (n=74)
MMR (n=58)	7	1	1	1	48
No MMR (n=32)	5	0	1	1*	26

Of the 17 patients found to have a SNP in OCT-1, only one was homozygous (G465R). R61C was the most common SNP (12/91 patients), while the C88R was not detected. The presence of a reduced- or loss-of-function SNP did not correlate with failure to achieve MMR, as shown in Table 1. Of the 8 patients with a SNP and OA data available, 4 (50%) were in the low OA group and 4 (50%) had a high OA.

Conclusion

The impact of SNPs has been classically associated with [³H]1-methyl-4-phenylpyridinium (MPP+) uptake. However it is known that some SNPs effect substrate recognition, hence a SNP that affects one drug may not necessarily affect another. In this study we cannot demonstrate that genetic variation in OCT-1 is the underlying cause of either the wide interpatient variability in OA or the response to imatinib observed in de novo chronic phase CML patients.

This research was supported by Novartis. The company had no role in analysing the data or preparing the abstract

A226

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Meeting Room 10

O129

1100

A Role for Klf5 as a Tumour Suppressor in AML

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Kruppel-like factor 5 (Klf5) is a zinc finger transcription factor with roles in proliferation, self-renewal, differentiation, and apoptosis. Klf5 displays oncogenic or tumour suppressor properties in a context-dependent manner, and has been implicated in numerous malignancies. We aim to investigate the role of Klf5 as a novel tumour suppressor in the myeloid system and to determine how aberrant regulation of Klf5 may contribute to the pathogenesis of Acute Myeloid Leukaemia (AML). We show that expression of Klf5 mRNA is up-regulated during granulocytic differentiation of murine myeloid cell lines, and this is confirmed in human and mouse primary haemopoietic systems from bioinformatic analysis of published microarray data. Accordingly, retroviral expression of Klf5 in the murine myeloid FDB1 cell line induces growth arrest and apoptosis, and concurrently drives differentiation. Enforced expression of Klf5 in mouse bone marrow progenitors similarly inhibits cell growth and enhances differentiation, with reduced colony forming ability observed in all cytokine combinations tested. Using a panel of 14 human AML samples, 7 leukaemia cell lines and 4 normal bone marrow controls we show that Klf5 mRNA expression is significantly lower in AML samples compared to normal controls. This finding agrees with our analysis of published microarray data sets evaluating larger AML patient cohorts (n=285). We have employed Sequenom MassARRAY quantitative technology to identify methylation of Klf5 Intron 1 as a potential mechanism for its down-regulation in AML. Treatment of leukaemia cell lines with the methyltransferase inhibitor 5-Azacytidine re-activates Klf5 expression indicating a functional role for the observed methylation. Additionally, retroviral expression of Klf5 in AML cell lines results in changes associated with myeloid differentiation. Collectively, these data are consistent with Klf5 acting as a myeloid tumour suppressor and re-activation of Klf5 may be important in the development of therapeutic strategies for AML.

No conflict of interest to disclose

Wednesday 21 October
HSANZ Free Communications 12

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Meeting Room 10

O130

1115

New Targets for the *Protein Kinase* Activity of PI3K: Implications for Deregulated Cell Survival in Acute Myeloid Leukaemia

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Cytokines are potent regulators of cell survival through their ability to activate intracellular signalling cascades via protein phosphorylation. We have previously shown that GM-CSF stimulation of haematopoietic cells results in serine 585 phosphorylation of the GM-CSF receptor, the binding of 14-3-3 family of scaffold proteins and regulation of cell survival¹⁻². We have also shown that Ser585 phosphorylation is deregulated in acute myeloid leukaemia¹. We have therefore sought to identify the kinase that phosphorylates Ser585 using a biochemical purification approach. We have isolated a Ser-585 kinase activity and determined its pharmacological profile against a panel of inhibitors. Strikingly the Ser585 kinase activity was only inhibited by PI3K inhibitors. We have confirmed that PI3K p110 alpha can directly phosphorylate Ser585 in vitro and that p110 alpha selective-inhibitors block Ser585 phosphorylation and abrogate haematopoietic cell survival not only of primary bone marrow progenitors but also in leukaemic stem/progenitor cells³. These results show that Ser585 of the GM-CSF receptor can be phosphorylated by the **protein kinase** activity of PI3K and that these events are deregulated in leukaemia leading to constitutive Ser585 phosphorylation and autonomous cell survival. Blocking PI3K **protein kinase** activity by alpha selective PI3K inhibitors may be a potential strategy in eradicating residual AML leukaemic blasts.

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No conflicts of interest to declare

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1130

Repression of *Gadd45a* by Activated FLT3 Receptors and DNA Methylation in AML

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The tumour suppressor gene Growth Arrest and DNA Damage Inducible (*Gadd45a*) is involved in induction of cell cycle arrest and DNA repair in normal cells in response to DNA damage. Down-regulation of *Gadd45a* is a hallmark of many solid tumours, such as breast and prostate. In AML, we have shown *Gadd45a* expression to be down-regulated across a broad range of karyotypes and cytogenetic abnormalities (Perugini *et al*, Leukemia, 2009). Our functional analysis is consistent with an important role for *Gadd45a* down-regulation in the continued growth and survival of AML cells. *Gadd45a* is most significantly down-regulated in AML patients harbouring MLL translocations or FLT3 receptor mutations, which together constitute >40% of AML cases. In FLT3-ITD+ cells we have shown regulation of *Gadd45a* downstream of sustained ERK1/2 signalling. Consistent with functional down-regulation of *Gadd45a* by FLT3-ITD we show that over-expression of *Gadd45a* in FLT3-ITD+ myeloid cell lines induces G1/S cell cycle arrest and increases apoptosis. *Gadd45a* expression in AML may also be regulated at least in part by DNA methylation. Using Sequenom Mass Array methodology we identified methylation of the *Gadd45a* promoter on CpG residues previously reported to be methylated in breast and prostate cancer. We found significant promoter methylation in 8/15 AML samples when compared to normal controls. In summary we propose that *Gadd45a* is an important regulator of myeloid cell growth and there are multiple mechanisms of *Gadd45a* down-regulation in AML, including activated receptor signalling and DNA methylation. We are now investigating whether the DNA methylation status and expression of *Gadd45a* is an important prognostic indicator in AML.

No conflict of interest to disclose

A229

Wednesday 21 October
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1100-1230
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O132

1145

Membrane Bound Phosphatase Genes Are Epigenetically Regulated in Acute Lymphoblastic Leukemia

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Aim

Gene promoter methylation is an important epigenetic abnormality associated with silencing of tumour-suppressor genes and cancer development. The methylation status of members of a phosphatase gene family was examined in Acute Lymphoblastic Leukemia (ALL).

Method

Methylation specific digestion of DNA derived from ALL patients was performed prior to hybridisation to an Agilent array. Promoter methylation was examined in cell lines and primary leukemia cells with pyrosequencing after bisulfite treatment of DNA. Gene expression was measured in cell lines with real-time PCR.

Result

Genome wide analysis of CpG island methylation in ALL identified abnormal promoter methylation in a large number of genes including members of a membrane bound phosphatase gene family. The methylation status of the promoter of seven phosphatase genes was examined in leukemia cell lines using pyrosequencing and promoter methylation was validated in four members of this gene family. The methylation status of these four validated genes was then examined in primary leukemia samples. The promoters of these four phosphatase genes were significantly methylated in 75% of primary ALL samples analysed (n=53) but promoter methylation was not identified in CLL (n=14), AML/MDS (n=28) or normal samples (n=11). Expression of all 4 phosphatase genes increased in Raji and ALL1 cell lines after *in vitro* exposure to decitabine suggesting that these genes may be epigenetically modified with demethylating therapy.

Conclusion

Four members of a phosphatase gene family were identified to be specifically methylated in ALL. These membrane bound phosphatases may represent tumour-suppressor genes and provide potential targets for demethylating therapy with drugs like azacytidine or decitabine.

No conflict of interest to declare

A230

Wednesday 21 October
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1200

A Distinct Set of MicroRNAs Differentiates Acute Promyelocytic Leukaemia According to FLT3 Mutation Status

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Aim

The PML-RARA fusion gene is central to the pathogenesis of acute promyelocytic leukaemia (APL), however is insufficient alone to induce leukaemia and co-operating mutations have been postulated as "second hit" leukaemogenic events. Foremost amongst these are internal tandem duplications (ITD) and point mutations (PM) in the FLT3 tyrosine kinase which occur frequently in APL (40-50%). Presumably, alternate mechanisms of gene dysregulation are operating in APL patients with only wild type FLT3. MicroRNAs are important regulators of gene expression and their deregulation is linked to the pathogenesis of haematological malignancy. To address what role microRNAs may be playing in the pathogenesis of APL we have examined microRNA expression patterns in FLT3 mutated and wild type APL specimens.

Methods

Total RNA from 24 diagnostic APL bone marrow aspirates (12 wild type FLT3; 9 FLT3 ITD; 3 FLT3 PM) were labelled and hybridized to Agilent microarrays encompassing probes for 723 human microRNAs sourced from miRbase version 10.1. Linear modelling analysis with a false discovery rate cut-off <0.05 was applied to determine differentially expressed microRNAs between FLT3 wild type and ITD samples. Differential expression was validated using RT-PCR (Applied Biosystems) in 17/24 microarrayed samples and a second set of 16 diagnostic APL bone marrow aspirates (8 FLT3 ITD mutation; 8 FLT3 wild type).

Results

Seven differentially expressed microRNAs were identified that were down-regulated (miR-155; miR-378 and miR378*) or up-regulated (miR-10a; miR-99a; miR-100; miR125b) in FLT3 wild type compared to ITD samples. Fold change differences ranged from 1.9 to 6.7. RT-PCR for these seven microRNAs validated these findings.

Conclusions

These microRNAs include several already known to play a role in normal and/or malignant haemopoiesis (miR-155; 10a; 125b). Bioinformatic studies are currently underway to provide insight into leukaemogenic mechanisms in APL by determining potential mRNA targets and biological pathways in which these microRNAs act.

This work was supported by the Cancer Institute of NSW Clinical Fellowship Programme

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A231

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1215

Up-regulated Expression of a Large MicroRNA Gene Cluster in Acute Promyelocytic Leukaemia

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MicroRNAs are small non-coding RNAs that regulate gene expression and have a central role in cellular differentiation. They act by binding to target messenger RNAs and inhibiting protein translation from that gene. We have been exploring the role of microRNAs in the leukaemogenesis of acute promyelocytic leukaemia (APL) and have used microarrays to determine microRNA expression patterns in 24 diagnostic APL bone marrow specimens. Unsupervised two-dimensional hierarchical clustering analysis of these data identifies a group of 47 microRNAs with 10-100-fold up-regulation in 17/24 APL bone marrow specimens. All of these microRNAs are encoded within a known gene cluster that forms part of a larger imprinted region (DLK1-DIO3) on chromosome 14q32. In mice, microRNA genes in this cluster are expressed predominantly in embryonic tissue, and adult brain and placenta. The two groups with high or low expression did not segregate on the basis of FLT3 mutation status, PML-RARA breakpoint region, white cell count at diagnosis, patient age or sex, or cytogenetic abnormalities additional to t(15;17). To further investigate this we selected four microRNAs encoded in the 14q32 cluster (miR-127; miR-337-3p; miR-299; miR-495) and used quantitative RT-PCR to determine their expression in bone marrow specimens from an independent set of newly diagnosed APL (n=12), other acute myeloid leukaemia (AML) patients (n=10), and from APL patients in molecular remission (n=5). Expression of the 14q32 cluster microRNAs was unique to the APL patients at diagnosis, with 10/12 samples showing up-regulation. Minimal expression was noted in the AML and APL remission samples. Deregulation of 14q32 cluster microRNA genes has been reported in other cancers and our findings suggest this region is also important for leukaemogenesis in APL. Mechanisms responsible for up-regulation of this cluster in the majority of APL patients are under investigation.

This work was supported by the Cancer Institute of NSW Clinical Fellowship Programme

No conflict of interest to disclose

A232

Wednesday 21 October**1100-1230****ANZSBT ASTH Combined Symposium: Anti-thrombotics and Anti-platelets: Problems They Cause****Hall B
1100****Reversal of Anticoagulants: Old and New. The Emerging Paradigm****Kate Burbury***Peter MacCallum Cancer Centre, East Melbourne, Victoria, Australia*

Thromboembolism (TE) is a major contributor to morbidity and mortality. More importantly, it is a preventable disease. Hence, with a growing number of indications, extended antithrombotic therapy use is increasing.

The vitamin K antagonist warfarin is the current conventional choice, but concerns surrounding the safety and practicalities of its use remain an important issue. Heparins, in particular low molecular weight, as alternatives are not without problems. This has not only limited the feasibility of thromboprophylaxis, but has spurred the development of novel parenteral and oral agents – which may offer a more reliable and convenient approach. Many of these emerging options, which predominantly target factor Xa and thrombin, are under phase III investigation and some have already been approved by regulatory bodies. However there are potential obstacles.

The most important complication of anticoagulant use is bleeding. If a patient presents with clinically relevant bleeding, or in situations of recognised risk, such as surgery or trauma - rapid and reliable reversal is required and ideally with a means to monitor the residual antithrombotic activity.

Warfarin is reversed by vitamin K and plasma-based products, but the optimal approach, despite widespread guidelines, remains varied. Apart from idrabioparinex, none of the newer agents have a specific antidote. “Non-specific” prohaemostatic agents, such as recombinant FVIIa, and modalities to assist clearance of these small molecules (e.g. haemodialysis) may have a role. However, there is no robust clinical data demonstrating utility, particularly in bleeding patients. Moreover, there is lack of validated assays for monitoring the antithrombotic effect of these new agents.

As efficacy and safety benchmarks are met, notwithstanding costs, the newer agents offer the potential to refine TE management. Lack of effective antidotes and means of monitoring remains a challenge. Local guidelines and strategies for management need to be considered, as use of these novel agents emerges.

No conflict of interest to disclose

Wednesday 21 October

1100-1230

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They Cause

Hall B
1130

Heparin-induced Thrombocytopenia in the Critical Setting

Lena Napolitano

Abstract not received at time of going to print

Wednesday 21 October

1100-1230

ANZSBT ASTH Combined Symposium: Anti-thrombotics and Anti-platelets: Problems They Cause

Hall B
1200

Novel Tests for Monitoring Haemostasis and Anticoagulants

Benny Sorensen

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Standard coagulation assays such as the activated clotting time (ACT), prothrombin time (PT/INR), or activated partial thromboplastin time (APTT) gives information only of the very initiation of clot formation. However, formation of a sufficient haemostatic plug is a continuous process with characteristic rate-specific properties. Routine coagulation assays, such as the PT and APTT are often performed using platelet poor plasma. The revised understanding of the haemostatic system has emphasized that all blood cells, platelets in particular, are important for the overall regulation of the haemostatic process.

A problem of special concern includes measurement of levels of fibrinogen a.m. Clauss (standard procedure in most coagulation laboratories). Hemodilution with colloids and dextran interfere with the measurement and reveal "false" high levels.

An ideal laboratory test would have the capacity to determine the clinical implication of a biochemical coagulation diagnosis and provide specific guidance in choice of effective haemostatic intervention.

Currently, there is no single haemostasis laboratory test that has the capacity to accurately illustrate the clinical effects of all types of pro- or anticoagulant interventions. Although the time course of thrombin generation in plasma and the endogenous thrombin potential (ETP) may be useful coagulation parameters, clotting involves components other than thrombin (e.g. platelets, fibrinogen). In particular it should be emphasized that normalization of thrombin generation has limited effect if the predominant characteristic of a coagulopathy is due to a dysfunctional fibrinogen polymerization (e.g. dilutional coagulopathy).

The continuous coagulation profiles of thrombelastometry may provide a more accurate reflection of *in vivo* biology, covering initiation, development and final clot firmness during whole blood clot formation. This method has helped to clarify the mechanism of action of whole blood clot formation, demonstrating the differences from clotting in plasma, and the importance of platelets and fibrinogen. Thrombelastometry has been used extensively in the clinic for monitoring the haemostatic system during cardiac and liver –surgery. It has also been used to investigate hypocoagulation (in haemophilia A, rare coagulation disorders, anticoagulant therapy and dilutional coagulopathy), and the effect of haemostatic interventions by e.g. fibrinogen, activated prothrombin complex concentrate, factor VIIa, factor VIII, factor XIII, and antifibrinolytics.

Evaluating levels of functional fibrinogen using thrombelastometry and commercial assays (e.g. FIBTEM®) do not overestimate levels of fibrinogen in cases of dilutional coagulopathy.

Overall, tailoring laboratory assays to illustrate and correlate with clinical phenotypes is essential for effective coagulation monitoring. Applying an algorithm of pre-, peri- and postoperative tests, including thrombelastometry and evaluation of whole blood platelet aggregation, may enable physicians to achieve this.