ROYAL COLLEGE OF PHYSICIANS OF EDINBURGH

CONSENSUS CONFERENCE ON PLATELET TRANSFUSION

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ABSTRACTS

Queen Mother Conference Centre
Royal College of Physicians
9 Queen Street
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Oral Presentations

(in order of programme)

Thursday, 27 November 1997

Therapeutic Platelet Transfusions

Platelet Transfusion in Cardiac Surgery

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Excessive bleeding following cardiopulmonary bypass (CPB) occurs in up to 5% of cases and is a major indication for post operative homologous blood transfusion. The mechanisms underlying defective post-operative haemostasis include surgical damage to blood vessels, unneutralized heparin, excessive protamine, fibrinolysis, thrombocytopaenia and defective platelet function. Platelet dysfunction lasts for up to 12 hours following CPB and is felt to be an important cause of inadequate platelet plug formation. Possible causes of the acquired platelet dysfunction include contact with foreign surfaces, physical trauma of the bypass and exposure to plasmin. Prophylactic administration of platelets following CPB is not indicated (BCSH Guideline 1992). We questioned whether peri-operative autologous plateletpheresis (PAP) using a BRAT2 (COBE Labs) and subsequent re-administration following the termination of bypass and reversal of heparin would reduce post-operative bleeding and homologous blood transfusion in patients undergoing cardiac surgery. 101 patients were randomly allocated to 2 groups; PAP or control. The groups were matched for age, sex distribution, operative procedure, pre-operative haemoglobin concentration and haematocrit. Of note the pre-operative platelet count was significantly lower in the PAP group. The mean volume of blood processed was 2.0 ± 0.3 litres and 1.96 ± 0.6 x 10^{11} platelets were autotransfused. Post-operative mediastinal blood loss was less on arrival in ITU, and at 1, 6 12 and 24 hours in the PAP group and reached significance ($P<0.05$) at 6 and 12 hours. Mean homologous blood exposure was 1.3 Units in the control group and 0.2 in the PAP group ($P<O.O1$). 12.2% of the PAP group and 42.3% of controls were transfused with homologous blood ($P<0.001$). By removing platelets from the CPB circuit, PAP offers a rational method to reduce post-operative bleeding and homologous blood exposure.

Platelet Transfusion in Liver Transplantation

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Thrombocytopaenia is universal during liver transplantation. Causes include hyper-splenism and increased turnover. Disorders of platelet function complicate this. Re-perfusion of the liver graft reduces platelet count by adhesion to endothelium, and Kupffer cell phagocytosis. Raised serum β-thromboglobulin and platelet factor 4 cause increased activation and turnover of platelets in liver disease/ transplantation. Reduced ADP/ristocetin-induced platelet aggregation correlates with reduced hepatocellular function. Antibodies directed against platelet surface glyco-proteins (PAIgG) further aggravate thrombocytopaenia. Platelet count reaches a nadir at day 5 following transplantation. Fall in platelet count is an independent predictor of survival. Platelet requirement in our first three hundred transplants was predicted by patient age, clinical evidence of fibrinolysis, acute hepatic failure, and year of transplantation. In the Birmingham series, massive transfusion and requirement for platelets was predicted by blood urea and pre-operative platelet count. In children, the only clear predictors are technical factors. Current median transfusion requirements (adults) are 8 units platelets, 8 blood, 10 FFP, 6 cryo - precipitate. This contrasts markedly with much higher historical values. The use of platelets and clotting factors are best guided by thromboelastography (TEG). This has been shown to correlate with better overall haemostasis, as well as more economical use of blood products. Therapeutic approaches include the serine protease inhibitor aprotinin, and infusion of prostaglandin E 1 which reduces platelet activation and turnover post re-perfusion. Recent studies have shown that platelet transfusion is the single most effective factor in correcting abnormal thromboelastogram (TEG) during liver transplantation. Transplantation without clotting factors is possible, provided platelet count is maintained in the normal range and anti-fibrinolytic drugs are used. It is likely that requirement for blood products during liver transplantation will steadily fall, although platelet transfusion is likely to remain a cornerstone.

Therapeutic Platelet Transfusions in ATP

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The use of platelet transfusions in ATP is controversial but while their use is relatively widespread no clear guidelines exist. Platelet life-span in ATP is usually less than 6 hours and that of transfused platelets less; although some claim that no appreciable increment is seen after infusion a beneficial clinical effect is often seen. However, at least one study has shown a worthwhile increment ($>20x10^9/l$) on one or more occasions in seven of 11 patients receiving platelet transfusions (Carr et al). A finding which, with other anecdotal cases, supports the contention that platelets may be used in actively bleeding or high risk patients. Patients with de-novo ATP or who are refractory to conventional treatment, with severe mucosal bleeding or if CNS bleeding is suspected, should receive platelet supplements until alternate treatments have had a beneficial effect. In general, larger than conventional doses, up to 10-15 units 4 to 6 hourly, should be given until bleeding stops. Alternate treatments should be considered, in particular IV immunoglobulin, high dose methylprednisolone and vincristine, all of which may show a response within hours in the de-novo patient. In the refractory patient the position is more problematic but the use of IVIG in association with random donor platelets has been shown to enhance the platelet response. The problem remains, however, that many patients receive platelets on the basis of their count rather than their clinical state and a recent survey of childhood ATP showed that platelets were given to 10.3%(44) of 427 children of whom 25 were classified as mild, with only bruising and petechiae (Bolton-Maggs&Moon). In conclusion, it can be argued that platelet transfusions may be effective in ATP (and in other cases of immune platelet destruction) and that the clinical response seen is paralleled by a haematological response. However, their use should be reserved for patients who are bleeding or who require urgent intervention (such as surgery) before alternate therapies will have had time to produce a platelet increment.

Trigger Factors for Prophylactic Platelet Transfusions

Trigger Factors for Platelet Transfusions in the Support of Patients with Haematological Maligancy

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Platelet transfusions in haematological malignancy have been traditionally administered when the platelet count falls below 20 X 10^9/1 thus reducing the haemorrhagic risk. In MRC leukaemia trials 4% of centres used the threshold of <5 X 10^9 /1, 46% a threshold of <10, 21% a threshold of <15 and 25% a threshold of 20 X 10^9 /1 or greater. In our own unit shifting the threshold from 20 down to 15 X 10^9/1 has shown a 20% overall reduction in platelet usage but an instance of major haemorrhage changing from 12% to 10% (p=NS). Two prospective randomised studies in relation to this problem have appeared recently. Heckman et al randomised 78 patients, both new and relapsed, undergoing induction therapy for AML. There was no difference in bleeding complications and there was approximately 35% less usage of platelets at the lower threshold level. A new Italian study just reported in the New England Journal of Medicine (Rebulla et al) randomised 135 newly diagnosed AML patients in 21 centres to a transfusion trigger of 10 X 10^9 /1 vs 20 X 10^9/1. The group of patients transfused at 10 X 10^9 /1 received 21.5% fewer transfusions and major bleeding occurred in 21.5 and 20% of the patients in the two groups respectively and on 3.1 and 1.9% of hospital days respectively in the groups 10 vs 20. If we in our own unit produced a 20% reduction in usage by shifting from a threshold of 15 down to 10 X 10^9/1, this would produce a saving in the costs of platelet transfusion of somewhere between £10-18,000 per month whilst in addition lowering the infectious risk.

Trigger Factors for Platelet Transfusion in the Elderly.

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Is there any reason to suppose that trigger factors in the elderly should be any different than in the young or middle aged? I can suggest some which might be adopted though never enunciated "Acute leukaemia in the old is never cured so we should not be too generous with the platelets for what at best will be a few months of extra life." "There can be no justification in prolonging the dying of a patient with unresponsive acute leukaemia by the intrusion of unwelcome platelet transfusion." Such attitudes often really mean "Old people are worth less and this expensive resource should not be wasted on them."

I believe that in elderly patients with leukaemia, transfusions of platelets are an important element of palliative care. The reason for such transfusions is to prevent bleeding and as in other situations the platelet count is the best gauge of whether bleeding is likely. Because so many leukaemias in the elderly have a dysplastic element, and thus poor platelet function, bleeding may occur at a higher count than in the young. Thus each decision needs to be taken in context. An overriding principle is "will this transfusion improve the quality of life of the recipient?" Factors that will impact on this will include the availability of vascular access, transfusion reactions and the need for iv hydrocortisone which might increase the risk of infection, particularly fungal infections; platelet refractoriness and the question of whether to proceed to HLA matched platelets; and the rate of progression of the acute leukaemia.

For many elderly patients with myelodysplastic syndrome or acute leukeamia the rate of progression of their disease is slow, and the cause of death is bone marrow failure. Platelet transfusion may be seen as a type of organ support similar to renal dialysis, which may be troublesome yet is undoubtedly life prolonging. The patient should be honestly involved in the decision over its use.

Trigger Factors for Prophylactic Platelet Transfusion in the Young.

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The majority of children receiving platelet transfusions (plt trans) will have haematological or solid malignancies for which they are receiving chemotherapy. A number of patients from both groups will subsequently undergo high dose chemo/radiotherapy with autologous or allogeneic stem cell rescue (transplant). Our practice is, in the absence of bleeding, surgical intervention or major sepsis, to give prophylactic plt trans only if the platelet count falls below 10 x 10^9/L. In the presence of other eventualities we would aim to keep the platelet count >50 x10^9/l. An exception is those in intensive care requiring ventilation ± other invasive intervention, eg IV/arterial line placement, BAL, etc., in whom prophylactic platelets are given if <20 x10^9/1 in the absence of bleeding. Leucodepleted platelet products are routinely given to infants (< 1 year), patients likely to require frequent or long term platelet support (eg AML or SAA) and those in whom an allogeneic bone marrow transplant may be required. Our policy in children with inherited (or acquired) platelet function defects is to give plt trans only for life threatening bleeding or for surgical intervention, as the development of alloimmunisation to absent platelet glycoproteins may prevent beneficial results from plt trans subsequently. Theoretically there is no role for plt trans in ITP, however in patients with platelets <10 x10^9/1 presenting with haemorrhage or massive oropharyngeal oozing/blisters or requiring splenectomy, it is difficult to avoid. Infants presenting with NAIT will require plt trans with maternal platelets (or donor platelets expressing the same platelet specific antigens as mother). Extracorporeal circuits: haemodialysis/filtration, cardiopulmonary bypass and ECMO are complicated by a reduction in platelet numbers (adhesion) and function (activation) which may necessitate transfusion. The use of prophylactic plt trans is an attempt to avoid devastating and permanent damage following a bleeding episode. There are, however, potential sequelae of plt trans: transfusion reaction, alloimmunisation and transfusion transmitted infection. The decision to administer prophylactic platelets has to be a balance of risks.

Safety and Efficacy of Platelet Transfusions

Bacterial Safety of Platelets

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The presence of bacteria in cellular blood products has been a problem for many decades and currently it is the most common cause of microbiologically-related transfusion-associated morbidity and mortality for transfusions of either platelets or red cells (the latter also include autologous units). The true prevalence of bacterial contamination of platelet concentrates (PCs) has not yet been established and a variety of approaches have been used to monitor the sterility of cellular blood components. Using automated equipment, the incidence of contamination of 31,610 random donor PCs collected over a 12-month period was recently determined in our Centre, with evidence for bacterial contamination found in 81. 16 were classified as "true positives", 17 as "unconfirmed positives" and 28 as "false positives". Thus the prevalence of contaminated PCs was found to be at least 51 per 100,000 PC units. A second study, done over an 18-month period, during which samples from routinely produced random donor PCs were cultured on both days 1 and 3 following preparation, found that of 16,290 PCs cultured on day 1, 10,065 (65%) were also available for culture on day 3. Four PCs were positive on both days 1 and 3; and a further 3 units were negative on day 1 but positive on day 3. This represents a day 3 "true positive" prevalence of 70 per 100,000 PC units. The concentrations of bacteria present ranged from 10^{-3} CFU/mL to 10^{7} CFU/mL. These data indicate that the bacterial contamination of PCs is relatively common and such units can contain sufficient numbers of bacteria to cause clinically significant transfusion-associated morbidity and mortality.

Viral Safety of Platelet Concentrates

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Immunosupression, and vulnerability to disease associated with viral infections, is relatively common in platelet recipients.

Increasingly, platelet concentrates are made exclusively from donations from repeat donors who have previously undergone serological tests and for markers of some transmissible infections: the frequency of transmissible HBV, HCV and HIV infection in these donations is low. Testing these donations for HBsAg, anti HCV and anti HIV with highly sensitive tests excludes most donations with these infections. However, a risk of infectious donations entering platelet production remains if seronegative, infectious donations are bled, or there are errors in the testing process. The same conditions determine the risk of CMV infection in platelets issued as CMV negative. Monitoring seroprevalence in donations, and seroconversions in repeat donors, enables estimation of the risk of an infectious donation entering platelet production: recent data suggest this risk is less than 0.2 per million donations for HIV and for HCV in England.

There are continuing dangers from viral infections not addressed by current serological testing. Vigilance of HBsAg negative HBV carriers, HBV mutants, and HCV and HIV variants is needed. Clinically significant transmissions of HAV and parvo B19 infection do occur, but are fortuitously rare in England due to low average incidence in donors, short periods of viraemia and high levels of immunity in recipients: concern is greatest during epidemic years and for recipients who are at particular danger of disease. Assessing the appropriateness of expanding the serological testing of all donations, or of donations destined for platelet production only, to include markers for other persistent viral infections, such as HTLV I/II, HGV and HHV8, needs to be comprehensive and responsive to new knowledge about the epidemiology, the serology and available tests, and the natural history and pathogenicity of the infection. Knowledge about the epidemiology of viral infections should also improve safety via donor selection. CJD remains contentious.

Other approaches with potential to improve the safety of platelet concentrates include white blood cell filtration - proven effective for CMV and proposed for HTLV I/II, the application of novel methods of inactivation - of which the psoralens seem most promising, and the introduction of nucleic acid testing of mini-pools - if completion of testing during the short shelf-life of platelets becomes feasible.

How Can We Best Evaluate Clinical Efficacy of Platelet Transfusion?

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Primary haemostasis is predominantly dependent upon platelet numbers, their function and von Willebrand factor. Whilst the corrected count platelet increment is useful for the evaluation of transfusion efficacy it provides no qualitative information. The only routinely available test of in vivo primary haemostasis is the skin bleeding time (BT). When performed in a standardised manner it is informative. The BT relates to severity of thrombocytopaenia and is also sensitive to qualitative platelet defects, including those due to medications. It is a useful tool in screening for disorders in primary haemostasis. There are, though, limited data to support its use as a predictor of haemorrhage. Bleeding from the skin may not correlate with that from other tissues, and the BT does not predict the extent of surgical haemorrhage. Repeated testing of subjects with cytopaenias may not be acceptable.
A recently described method for testing the 'bleeding time' ex vivo (Thrombostat 4000/2) depends upon the passage of venous blood through a capillary and collagen-coated filter and measures time to occlusion. It correlates well with conventional tests of platelet reactivity and has been used to monitor the response to platelet transfusion. Prospective studies are required to fully evaluate this novel approach.

Ideal Platelet Prescription

Assessing Hemostatic Function of Transfused Platelets Experimentally

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The ultimate reason to transfuse allogeneic platelets to bleeding thrombocytopenic patients is to improve their hemostatic function. Alterations to the standard preparative and storage parameters of platelet concentrates (PCs) may result in preparations with retained *in vitro* function but which do not manifest *in vivo* hemostatic function when transfused to a recipient. An ear bleeding time model in experimental animals has been developed to evaluate the hemostatic function of human PCs. In this model, New Zealand White rabbits are made thrombocytopenic using a combination of y-irradiation and the infusion of heterologous IgG to rabbit platelets. This results in a severely thrombocytopenic rabbit with a platelet count of 5-10 X 10^9/L. In order to be able to assess the *in vivo* hemostatic function of *human* platelets in such rabbits, they are also treated with a reticuloendothelial (RE) blocking agent, ethyl palmitate. Thus, when fresh human PCs are transfused into such RE-blocked thrombocytopenic rabbits, the platelets remain in the circulation with a half life of ~6 hours. During this time frame, the hemostatic function of the human platelets, remaining in the circulation, can be ascertained. This model has proven very robust for the evaluation of the *in vivo* hemostatic function of liquid stored (22°C and 4°C), frozen, UVA-treated, and lyophilized human platelets.

Blajchman MA, Lee DH. The thrombocytopenic rabbit bleeding time model to evaluate the *in vivo* hemostatic efficacy of platelets and platelet substitutes. Transfusion Medicine Reviews 1997:11:95-105.

Platelet Product Specifications - In vitro Parameters and Dose

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Platelet transfusion efficacy is dependent upon product content, post transfusion recovery and the biologic function of stored platelets. Platelets may be prepared from whole blood donations by the platelet rich plasma (PRP) or buffy coat (BC) method. BC are often pooled in groups to 4 or 6 during preparation to maximize recovery and plasma harvest. Alternatively platelets may be prepared by apheresis. Representative product contents include:

Method (N)	Platelets ($x10^{11}$)	WBC ($x10^{6}$)	(mL)
PRP-single (100)	0.78 ± 23	181 ± 267	52 ± 3
BC-single (100)	0.54 ± 14	6 ± 8	51 ± 7
BC- 4 pool (100)	3.32 ± .39	44 ± 36	334 ± 14
BC- 6 pool (100)	4.33 ± .43	37 ± 20	319 ± 18
SDP-standard (78)	3.5 ± 7	.79(.02-43)	277 ± 44
SDP-LD (58)	3.5 ± .5	.03(.006-.45)	232 ± 19

During storage with agitation in gas permeable containers at 22°C storage induced loss of viability occurs:

Days Stored (N)	1-Day (32)	5-Days (276)	7-Days (15)	10-Days (4}
%Recovery	59 ±10	51 ± 13	41 ± 11	24 ± 8
Survival (hours)	186 ± 21	151 ± 38	121 ± 40	82 ± 38

Commonly used measures of in vitro platelet quality include pH, morphology (or Extent of Shape Change), Hypotonic Shock Response and ATP levels. In vivo/in vitro correlations are shown:

(N=97-373)	pH(<7)	ATP(<4um/10^{9})	ESC(<20%)	HSR(<60%)
% Recovery	.39	0.17	0.59	0.47
Survival	40	0.09 (p=.1)	0.48	0.43

The best in vitro predictor of in vivo recovery is lactate production, r =.62, n=369. Through regression analysis, an independent relationship between storage duration, in vitro values and in vivo efficacy may be developed which allows prediction of in vivo efficacy. A ratio of platelet content, plasma volume and container oxygen permeability may be defined to maximize platelet product efficacy

Biological Mediators in Platelet Transfusion Reactions.

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A variety of Biological Response Modifiers (BRM) play a role in the pathogenesis of transfusion reactions (TR). Febrile reactions arise from donor-recipient interactions between HLA or platelet specific antibodies and their corresponding antigens. Too, soluble allergens are implicated in urticarial reactions while antibodies to IgA may cause anaphylactoid reactions. Other classes of BRM which may be involved in TR include: activated complement fragments C3a and C5a; cytokines IL-1, IL-8, and TNF-α; histamine; lipid moieties; and bradykinin. While under-reporting is likely,the incidence of platelet TR has been estimated to be as high as 30%. The use of some bedside leukoreduction filters (LRFs), specifically those with a negatively charged filter media has been associated with a small but serious group of anaphylactic reactions to transfused platelets and plasma. The incidence is more frequent and clinical symptoms more serious when the recipient is taking an ACE inhibitor. Links have been reported between exposure to some biomaterials and: activation of complement, release of histamine from mast cells, and activation of lipids which can in turn activate granulocytes to produce TRALI-like symptoms. Febrile non-hemolytic TR due to infusion of platelets have been linked to the platelet-poor plasma portion, due to soluble BRM (cytokines and chemokines) contained in non-prestorage leukodepleted platelet concentrates. Even prestorage platelet leukoreduction, however, does not preclude generation of activated complement in the platelet-poor plasma fraction of the concentrate. Some LRFs, have been reported to scavenge a portion of these BRMs. Although the etiology of platelet TRs is becoming clearer, attempts at prevention may produce other reactions. This situation further supports the need to ensure a valid indication for the transfusion of any blood product.

Oral Presentations

(in order of programme)

Friday, 28 November 1997

Management of Patients Refractory to Platelet Transfusions

Differential Diagnosis of Refractoriness to Platelet Transfusions.

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The repeated failure to obtain satisfactory responses to platelet transfusions, or platelet refractoriness, is a major problem in patients with bone marrow failure, occurring in many patients receiving multiple transfusions. Before considering the causes, it is important to define platelet refractoriness, and the key to this is a clear distinction between normal and poor responses to platelet transfusions. Whereas the most important measure of the effectiveness of a therapeutic platelet transfusion is the cessation of bleeding, an indirect approach is required for the assessment of responses to prophylactic platelet transfusions, and many parameters have been used including the corrected count increment and platelet recovery. The particular parameter selected by an individual centre is perhaps of secondary importance to the routine assessment of patients' responses to platelet transfusions for the identification of refractory patients, who may require a different approach to platelet transfusion therapy after the cause of platelet refractoriness has been established.

Platelet refractoriness may be due to immune and non-immune mechanisms. Platelet alloimmunisation is the most important of the immune causes, but platelet autoantibodies, ABO antibodies, drug-related antibodies and circulating immune complexes have also been associated with refractoriness. There is an increasing recognition of the importance of non-immune mechanisms of refractoriness. Patients requiring multiple platelet transfusions often have complex clinical problems which may influence the survival of transfused platelets; well recognised factors include infection, disseminated intravascular coagulation and splenomegaly.

The initial investigation of platelet refractoriness should involve a clinical evaluation for the presence of factors associated with non-immune platelet consumption, and testing the patient's serum for HLA antibodies. If the cause of refractoriness remains uncertain, further serological testing is indicated including testing for platelet-specific antibodies. In some cases, no obvious cause is found for the refractoriness, but more often there are multiple factors which could account for poor responses to platelet transfusions.

Management of Platelet Transfusion Refractoriness

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Definition:
Platelet transfusion refractoriness can be defined as lack of expected increment or even decrement after platelet transfusions from random donors. When clinical reasons for increased platelet consumption are not obvious, antibodies are evaluated by serology.
Diagnosis
Preferentially a screening-test(s) followed by an algorithm to distinguish between ABO, HLA, HPA-allo-antibodies and non-specific auto-or drug-induced antibodies is used.
Depending on prior allogenic contacts (transfusions and pregnancy) and whether a leukoreduced transfusion regime is used, in <50% of the refractory patients antibodies are found. HLA antibodies are still the major cause of immunological refractoriness, followed by ABO antibodies. Platelet alloantibodies are rare (<5%) when the whole population of platelet transfusion dependent patients is considered but show an increased incidence in HLA-allosensitized patients.
Treatment:
When, in case of HLA-immunization, HLA split-antigen compatible donors are used, matching results in >80% of the transfusions in sufficient platelet increments.
In case the patient has a rare HLA-type, the available donor pool can be increased by determination of acceptable mismatches. Transfusion-failures with HLA matched platelets are analyzed for antibodies against low frequency platelet-specific antigens.
When sufficient HLA-typed donors are not available or HPA-antibodies against high frequency antigens are present the approach of donor-selection, crossmatching and additional interventions must be individualized.

Haemostatic Support for Refractory Patients

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In the acute situation what pharmacological agents are presently available which may help to maintain haemostatic control? Aprotinin, a broad based protease inhibitor of bovine origin, inhibits trypsin, kallikrein and plasmin activity and may also have a direct effect in maintaining platelet function. Although primarily used to prevent bleeding during high risk procedures, aprotinin is useful when excessive bleeding is not controllable in the peri or immediate post-operative period. Other agents are not generally of proven benefit but may be helpful in specific circumstances. The fibrinolytic inhibitor tranexamic acid at a dose of up to 1 g IV slowly may be beneficial to control excessive primary fibrinolysis, particularly with bleeding following prostatectomy or thrombolytic therapy. The local application of a fibrin sealant or glue may help to control generalised parenchymal bleeding (i.e., during liver surgery) either by directly spraying the fibrinogen and thrombin solutions onto the bleeding surfaces or by covering the area with an impregnated swab. An infusion of the vasopressin analogue, DDAVP at a dose of 0.4 μg/kg in 100 ml of normal saline IV slowly, may also help by increasing circulating factor VIII-von Willebrand factor activity in those patients with low baseline vWF levels (i.e., ≤1.5 u/ml but within normal range) and non-specifically improving platelet function in the generalised post-operative bleeding state. Attempts to modify immunological refractoriness with human immunoglobulin infusions or antibody removal on an ion-exchange column may increase platelet survival to some extent in chronic situations.

Future Developments

Thrombopoietin: From Theory to Practice

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Although predicted to exist over 40 years ago, "thrombopoietin" (TPO) was only recently purified and cloned. The amino-terminal 153 amino acids of TPO contain the region that binds to the thrombopoietin receptor, Mpl, and are 50% similar to those in erythropoietin. The rest of the protein is a carbohydrate-rich region that increases the stability of the molecule in the circulation. Two recombinant TPO molecules are currently under intensive study: a full-length, glycosylated molecule (rTPO) and a truncated (aa 1-163), non-glycosylated, pegylated molecule (PEG-rHuMGDF). Both molecules stimulate megakaryocyte growth and increase platelet production up to 6 fold in vivo. Early, but not late, progenitors of all lineages are also stimulated. Clinical sudies have demonstrated that PEG-MGDF is able to shorten the duration, and sometimes the extent, of chemotherapy-induced thrombocytopenia. Unfortunately there is no clinical benefit when administered after treatment for myeloid leukemia of after bone marrow transplantation. PEG-rHuMGDF does increase the number of circulating peripheral blood progenitor cells several logs. Also, in platelet donors, PEG-rHuMGDF increases the platelet count and apheresis yield. The exact role of TPO in the clinical arena remains to be determined by future studies.

Platelet Alternatives

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Two alternatives to platelet therapy in current clinical trials include infusible platelet membrane (IPM) and Mpl ligand (thrombopoietin) therapy. The potential roles of each of these inpatients undergoing stem cell transplantation will be summarized briefly.

IPM is a soluble platelet membrane preparation containing platelet Ib receptors but lacking Class I HLA antigens. IPM has shown efficacy in shortening bleeding times in thrombocytopenic rabbits at a dose of 2mg/kg. Slow infusion IPM demonstrated no significant detectable toxicity, including analysis by Wessler thrombogenicity assay. Human studies conducted on 15 normal volunteers have demonstrated no formation of antibodies to platelets, HLA class I antigens, or IPM. Subsequent phase I studies have been performed to assess safety, tolerance, and pharmacodynamic effects of single, ascending, (2 to 6 mg/kg) intravenous doses in thrombocytopenic patients with minor breeding episodes(1). Summary results:

	Treatment	Positive Response	Total
All Patients	IPM	17	26
	Platelets	3	5

No differences in efficacy were noted among three dosage levels. These preliminary results show efficacy of IPM in cessation of minor bleeding episodes in thrombocytopenic patients.

PEG-rHuMGDF, a peglygated truncated Mpl ligand, increases circulating platelet counts in preclinical models and in cancer patients.We have analyzed the relationship between donor platelet counts and platelet product yields in a large hospital-based platelet apheresis program in order to define the potential role of recombinant Mpl ligands in this setting. In 708 consecutive procedures, preapheresis platelet counts were $237 \pm 49 \times 10^3/mm^3$ ($m \pm sd$), and mean yield was $4.24 \pm 1.09 \times 10^{11}$ platelets. In thirty eight (5.4%) procedures, the yield exceeded 6×10^{11} platelets. Sixty five percent of donors had platelet counts $< 250 \times 10^3/mm^3$, and accounted for only 7.9% of the "split" products with $> 6 \times 10^{11}$ platelets; in contrast, the 10% of donors who had platelet counts $\geq 300 \times 10^3/mm^3$ accounted for 63% of the products with $> 6 \times 10^{11}$ platelets. We conclude that the donor platelet count, and the potential role of PEGrHuMGDF, has important clinical and economic consequences for platelet apheresis programs.

1. Goodnough LT, Kolodzie J, Ehlenbach. Blood 1996 ;86:610a.
2. Goodnough LT, DiPersio J, McCullough J, et al. Transfusion (abstract) 1997, In Press

Inactivation of Viruses, Bacteria, and Leukocytes in Platelet Concentrates

L Corash, L. Lin, G. Cimino, D. Hei, G. Wiesehahn, J. Grass, S. Wallowitz, D.Cook, and S. Isaaes. Cerus Corporation, Concord, CA, USA.

Despite improved pre-transfusion donor evaluation and testing, risk of transfusion-associated infectious disease persists. Importantly, other infectious pathogens including bacteria, protozoa, and non-enveloped viruses are not detected in current testing programs. A robust pathogen inactivation process could provide prospective protection against new pathogens. The psoralen S-59 and long wavelength ultraviolet light inactivates cell-free and cell-associated viruses, integrated pro-viral sequences, bacteria, and protozoa. When platelet concentrates (PC) containing 3 to 6 x 10^{11} platelets in 300 mL of 35% autologous plasma and 65% platelet additive solution (PASIII) were treated, the following levels of pathogen inactivation were achieved: > $10^{6.7}$ plaque forming units per mL (pfu/mL) of cell-free human immunodeficiency virus (HIV-1), > $10^{6.6}$ pfu/mL of cell-associated HIV-1, >$10^{6.8}$ infectious doses per mL (ID_{50}/mL) of duck hepatitis B virus (DHBV), >$10^{6.5}$ pfu/mL of bovine viral diarrhea virus (BVDV), >$10^{6.6}$ colony forming units (cfu) of *Staphylococcus epidermidis,* and >$10^{5.6}$ cfu of *Klebsiella pneumoniae.* Expression of proviral HIV-1 was abolished at a dose of S-59 1500-fold lower than that for viral and bacterial inactivation. *In vitro* platelet function was maintained over 7 days of storage following PCT. *In vivo* post-transfusion recovery and life-span of PCT treated primate PC (77.8 ± 16.4%, 99.2 ± 30.4 hours, n=11) were comparable to that of untreated PC (82.6±15.2%, 103±19 hours, n=4). Additionally, S-59 mediated PCT inactivates leukocytes more effectively than gamma irradiation, and can prevent transfusion associated graft versus host disease in animal model studies. Previous studies using virucidal and bactericidal conditions demonstrated inactivation of >$10^{5.4}$ T cells/mL as measured by an in vitro clonogenic T cell expansion assay (Blood 86:542a, 1995). In addition, PCT prevented TA-GVHD in a well characterized in vivo murine model in which parental A (H-2a) splenocytes were transfused into hybrid B6AF1 (H-2a/b) recipients (Blood 88:627a, 1996). If successful, PCT could result in the production of platelet components substantially free of viruses, bacteria, protozoa, and functional leukocytes; and may lead to significant reductions in the cost of platelet transfusion.

Tim Goodenough - Cypress
Membrane fragments - heated & lysed cells the expired platelet material
Blajchman - thrombotypic agents
Blood Abstract - Dec AsH meeting.
70% - 100% response rate (even in clin. refractory patients) / Haemostatic parameters only / elite platelet product not covered
(correction of minor bleeding (mucosal bleeding)
?? Use of MGDF (megakaryocyte growth & Development Factor)
? Significant increasing problems.

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Poster Presentations

Therapeutic Platelet Transfusions

P5

Auto-Immune Thrombocytopenic Purpura in Pregnancy with Positive IgM Platelet-Associated Immunoglobulin.

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Management problems in dealing with Auto-Immune Thrombocytopenic Purpura (AITP) and pregnancy are to deal with possible haemostatic problems in the mother and to identify the risk of having an infant with severe thrombocytopenia. We report 6 cases of AITP in pregnancy with raised IgM, platelet-associated immunoglobulin (PAIg), all of whom gave birth to infants with a normal platelet count. Many have studied the correlation of PAIgG and neonatal thrombocytopenia in pregnancy, but little has been written about the relationship of IgM PAIg and the outcome, in infants. Although the incidence of raised PAIgM only in AITP is not known, PAIgM alone has been shown to cause complement mediated AITP. Out of 6 cases, 4 mothers (cases 1,2,3&6) were treated for maternal thrombocytopenia in late pregnancy either with intravenous immunoglobulin (IVIg) or IVIg followed by steroids. There was no response to therapy. Four cases (Nos.2,3,4&6) were given prophylactic platelet transfusion prior to and during Caesarian section/labour, which effectively prevented excessive bleeding. Our observation indicated that AITP in pregnancy with raised IgM, PAIg is a predicting factor indicating that the infants are at no risk of thrombocytopenia at birth. Response to therapy with IVIg/steroids is disappointing but prophylactic platelet transfusion seems to be safe and effective for prevention of excessive bleeding secondary to thrombocytopenia at the time of delivery.

P9

The Effect of Autologous Platelet Dose on Allogeneic Blood Transfusion in Cardiac Surgery.

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The collection of autologous platelets prior to commencement of cardiopulmonary bypass with transfusion after protamine neutralization has been suggested to abrogate the platelet dysfunction associated with cardiac surgery. There is controversy, however, with regard to the benefit of this procedure and little data regarding the relationship between potency and therapeutic benefit.
Autologous platelet harvests were performed prior to cardiac surgery using a Haemonetics Cell Saver 5. Data was recorded for pre operative blood counts, allogeneic blood transfusion, discharge blood counts, and platelet harvest yields.
690 autologous platelet collections were performed over a two year period. The median platelet yield was 1.9×10^{11}. The products were separated into a low yield group ($<2 \times 10^{11}$) and a high yield group ($>2 \times 10^{11}$). Data is the mean ± SD.

	Low Yield	High Yield	p
n	371	319	
plt yield (10^{11})	1.4 ± 0.4	2.6 ± 0.6	<0.01
RBC Transfused (U)	3.6 ± 4.5	3.7 ± 4.5	0.3
Plt Transfused (U)	2.1 ± 60	1.3 ± 3.6	0.02
Plasma Transfused (U)	1.0 ± 3	0.7 ± 2	0.14
Cryo. Transfused (U)	0.6 ± 2.4	0.4 ± 1.9	0.06
Pre-operative Hb (g/L)	138 ± 14	134 ± 16	< 0.01
Discharge Hb (g/L)	104 ± 11	104 ± 14	0.45

Despite lower preoperative Hb, the high yield group showed similar allogeneic red cell transfusions and less transfusions of allogeneic platelets, plasma and cryoprecipitate. The data is suggestive of a dosage effect of autologous liquid platelets. The low potency of products reported in previous studies could, therefore, explain the lack of efficacy.

P14

Collection of Double Dose Leucodepleted Platelets Using the Haemonetics MCS+ Machine.

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The MCS+LDP incorporates automatic leucocyte filtration during the final return cycle of platelet collection. Our aim was to collect double doses ($\geq 4.8 \times 10^{11}$) of leucodepleted ($\leq 5 \times 10^{6}$ WBC per dose) platelets in 90-100 minutes from selected donors and compare ACDA and Acid CPD as anticoagulant. 30 donations were collected from 17 females and 13 males with previous platelet counts $>280 \times 10^{9}$/L. Collection times were monitored. 20 donations were collected in ACDA and 10 in Acid CPD. All donations were divided into 2 doses, 12-24 hours after collection. Platelet yield, WBC content, pH, swirling, MPV and dMPV/dPlt were measured and these tests were repeated at 5 days for 7 donations in ACDA and 5 in Acid CPD.
All 30 donations had a WBC content of $<1 \times 10^{6}$. Platelet yield was $> 4.8 \times 10^{11}$ in 25/30 (83.3%) . Mean yield was 5.45×10^{11} and collection time, 104.6 mins. The procedure was acceptable to all donors. In ACDA, mean pH was 7.08, day 1 and 7.22, day 5 . Mean pH in Acid CPD, day 1 was 6.75 and day 5, 6.81. Smaller platelets are selected out by this system (low MPV). dMPV showed good platelet activity in 26/30 donations (day 1). dPlt results showed acceptable levels of reversible aggregates. Double doses of leucodepleted platelets can be collected from selected donors using the MCS+ LDP. ACDA provides a more suitable pH than Acid CPD, though platelets stored well in both anticoagulants for up to 5 days.

Trigger Factors for Prophylactic Platelet Transfusions

P11

Survey of Use of Platelet Transfusions in Centres Participating in the MRC Leukaemia Trials.

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Platelet transfusion therapy has played a major role in the supportive care of patients with acute leukaemia for about 30 years. However, there have been few attempts at controlled trials, and debate continues about the value of prophylactic platelet transfusions and the optimal threshold of platelet count for their use. There are data to suggest that the previously commonly used threshold level of 20 x 10^9/L may be safely reduced without increasing the risk of bleeding. Use of a lower threshold has been associated with a considerable decrease in the use of platelet concentrates, and has the potential to reduce complications of platelet transfusion therapy such as alloimmune platelet refractoriness and transmission of infection. However, the extent to which hospitals in the UK have changed their policy for prophylactic platelet transfusions is not known.

In the planning of a proposal to study different regimens of prophylactic platelet transfusion support in a future MRC trial of AML in the elderly, hospitals participating in MRC leukaemia trials were sent a questionnaire asking for details about their threshold for prophylactic platelet transfusions and if they used different thresholds for young and elderly patients.

There were responses from 99 hospitals. The threshold for prophylactic platelet transfusions was <5 x 10^9/L in 4 (4%) hospitals, <10 x 10^9/L in 46 (46%), <15 x 10^9/L in 21 (21%), <20 x 10^9/L in 25 (25%) and prophylactic transfusions were not used in 3 (3%) hospitals. 3/4 (75%) hospitals with a threshold of <5 x 10^9/L, 18/46 (39%) hospitals with a threshold of <10 x 10^9/L, and 2/21 (10%) with a threshold of <15 x 10^9/L increased the threshold in the presence of fever, sepsis, coagulopathy or recent bleeding. Only 3 hospitals used a different threshold for young and elderly patients.

This study shows that there is considerable variation in platelet transfusion practice amongst hospitals participating in MRC leukaemia trials, providing justification for a large prospective, randomised controlled trial of different prophylactic platelet transfusion regimens in acute leukaemia.

Safety and Efficacy of Platelet Transfusions

P2

ABO-Incompatible Plasma in Apheresis Platelet Transfusions: A Seven Year Retrospective Analysis.

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Background: There is a remaining controversy on the safe and efficient ABO-constellation between donor and recipient in platelet transfusions, particularly if HLA-matching prevails over ABO-match. Whereas corrected count increments are now known to depend on ABH-expression on platelets, side effects from plasma incompatibility have been poorly analyzed. Methods: Between September 1987 and August 1994 a total of 2446 platelet concentrates by apheresis (PCA), normally suspended in 240-400 ml plasma, have been collected of which 230 were transfused as "ABO plasma incompatible" (175 x 0 in A, 46 x 0 in B, 9 x 0 in AB).
Results: The retrospective analysis of 230 "plasma-incompatible" PCA transfusions revealed that 198 (86%) were without any side effects, 22 (9.6%) medical records were missing, 10 (4.3%) PCA (9 x 0 in A, 1 x 0 in B) caused complications, classified as surely related in 5 (2.2%), probably in 1 (0.4%), and possibly in 4 (1.7%) cases. PCA from a donor of blood group (BG) O given to a patient with BG A caused chills, fever, and acute hemolysis despite a low anti-A isohemolysin titer of 1:2. There was no difference in the distribution of the isohemolysin titers and duration of storage between PCA transfusions without and with complications. The distribution of isoagglutinins revealed a shift towards significant higher titers in PCA with side effects.
Conclusions: These data show that transfusion of PCA with ABO incompatible plasma can cause significant side effects and support, when ever possible, the transfusion of ABO-identical platelets.

P3

Identification and Analysis of Transfused Platelets Using Whole Blood Two-Colour Flow Cytometry.

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Background: Platelets manufactured by different methods show variable expression of activation markers (1), but few data exist on these changes following transfusion. We describe a whole blood method for the differentiation and analysis of donor and patient platelets by flow cytometry in post transfusion samples.
Method: HLA-A2 positive haematology patients were transfused with HLA-A2 negative apheresis platelets. Citrated whole blood samples were taken at appropriate post transfusion intervals, diluted 1:10 in HEPES buffer, and incubated with a FITC-labelled monoclonal HLA-A2 antibody and biotin/streptavidin labelled monoclonal antibodies to P-selectin, GPIIb/IIIa, GPIbα, GP IX, GP V, and fibrinogen, and labelled annexin V. Two-colour flow cytometric analysis allowed simultaneous identification of the transfused platelet population and analysis of activation markers.
Results: In vitro mixing experiments established a detection limit of 3% A2 positive or negative platelets, without interference with detection of activation markers. Preliminary post-transfusion data showed reversal of P-selectin expression from 26% to 7% in the transfused population by 6 hours, and fibrinogen binding from 15% to <1% by 1 hour. GP IIb/IIIa expression increased by 25% in the first hour, while changes in other markers were minor or less consistent.
Conclusion: Transfused platelets can be 'tracked' and analysed in patients without the need for direct platelet labelling. This system provides a convenient, non-invasive post-transfusion model for assessment of surface changes in novel platelet products.

(1) Metcalfe P et al Brit J Haem 1997;98:86-95.

P6

Transfusion Support with Filtered Washed Red Cells to Prevent Relapse of Post-Transfusion Purpura.

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Post-transfusion purpura (PTP) may recur after subsequent transfusion with antigen positive platelets. Further transfusion support requires special attention. We report 2 cases of this kind, transfused with filtered/washed red cells uneventfully. Both were diagnosed PTP 8 and 11 years ago with persistence of anti-HPA-1a antibodies. SAG-M red cell units were used for filtration and washing. Sterile connecting device was used to connect Pall BPF-4 leucocyte removal filter. Red cells were washed three times with normal saline. To evaluate the residual platelet count in filtered washed units, we determined the platelet count before and after filtration/washing of ten SAG-M red cell units. Sysmex E-5000 analyser was used for platelet counting. Mean platelet count pre-filter/wash was $227x10^9$/L, post-filter/wash was $2x10^9$/L, respectively. Re-exposure to antigen positive blood products can provoke recurrence of PTP even years after recovery. The presence of HPA-1a antigen / Antigen-Antibody complex can enhance destruction and clearance of HPA-1a negative autologous platelets. Therefore the best option is to provide HPA-1a negative cross-matched compatible red cell units. If such blood is not readily available, our cases demonstrate that transfusion with filtered washed red cells can prevent recurrence of PTP. Leucodepletion can effectively prevent complement mediated red cell lysis in paroxysmal nocturnal haemoglobinuria. Washed filtered red cells not only reduced the platelet antigen load, but leucodepletion might also eradicate the component of complement mediated-lysis of the autologous platelets in PTP. The threshold level of platelet antigen antigen necessary to initiate PTP is not known, therefore caution has to be taken and the platelet count should be checked immediate post transfusion and 7-10 days after the transfusion of filtered washed red cells.

P7

Buffy Coat Derived Platelet Concentrates: Progressive Decrease in Platelet Function During the Shelf Life.

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Platelet concentrate (PC) transfusions are useful in the maintenance of haemostasis in a variety of clinical situations. In recent years, preparation of PC from the buffy coat has been introduced into routine use. The function of transfused platelets is of critical importance and changes on storage of buffy coat prepared PC may influence their haemostatic potential. We studied platelet aggregation responses and total platelet adenine nucleotide content serially in PCs (n=7) stored under ideal blood bank conditions over the stipulated shelf life of 5 days. PCs were obtained from normal donors at the National Blood Transfusion Service. Mean Platelet Volume (MPV) and platelet counts were also quantified. Responses (% max of height, mean ± sem) to 10μM ADP decreased gradually from 18.24 ± 5.19% to 9.20 ± 0.95 (NS, day (d)1 v d4) and 4.67 ± 2.25% ($p < 0.02$, dl v d5) while the decrease in response was more pronounced for 4 μg/ml collagen: 50.24 ± 13.02% to 7.31 ± 7.31 ($p < 0.01$, dl v d4) and 2.13 ± 2.13% ($p < 0.001$, dl v d5). Total platelet ADP decreased from 4.45 ± 0.78 to 3.71 ± 0.69 nmoles/10^8 platelets ($p < 0.01$, dl v d5). MPV also decreased progressively over the shelf life period from 7.30 ± 0.25 to 5.33 ± 0.36 fL ($p < 0.005$, dl v d5). There was also a decrease in platelet count (878 ± 53 x 10^9/L to 640 ± 89 x 10^9/L, $p < 0.02$, dl v d5) over the same period. These data indicate decreased platelet function characterised by significant decreases in platelet aggregation responses, which are maximal by d4 - d5 and an acquired storage pool defect. The storage changes may be of clinical significance when buffy coat derived PCs are transfused towards the end of their shelf life.

P8

Comparison of Effect of Blood Bank Versus Bedside Filtration on Platelet Transfusion Reactions

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In a retrospective analysis two methods for preventing platelet transfusion reactions are compared. The first group of patients received platelets filtered in the hospital blood bank and were subsequently given filtered and washed platelets if they developed reactions. The second group received platelets filtered at the bedside.
In the group receiving platelets filtered in the hospital blood bank there was a very low rate of reaction (2/101 = 2%) and no patients receiving filtered and washed platelets (0/91) developed a reaction. In the group receiving platelets filtered at the bedside there was a reaction rate of 19/104 (18.3%). Further, two of these reactions were categorised as very severe, the patients showing hypotensive collapse.
We conclude that filtering platelets in the hospital blood bank is significantly more effective than bedside filtration in preventing platelet transfusion reactions.

P13

Microvesiculation of Platelet Concentrates During Storage

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The ability of platelets to shed microvesicles on the site of injury is important for the formation of haemostatic plug. During processing and storage of platelet concentrates various degrees of microvesiculation occur which may cause deficiency in platelet function. We have investigated both the presence of microvesicles and the ability of stored platelets to undergo further microvesiculation upon activation with Ca ionophore. Microvesicles were measured by flow cytometry and expressed as percentage of total population. Storage induced cellular injury and release reactions were measured by annexin V, PF4, ,ßTG and thrombospondin. All testings were performed at the beginning and the end of shelf life. At the beginning of shelf life the level of microvesicles varied from 1 to 5% and the products responded well to Ca ionophore as measured by aggregometry (slope 46, maximal aggregation 64%). At the end of shelf life the level of microvesicles increased in a platelet concentration dependent manner (5-14%), with a concomitant decrease in response to Ca ionophore (slope 3, aggregation 4%). At the beginning the levels of markers of cellular injury and release reactions expressed as per 10E9 platelets were: annexin V 4.5 ng; PF4 2.2 µg; ßTG 3.2 µg; thrombospondin 157 µg. These increased 2-3 fold during storage. Platelets which had undergone a higher degree of microvesiculation, release reactions and platelet injury responded poorly to Ca ionophore. We conclude that the optimal storage condition should be reappraised on the basis of the induced and inducable microvesiculation.

P19

Screening for Bacteria in Platelet Concentrates

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During the last years an increased demand for platelet transfusions has occurred mainly because of improvement in treatment of blood malignancy. Because platelet concentrates are stored at 22°C the risk for a bacterial growth in contaminated concentrates is high. This fact has lead to the recommendation not to store concentrates longer than 5 days. However, an increasing demand of platelet transfusion also need a bigger stock of platelet concentrates which increases the risk for a large number of concentrates to be outdated.

In order to increase the security for platelet transfusion we have been monitoring the concentrates for bacterial contamination using the BacT/Alert® system (Organon Teknika). This measure also provided us to prolong the storagetime to six days which increased the flexibility to meet the increased demand for platelet concentrates.

From March 1994 to February 1997, 8281 platelet concentrates have been monitored. In 8199 (99 %) concentrates no bacterial growth was seen while in 82 (1%) of the concentrates a positive indication of bacterial growth was detected. After bacterial control cultures, 48 (0.58 %) of the positive indications were regarded as true positive, 33 (0.40 %) regarded as not confirmed and one (0.01%) regarded as false positive. Of the 48 true positive indications mainly skin bacteria as coagulase-negative staphylococci was cultured.

Our conclusion is that platelet concentrates stored for six days can be safely transfused if there is a negative bacterial screen result.

A positive economical benefit was also obtained due to a decrease in outdated concentrates and to a decrease in transfusion reactions.

Ideal Platelet Prescription

P1

Platelet Support for Orthoptic Liver Transplantation.

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Orthoptic liver transplantation is used in the treatment of fulminant hepatic failure, as well as end-stage liver disease resulting from a variety of congenital and acquired disorders. The aim of this study was to determine the influence of disease category on platelet transfusions in liver transplantation. One hundred and ten patients underwent OLT at the Royal Free Hospital between December 1995 and April 1997. Thirty-six of these had hepatitis-related disease (HBV and HCV), 22 had alcoholic liver disease/cirrhosis, 14 had primary biliary cirrhosis, 10 had primary sclerosing cholangitis, 11 had second or subsequent transplants, and the remaining 17 had OLT for miscellaneous disorders including Wilson's disease, Budd Chiari syndrome and Carcinoid syndrome. An average of 2.8 pools of platelets were used in the first 24 hours after surgery, with 2.9 pools used over 72 hours. This compares with historical figures of 3.3 pools at 24 hours and 5.1 pools at 72 hours, in 60 patients who received liver transplants in 1988-1991. A marked difference between the disease groups was seen with regard to platelet transfusions. Over 24 hours, patients with HBV and HCV-related conditions and alcoholic liver disease used significantly more platelets (2.6±2.1, range 0-12, and 2.4±2.3, range 0-10 pools respectively than patients with either primary biliary cirrhosis or primary sclerosing cholangitis (0.9±1.0, range 0-9 for both). Our previous policy has been to provide 4 pools of platelets for each transplant. Based on these data, however, we have amended this so that we no longer issue platelets for patients with primary biliary sclerosis/sclerosing cholangitis routinely, but only when requested. Higher platelet transfusion were associated with more red cell transfusions, rather than a lower preoperative platelet count.

P4

Glucose Determination and Evidence of Depletion in Pooled Buffy Coat Platelets.

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Glucose in anticoagulated plasma is the major fuel metabolised to produce ATP and maintain shape and function of platelets during routine storage. The resting rate of glucose utilisation is $=0.17\text{mmoles}/10^{11}$ platelets/day. 250ml plasma with a glucose concentration of 15mM is considered adequate for the storage of $3\text{x}10^{11}$ platelets for 5 days at 22°C. Rapid determination of the glucose level immediately prior to issue, provides metabolic information on the platelet quality. Samples of pooled buffy coat platelets taken from stripped and isolated segments of tubing were assayed for glucose during storage. A photometric hand-held blood glucose analyser which utilises a two-step enzymatic assay, resulting in the formation of molybdenum blue, was compared with a plasma hexokinase assay. The assay methods showed excellent correlation ($p<0.001$, $r=0.945$, $n=131$). Glucose levels in random platelet concentrates during storage are tabulated below: *Day1=15.0 ± 0.91 mM; n=6

GLUCOSE	Day 4	Day 5	Day 6	Day 7
Mean mM*	11.36	9.06	6.28	4.36
<4mM	0/16	3/22	12/33	16/26
>4mM<8mM	0/16	3/22	9/33	3/26
>8mM	16/16	16/22	12/33	7/26

By day 5 (the final day for issue), 14% PCs had <4mM glucose remaining, and by day 6, 36% of all PCs tested had <4mM glucose available for metabolic processes, with 24% below the analyser limit (1.1mM). The hand held meter routinely used by diabetics for whole blood glucose determination provides a means of quality controlling PCs for likely transfusion effectiveness. Of concern is the number of units, following pooling, with abnormal glucose metabolism. In deciding the ideal platelet prescription, the age and quality of the platelets should be assessed and the limitations on the issue life of pooled buffy coat derived platelets considered.

Management of Patients Refractory to Platelet Transfusions

P10

Response to HLA-Matched Platelet Transfusions in Patients Refractory to Random Donor Platelets.

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Frequent pooled random donor platelet transfusions result in a high incidence of alloimunisation. Most studies suggest that alloimmunised patients will benefit from the transfusion of HLA matched platelets. We analysed 402 HLA matched transfusions to 13 thrombocytopenic refractory patients in order to assess the importance of the degree of HLA compatibility on clinical response. Clinical data including fever, infection, bleeding and splenomegaly at the time of transfusion were noted. The response to HLA-matched transfusion was assessed by the corrected count increment at 24 hours post transfusion and graded as excellent (>$10x10^9$/l), good (>$5x10^9$/l), fair (>$2.5x10^9$/l) and poor (<$2.5x10^9$/l). 30% of donors fully matched for HLA-A and-B loci (A-matched) and 12% of donors mismatched with the recipient for two or more HLA antigens (D-matched) showed an excellent response; 11.5% of A-matched and 42.6% of D-matched donors showed a poor response. Amongst all groups analysed only A-matched donor recipient pairs showed significantly higher increments than other degrees of HLA matching ($p<0.01$). The results of this study suggest that HLA matched platelets are of benefit in selected cases, however, HLA matching does not reliably predict platelet transfusion response, even in stable patients. Non-immune factors also play an important role in determining transfusion outcome.

P16

Platelet Support of HLA Alloimunised Patients from Ethnic Minorities

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The Histocompatibility and Immunogenetics laboratories within the NBS London and South-East Zone are responsible for the selection and provision of HLA matched platelets. In London this service is provided for a patient population consisting of a variety of ethnic groups but the donors are predominately of British Caucasoid origin. In this study we have analysed the HLA antigen frequencies of the donor and the patient populations and examined how this can affect the management of these patients. The donor panel consisted of over 95% Caucasoids and the remaining 5% were of Asian and African origin. However the patient group consisted of a higher proportion of non-European, with 22% of Asian origin and only 72% were Caucasoid. When we compared the frequency of HLA antigens in the patients with those in the donors, we found HLA-A1, a typical Caucasoid antigen, was decreased in the patients; HLA-A3, A19 and B14 were increased in the patients. HLA - B42, an antigen found typically in individuals with Black African ancestry, was restricted to the patient group . One of the important features of the HLA system apart from its extensive polymorphism is the high degree of epitope sharing amongst gene products of the same locus. Matching for antigens within the same crossreactive group has enabled the use of Caucasoid donors for non-Caucasoid patients. We have determined that donors that are homozygous for the most common HLA-A, B haplotypes in British Caucasoids HLA-A1, B8 and HLA- A2, B44, can provide well matched (A or B1) transfusions for 30% of our patient group illustrating the value of these HLA homozygous donors but also highlighting the need to recruit more donors from ethnic minorities.

P17

Techniques Used for the Detection of HLA Specific Antibodies in Patients Under Investigation for Immunological Refractoriness.

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The presence of donor reactive lymphocytotoxic HLA specific antibodies in the patient's serum is the most significant cause of immune refractoriness. It is important in the diagnosis of immune refractoriness that the presence or absence of these antibodies is accurately confirmed . In this study we have evaluated two techniques used routinely for the detection of HLA specific antibodies: The extended incubation NIH complement dependent microlymphocytotoxicity test (LCT) with and without DTT (dithiothreitol) and a solid phase ELISA based technique. In this technique soluble HLA molecules immobilised on microwell plates serve as a target for patient antibody which, if bound, are detected with an enzyme conjugated anti-human immunoglobulin.
104 serum samples from patients receiving platelet transfusions were tested and 50% of sera were positive by LCT but only 40% were positive when screened with DTT illustrating the importance of screening with DTT to remove irrelevant IgM, non-HLA, lymphocytotoxic antibodies(10%).
42% of the sera were positive by ELISA. The addditional 2% positivity detected by ELISA is most likely to be due to the detection of non-cytotoxic HLA specific antibodies. The results show that the ELISA is a technique that is HLA specific, detecting both cytotoxic and non-cytotoxic antibodies and when used in conjunction with the LCT to determine HLA antigen specificity it is a more effective method of screening for HLA specific antibodies.

P18 *(combined)*

The Frequency of HLA Specific Antibodies in Patients Investigated for Platelet Refractoriness in London.

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The laboratory management of patients refractory to random donor platelet transfusions involves the tissue typing of patients and donors, the screening of patient sera for platelet reactive antibodies (primarily HLA specific) and the selection and provision of HLA matched platelets. In this study we have analysed the HLA antigen frequencies in the donor and patient populations and looked for any relationship with the HLA antibody profiles of the patients.
324 patient's sera were screened for the presence of HLA specific antibody using the microlymphocytotoxicity technique using an extended incubation with and without dithiothreitol (DTT). 158(49%) sera tested positive and in 39% of these sera it was possible to define the HLA specificity of the antibodies present. The results also show that patients tended to make antibodies against the antigens present at high frequency in the donors. HLA-A2 & B12 occurred at frequencies of 47% & 28% in the donors and at 48%, & 24% in the patients respectively. The frequency of HLA-A2 and B12 specific antibodies in the patients was 36% and 23% respectively. Interestingly the antigen frequency of HLA-A3 was 26% and 34% in the donors and patients respectively but only 3% of HLA-A3 specific antibody could be detected in the patients. Furthermore the frequency of HLA-A28 in both patients and donors was around 5% but accounted for 10% of the HLA specific antibody found and is probably due to the high level of crossreactivity between HLA-A28 with A2. Antibody production is influenced by a number of factors including the level of mismatch between patient and donor, their genetic background and the immunogenicity of specific HLA antigens. These are important factors, as highlighted by these results, to consider in order to avoid sensitisation to antigens that are common in the donor panel.

P18 *(combined)*

HLA Profile of Patients Receiving HLA Matched Platelets in the South Thames Region.

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We have analysed the HLA antigen frequencies and HLA antibody status in patients receiving HLA matched platelets, and the donor platelets transfused, in the South Thames region between 1995 and June 1997. A total of 198 patients, with a number of diseases including aplastic anaemia, myeloma, CML, AML, ALL, and MDS, were included in the study. The most frequent A locus antigen in our patient group was HLA-A2 (23.6%). The frequency of Al9 was raised in this patient group (19.2%), reflecting the high proportion of patients from ethnic minorities in this cohort. The antigens, A1 and A3 were also present at frequencies greater than 10% (14.6% and 13.5% respectively). HLA-B12 was the most frequent B locus antigen (14.5%) with B7, B8 and B35 the next most frequent (12.5%, 9.9% and 9.1% respectively). HLA antibody status, assessed by lymphocytotoxicity, was available for 154 patients, of which 64 had multispecific antibodies and 39 were HLA antibody negative. Where HLA antibody specificity could be defined, the most frequent antibodies were anti-Al, A2, B12, B7 and B8 (24.1%, 20.4%, 18.9%, 16.2% and 13.5% respectively). The frequencies of HLA antibodies identified in this patient group reflect the antigen distribution in both patients and donor platelets provided. There were two notable exceptions: HLA-A19 was present in 19.2% of patients and 12.4% of platelets provided. However, specific anti-A19 antibodies were only detected in 1.9% of patients. This may be as a result of the increased A19 frequency in this patient population, thus reducing the incidence of A19 antibody production. A similar situation was seen with HLA-B35, where 9.1% of patients carried this antigen and 8.7% of platelet donors, however, no anti-B35 specific antibodies were identified in this group.

P20

A Policy for the Management of Immunological Refractoriness to Random Platelet Transfusions.

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HLA and, to a lesser extent ABO and platelet specific antibodies (abs) have been implicated in immunological refractoriness to platelet transfusions. In the London and South East Zone patients suspected to be immunologically refractory to random platelets are managed using the following protocol: (i) The transfusion of less than 48 hours old random, ABO identical platelet concentrate is advised initially. If poor/no increments are obtained, a serum sample is tested for HLA specific abs using an extended incubation lymphocytoxicity test (LCT) +/-DTT and ELISA to identify cytotoxic and non-cytotoxic abs. (ii) If HLA specific abs are detected, HLA matched platelets are provided. If good increments are obtained, this treatment is maintained. If poor/no increments are obtained, a serum sample is tested for HPA abs and the likelihood of non-immune platelet consumption is re-assessed. If HPA abs are detected, HLA and HPA matched platelets are provided, where possible. If this treatment is ineffective, solid phase cross match-negative platelets are given. (iii) If no HLA antibodies are detected, a trial with HLA matched platelets is initiated. If response is poor, an investigation for HPA abs is undertaken and whenever present, HPA matched platelets are provided if possible. However, if poor or no increments are observed, solid phase cross match-negative platelets are tried. All patients are screened for HLA abs four weekly.

If none of these approaches is successful, alternative therapies, including IVIG, are considered in discussions with the hospital clinicians.

Future Developments

P12

Storage Stability of Combined Hyper-and Regular Platelet Concentrates Prepared by Cobe SpectraLRS

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Volume reduced leucodepleted platelet concentrates (hyperconcentrate-HC) is needed for fetal transfusion. Currently, this product can be obtained in combination with regular platelet concentrate (R) without additional leucocyte filtration and concentration steps, using Cobe Spectra LRS. However, there is limited information on the storage stability of such a product. We evaluated the storage stability of HC as compared with R of the same origin. HC and R were kept in a nominal plasma volume of 60 and 200 ml, respectively. A similar surface-to-volume ratio was maintained and both were stored under a conventional condition. Storage stability was assessed by a comprehensive panel of markers of platelet storage lesion: i) cellular integrity, aggregation states, microvesiculation by an automated cell counter and soluble annexin V; ii) surface activation markers (GPIb, GPIIbIIIa, P-selectin, bound-fibrinogen, phosphatidylserine exposure) by flow cytometry; iii) release reaction (PF4, ßTG, thrombospondin) by ELISA; and iv) metabolic activity (pH, pO_2. pCO_2). Products were tested at days 1 and 3. On average platelet concentration of H was 3 fold higher than R (3032 vs 1320 x10E9/L). At day 1, H and R counterparts showed equivalent results of all markers except H contained more aggregates and slightly lower mean platelet volume, as compared to R. At day 3, products with platelets more than 3000 X1O E9/L failed pH and showed the highest level of all markers. Based on these findings we conclude H store well at least up to day 3. However, modification of storage condition is required for products which contain more than 3000 x1OE9/L.

P15

Development of a Novel Platelet Substitute, Synthocytes™ for the Treatment of Thrombocytopenia

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Synthocytes™ are being developed as a platelet substitute with the aim of overcoming problems associated with conventional platelet therapy. The product for intravenous administration is composed of fibrinogen immobilised on insoluble human albumin microcapsules of defined size. It is hypothesised that thrombin will cleave the immobilised fibrinogen to produce a fibrin bridge between the microcapsules, also the immobilised fibrinogen will bridge to activated platelets. *In* vitro and *in* vivo studies are being performed to establish the precise mechanism of action. Addition of thrombin to Synthocytes™ induces an aggregation response. Synthocytes™ also enhance aggregation of platelets activated with 1µM ADP. When Synthocytes™ are mixed with citrated whole blood and passed over a collagen type III surface in a perfusion chamber there is a 50% increase in platelet/Synthocytes™ deposition. The prolonged bleeding in rabbits rendered thrombocytopenic using an antiplatelet antibody was corrected using Synthocytes™ from >20 minutes to 5 ± 1.7 minutes (n=6). In a preliminary modified Wessler study, Synthocytes™ did not cause an increase in thrombus growth of an experimental rabbit jugular vein thrombosis indicative of no enhanced thrombosis risk. Distribution of ^{131}I-labelled Synthocytes™ in a normal rabbit shows a circulation time of at least 24 hours.