BLOOD ISSUES

Platelet Transfusion Refractoriness and Management of Platelet Support in Refractory Patients

A proportion of patients who receive regular platelet transfusions will become refractory or non responsive. A number of approaches are available to support these patients. The provision of HLA matched platelets has been the traditional approach to management often used in conjunction with platelet crossmatching procedures. These approaches are effective but present a number of logistical problems in small countries such as New Zealand. NZBS has recently adopted a new approach based on 'antibody specificity prediction' and provision of 'antigen negative platelets' for these patients. This review discusses the management of patients with platelet refractoriness and outlines the way that NZBS currently supports them.

Definition of Refractoriness: Refractoriness to platelet transfusions can be best defined as a failure to achieve the expected platelet increment after two or more consecutive platelet transfusions.

Diagnosis and Aetiology: Several formulas are used to estimate the effectiveness of platelet transfusion. One of the accepted but probably less commonly used approaches here in New Zealand is to calculate the corrected count increment (CCI) with knowledge of the patient's body surface area (BSA), as follows:

$$CCI = \frac{\text{platelet increment } (10^9/L) \times BSA \ (m^2)}{10^{11} \text{ platelets transfused}}$$

The response to platelet transfusions can also be measured as the % recovery of platelets transfused at a defined time post-transfusion:

$$\% \text{ recovery} = \frac{\text{platelet count increment/ml} \times \text{blood volume}}{\text{No. platelets transfused}}$$

It is considered ideal to determine the platelet count immediately before and 15 minutes, 60 minutes, and/or 24 hours after transfusion.

The platelet transfusion outcome is considered a failure if the CCI at 1 hour is <7.5 or the % recovery at 1 hour is <15-20, or if the CCI at 20-24 hours is <4.5.

A simplified approach based upon the above formula is an expected immediate rise of at least 40 x 10^9/L after a transfusion of pooled or apheresis units.

Non-immune mechanisms are responsible for about 80% of transfusion failures. In refractory patients with malignancy or haematological diseases, non-immune factors are present in 72-88% and HLA antibodies in 25-39%. Thus the diagnosis of immunological refractoriness to platelet transfusions is established after having excluded non-immune causes. Important non-immune factors include infection and its treatment, especially amphotericin B, splenomegaly, bleeding and disseminated intravascular coagulation (DIC).

HLA alloimmunisation is the most important cause of immune mediated platelet refractoriness. There is a strong association between the presence of HLA antibodies in the transfusion recipient and platelet refractoriness, but the relation between platelet-specific antibodies and refractoriness is weaker.

There is no available New Zealand data on the incidence of immune platelet refractoriness. Studies have shown that prior to the widespread use of leucocyte reduced blood components 45-70% of chronically transfused patients developed antibodies to HLA class I antigens. Whether universal leucodepletion has reduced this incidence is unknown.

ABO antibodies are also responsible for reduced platelet survival. Platelets express blood group A and B antigens and it is not unusual for patients to receive ABO-incompatible platelets because of low platelet inventories.

Investigation of the alloimmunised patient: All investigations for immune refractoriness are performed at the NZBS National Tissue Typing Laboratory in Auckland. It is recommended that a sample of the patient's serum be tested for the presence of HLA and platelet specific antibodies. An HLA type if not already done should also be requested.

NZBS has introduced a new methodology for the detection of HLA Class I antibodies. This is a flow cytometric platform based upon Luminex Microsphere (bead) technology. When the coloured beads are coated with purified Class I antigens, they can be used for screening and identification of HLA antibody specificities. This technology has replaced HLA antibody screening by lymphocytotoxicity an indirect method for detecting platelet alloimmunisation. This method has been traditionally used to determine the breadth of immunisation expressed as the percent reactive antibody (PRA) from 1 to 100%. Patients are classified as mildly (1-40% PRA), moderately (40-70% PRA) and severely (PRA >70%) immunised.

Transfusion Support of the Alloimmunised Patient: Three main strategies can be used to transfuse alloimmunised patients: matching donor-recipient HLA antigens, crossmatching platelets and by determining antibody specificities and then providing antigen-negative platelets. The third strategy is similar to the way antigen negative red cells are provided when antibodies are present.

HLA-Matched Platelets: Where the patient's HLA-type is already known, it is possible to identify matched donors from the available inventory. The disadvantage is that the registry requires the availability of large numbers of HLA-typed donors. A registry of about 18,000-25,000 HLA-typed donors is needed to provide at least five HLA-A and HLA-B matched donors for 80% of white patients. It is not possible for New Zealand to have an inventory of this size. NZBS records indicate that we have about 1600 HLA-typed donors, of which a smaller number are regularly donating platelets. In this situation patients can be transfused with partially matched platelets by a system where the donor and recipient are matched by assigning HLA-A and HLA-B antigens with shared public epitopes to clusters called cross-reactive groups (CREGs).

Crossmatch Compatible Platelets: Crossmatching test plasma from an alloimmunised patient against platelets available for transfusion or aliquots of platelets from potential donors that have been frozen for this purpose is another way to identify compatible platelets. We would now rarely use this method for identifying donors since it does not significantly increase the number of compatible donors particularly for the highly alloimmunised patient. HLA-matched and crossmatch compatible platelets are equally effective. For highly immunised patients and those with rare HLA types both methods have serious limitations in identifying adequate number of matches.

Provision of Antigen Negative Platelets: This approach was evaluated by Petz and Garratty in a large number of alloimmunised patients. The specificity of the antibodies are determined and antigen-negative platelets are selected from a HLA-typed donor pool. We use this approach quite often, utilising the Luminex technology, and have found it possible to identify a larger number of compatible donors for most patients. For us this has been very useful in the highly immunised patient. The risk of forming additional antibodies appears to be relatively low even when many units are transfused. Most platelet refractory patients will establish a stable breadth of HLA alloimmunisation without forming further antibodies. However, the technology is expensive but presently is cheaper than providing crossmatch compatible platelets.

Patient Follow-up: A majority of patients (60-90%) will have acceptable platelet count increments after receiving matched platelets. It is again appropriate to determine immediate (10 minutes) post-transfusion platelet recovery. The Tissue Typing Laboratory or a Transfusion Medicine Specialist must be notified about matched platelet transfusion failures. This information is useful in excluding the donors from the initial match list. About 10-20% of patients will have significant weakening of the antibodies and a few will totally lose them over a period of time. Three monthly repeat antibody screening is recommended.

The Antibody Screen and Issuing of Red Cell Components

Prior to the issue of red cell components to a recipient by a Blood Bank, both a blood group and an antibody screen are performed. The blood group includes testing for the A, B and Rh(D) antigen on the red cells and also determining the presence of anti-A and anti-B in their serum. These antibodies are "naturally occurring" meaning that they are present in every individual who does not have the antigen i.e. those who are group A have anti-B antibodies and so on. Most other antibodies are not "naturally occurring" and are "acquired" when the person is exposed to foreign red cells, either through transfusion or pregnancy. Antibodies that people make to foreign red cells are called alloantibodies. However, people can also make antibodies to their own red cells and these are labelled autoantibodies. The ability to form alloantibodies in response to recent blood transfusion and pregnancy are the basis of the "72 hour rule" - the length of time a group and screen is valid for in these patient groups.

Pre-formed alloantibodies to red cell antigens may be of clinical significance both in the context of reactions to transfused red cells and haemolytic disease of the foetus and newborn (HDFN). The clinical significance of alloantibodies can be predicted by a number of factors. Firstly, what temperature the antibody binds the antigen. Those that only react at temperatures well below 37˚C are less likely to be clinically significant. Secondly, whether the antibody is IgM or IgG. IgM is unable to cross the placenta, so is unlikely to cause HDFN. Thirdly, whether the antibody-antigen combination is able to "fix complement". These reactions may be associated with acute intravascular haemolytic reactions. Finally, historic information is the most useful predictor, with certain antibodies being associated with certain types of reactions.

Antibodies are initially detected by the screen method, where the recipient's serum is crossmatched to a set of blood group O red cells with known antigen expression. In New Zealand, the Blood Banks

use a set of three red cells, which cover the range of clinically significant antibody combinations. If there is no reaction between the serum and the cells this is labelled a negative antibody screen and blood may be issued to the recipient based on their blood group. This is achieved either by an 'electronic crossmatch' (the units are selected as compatible by a computer) or by an 'immediate spin' crossmatch (units are confirmed as ABO compatible by laboratory testing). Issuing based on blood group is required to avoid the predictable acute haemolytic reactions mediated by anti-A and anti-B.

A positive antibody screen requires the identification of the antibody(ies) present. To achieve this, the Blood Bank uses a panel of group O cells with known antigen expression. Using the patterns of reactions, the antibody can be identified. Where this panel fails to clearly identify the antibody(ies) samples are sent to the Red Cell Serology Reference Laboratory of the New Zealand Blood Service in Auckland. Further panels of cells are then used to help in the identification process.

When an antibody is identified, and deemed clinically significant, the safe provision of blood is based on providing blood units that do not express the corresponding antigen. Where the antigen is rare in the donor population, antigen negative units are common and compatible units can usually be found in the Blood Bank. However, finding antigen-negative units where the antigen is common can be a challenge. In very extreme examples, compatible units cannot be found within New Zealand and are sourced from the International Rare Donor Panel.

Approximately 0.2 to 2% of the population have antibodies to red cell antigens. In the majority, the antibody is easily identified and compatible units can be found. However there may be a significant delay in the provision of blood. For this reason, the Blood Bank requires notice when patients with known antibodies are undergoing procedures where blood product support is likely. Furthermore, the clinical team need to anticipate when the current group and screen expires. Finally, it is not uncommon to require additional serum to resolve difficult antibody identifications.

When blood is required urgently and either the antibody has not yet been identified or compatible units have not been found, the risks and benefits of immediate transfusion should be discussed with a Transfusion Medicine Specialist. They are contacted via the hospital Blood Bank.

Sample and Request Form Labelling Errors

International transfusion literature has shown that labelling errors and misidentification associated with pretransfusion samples significantly increases the risk of transfusion errors, particularly the transfusion of ABO incompatible blood.

The New Zealand Blood Service (NZBS) policy is based on the requirements of the ANZSBT "Guidelines for Pretransfusion Testing" (2005). The policy defines the acceptance criteria for samples and request forms submitted to the six NZBS Blood Banks for pretransfusion testing.

The request form must include the following:

- Patient family name and given name/s
- NHI number and/or date of birth
- Name of requesting practitioner
- Signature of the person completing the request form
- Date and time of the sample collection
- Details of request
- Date and time components are required
- Signed declaration by the sample collector confirming that the patient was positively identified at the time of collection and the samples were labelled before leaving the patient

Labelling of the patient details on the sample must be hand written and the information must be legible. Details that must be included on the sample include:

- Patient family name and one or more given name
- Patient's NHI number and/or date of birth
- Signature or initials of the collector

The policy details the actions to be taken when the specimen and request forms fail to meet NZBS requirements. For minor errors the collector may be able to correct the information on the sample or form but must sign a declaration accepting full responsibility for the corrections made to the form/specimen. If the discrepancy is unresolved after 24 hours, the patient care area is informed that a new specimen and request form is needed if testing is still required.

More significant errors require the Blood Banks to discard the specimen and request a new sample and request form. The patient care area or in instances that the request is urgent, the requesting practitioner is informed of the requirement of a new sample and request form.

If blood is required for transfusion while discrepancies remain unresolved only 'emergency' O Rh(D) negative red cells will be issued until a new sample has been received and testing is complete.

On 1 May 2006 NZBS began collecting standardised national data regarding sample and request form labelling errors at the six NZBS Blood Banks. Each site records instances of a range of predetermined errors and the associated corrective actions. Data is entered into a Microsoft AccessTM database for subsequent analysis.

Between May 2006 and 31 December 2007 a total of 247427 requests were received by the six NZBS Blood Banks. The chart below shows the monthly mean of errors per 1000 requests received by the six NZBS Blood Banks. The overall error rate during the period was 4.1%. The error rate per 1000 samples ranged from 24 – 103 for the six Blood Banks.

Errors Rate Per 1000 Samples
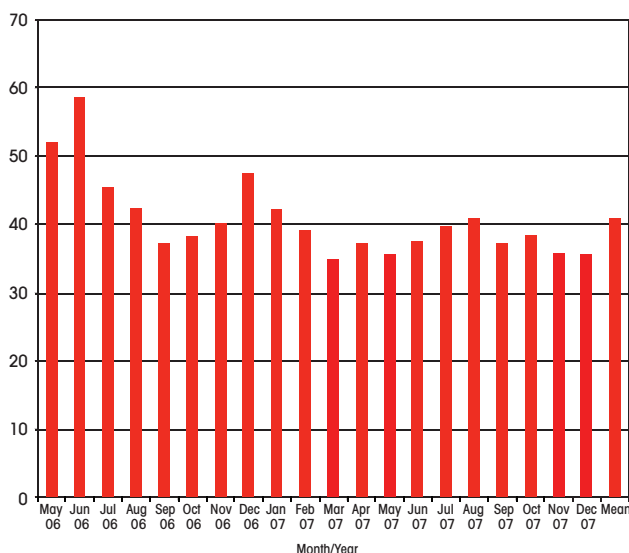
Of the specific errors reported, 82.9% were due to the five most prevalent errors:

- Missing or incomplete patient details (19.2%)
- Patient details – discrepancy between sample and form (18.0%)
- Declaration not signed (12.8%)
- Sample not signed (17.4%)
- Sticky label on sample (15.5%)

Overall 10095 of the 247427 requests had an error either of the request form or the sample associated with them. 3661 of these did not meet NZBS requirements and a new sample and request form were required for pretransfusion testing. Hence 36.3% of samples with errors or 1.5% of overall samples required recollection. These requests for recollection of a blood sample meant that the patient was subjected to a second venepuncture and potential delays in the provision of blood components.

The above data compare well with published international experience. A study from the UK involving 185 hospitals showed a median recollection rate of 2.45%.

Wrong Blood in Tube (WBIT)

The detection of WBIT is dependent on a Blood Bank being able to identify patients and compare the current result with the historical records. The NZBS Blood Management System, Progesa, allows New Zealand Blood Banks access to historical pretransfusion testing results and the transfusion history of any patient, irrespective of where the investigation were carried out. This capability assists in detection of WBIT.

Because WBIT can only be detected by a discrepancy between the current sample result and that from a prior test, chance alone might produce a result in the correct ABO Rh(D) groups even if the wrong patient's blood is taken, a correction factor was applied in the study to determine the actual WBIT (1.6 in NZ).

The table provides information on the incidence of WBIT for the period 1 May 2006 to 31 December 2007 for the six NZBS Blood Banks.

WBIT rate for all NZBS Blood Banks

	May 2006 – Dec 2007		
	Historical Blood Groups	WBIT	Corrected Frequency*
NZBS Blood Banks	116476	18	1:10353

Corrected to account for silent errors (correction factor 1.6)

This compares well with published data. In 2003 an international study compared sample error rates. This showed a median rate of wrong blood in tube of 1 in 2000.

Details of NZBS requirements for request forms and specimen labelling for pretransfusion testing and diagnostic testing can be obtained from the Sample Collection Manual (136I120) available from the NZBS website at www.nzblood.co.nz under Technical Services.

Progesa – an Update

NZBS plans to move the current version of Progesa onto new hardware during May 2009. This will reduce risks associated with the age of the current hardware. The plans have been reviewed and endorsed by external project specialists.

At this stage we are on track to undertake the migration on the weekend of the 16th and 17th of May with Go live on Monday the 18th of May. Further updates on project progress will be made available to all Progesa users closer to the scheduled go live date with details of any scheduled outages and contingency plans.