New Zealand Blood Service
The gift of life

BLOOD ISSUES

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Bacterial Contamination of Platelet Concentrates

Bacterial contamination of blood components was first recognised as a complication of transfusion over 60 years ago. The storage temperature of platelets of 22±2°C facilitates the growth of bacteria in contaminated units more often than Red Blood Cells and Fresh Frozen Plasma. The rate of bacterial contamination of platelet concentrates varies widely across studies but averages approximately 1 in 2000. Sources of contamination include the skin of the donor, donor bacteraemia, leaky seals in the blood collection packs, damaged tubing or micropunctures in collection bags or contamination during blood processing. The contaminating organisms are usually aerobic and the organisms that are most frequently implicated in clinical cases of transfusion-associated sepsis include *Staphylococcus, Streptococcus, Bacillus cereus, E. coli, Salmonella* and *Serratia*.

Key factors for the survival and multiplication of microorganisms in fresh blood components include the size of the inoculum, the microbial species/strain and the storage conditions. The presence of up to 10^4 Colony Forming Units (CFU)/ml coagulase negative Staphylococci in a transfused component is easily tolerated and cleared by most patients.

Platelets stored for longer than 3 days have a higher contamination rate due to the exponential growth of bacteria. The concentration of bacteria in the components may increase up to the order of 10^9 CFU/ml by the end of the platelet storage period.

The severity of clinically observed reactions is influenced by the bacterial load, the organism (Gram negative organisms are associated with more severe reactions), endotoxin release from bacteria causing septic shock and factors associated with the recipient such as immunosuppression, leucopenia, underlying disease and concomitant antibiotic therapy.

Fatalities

There were 18 deaths in France from 1994 to March 1998 due to the transfusion of blood components contaminated with bacteria and ten of these deaths were associated with platelet concentrates. In the US there were 6 deaths from transfusion of contaminated platelets between January 1998 and December 2000. The UK reported 29 bacterial transfusion-transmitted infections from 1995 to 2003 of which 25 were from platelet concentrates. There were 7 deaths reported, 6 of which were related to platelet concentrates (Bacillus cereus, Enterobacter aerogenes, E. coli, Staph aureus, Staph epidermidis) and one relating to red cells (Yersinia enterocolitica) The reported fatality rate is within the range of 1 in 50,000 to 1 in 500,000 platelet concentrates transfused.

Signs and Symptoms of Transfusion-Associated Bacterial Sepsis

Not all contaminated blood components cause symptoms in the recipient and the severity of the reaction is variable. Fever, chills, rigors, tachycardia, hypertension or hypotension during or soon after transfusion should be investigated. Shock, oliguria and multiple organ dysfunction syndrome can occur. Endotoxin can induce disseminated intravascular coagulopathy and lead to bleeding. Other symptoms include nausea, vomiting, diarrhoea and dyspnoea.

Strategies to Reduce Bacterial Contamination

Detailed donor screening with deferral of those identified to be at risk of bacteraemia is vital. Optimal disinfection of the venepuncture site will reduce the entry of skin flora into the unit. Iodine, povidone iodine and chlorhexidine have all been shown to be efficacious in combination with alcohol. Diversion of the first 10-40ml of blood collected prior to collection of the unit has been shown to significantly reduce the rate of bacteria contamination in several studies. This was introduced in New Zealand during 2002. A number of pathogen inactivation methods involving photochemical treatment (PCT) are under development. A combination of psoralen (amotosalen) and UVA light targets nucleic acid and inactivates a broad spectrum of viruses, bacteria, protozoa as well as white blood cells. Other PCTs include riboflavin and visible light, UVB irradiation and the addition of methylene blue or pthalocyanines.

Bacterial Detection Systems

In the US, the AABB require mandatory testing of all platelet components. This requirement was introduced in March 2004. There are a number of approaches that can be undertaken. The FDA has approved BacT/ALERT (BioMerieux) and Pall BDS for testing. BacT/ALERT is a sensitive automated blood culture system that detects 10-10^2 CFU/ml. The majority of positive cultures occur within 24 hours of inoculation of the culture bottle. Both systems are sufficiently sensitive to be used on a sample size of 5 to 6 mls that has been obtained a minimum of 24 hours post-collection.

A pilot study in Northern Ireland used the BacT/ALERT to test 4885 platelet concentrates on day 2, over a 1 year period. The ratio of pooled to apheresis platelets was 60:40 and platelets were available for issue after labelling. There were 13 reproducible positive cultures (1 apheresis and 12 pooled platelets). 86% of the negative cultures sampled at Day 8 met the specifications of a platelet count of $2.4x10^{11}$/unit and ph of 6.4 - 7.4. The authors concluded that routine bacterial testing with day 2 sampling and a negative culture result after 24 hours as a mandatory release criterion would improve transfusion safety. (Transfusion Med 2003, 13:189-195).

In Belgium (February 1998) and the Netherlands (November 2001) all Platelet Concentrates are cultured, quarantined and released into stock only if the cultures are negative 24-48 hours following inoculation. The storage period for Platelet Concentrates in these countries has been extended from 5 to 7 days. The Canadian and Welsh Blood Services have also implemented 100% screening of Platelet Concentrates.

The Council of Europe (15th edition) states that "Viability of platelets is preserved up to 7 days under optimal conditions. However >5 days storage is not currently recommended unless a validated system has assured absence of bacterial contamination". Each country needs to analyse their current risk of bacterial contamination. The cost of testing Platelet Concentrates may be offset by extension of the shelf life and this could improve the supply of Platelet Concentrates and reduce expiry, in addition to improving transfusion safety. Dipstick testing for low ph, low glucose and disappearance of platelet swirling are rapid and relatively inexpensive methods. However they lack sensitivity (detect 10^6-10^7 CFU/ml) and are not specific. Similarly, Gram Stain and microscopic examination of a smear is likely to detect 10^6 CFU/ml.

More sensitive methods under development include semiautomated fluorescent microscopy/cytometry, molecular techniques such as detection of ribosomal RNA, DNA using PCR amplification and immunological methods that use gold-conjugated antibodies against bacterial wall antigens eg lipoteichoic acid (Gram-positive bacteria) and lipopolysaccharide (Gram negative bacteria)

NZBS Pilot Study on bacterial contamination of Platelet Concentrates

A pilot study to determine the contamination rate of platelet concentrates produced by NZBS was commenced in Auckland during 2004. Waikato, Christchurch and Wellington are now also contributing to the trial which involves two different bacterial detection systems. 5000 platelet doses are being analysed using the BacT/ALERT system (Biomerieux). 1000 of these are also being tested using the Pall eBDS system. The trial is designed to avoid destructive sampling of platelet concentrates and to have no impact on the availability of platelets for clinical use.

Samples are taken on Day 2 (Day of collection = Day 0). With the BacT/Alert system, 12 ml of a platelet dose is sampled into a pouch. 6ml of this sample is inoculated into an aerobic culture bottle that contains culture medium and a colourimetric sensor which is then incubated at 36°C. Bacteria present in the sample metabolise the culture medium, producing carbon dioxide that lowers pH and leads to a colour change in a sensor disk at the base of the inoculated bottle. The bottles are continuously monitored and positivity is indicated by an alteration in light deflection. The instrument monitors this change and the user is alerted to a positive result. The bottles remain in the incubator for 7 days unless flagged as positive. The remaining 6ml of sample is retained for follow-up in the event of a positive result.

The Pall eBDS system measures oxygen concentration of air within the sample pouches as a surrogate marker for bacterial growth. 3ml of a platelet dose is sampled into an eBDS sample pouch that contains two tablets. These tablets contain TSB (Trypticase Soy Broth) which is a nutrient that enhances bacterial growth and Sodium Polyethanol Sulphonate (SPS) that inhibits normal bactericidal agents in plasma and causes platelets to aggregate, reducing their oxygen consumption. The samples are incubated for 24 hours at 35°C in an agitating incubator. The analyser measures the percentage oxygen in the headspace of the sample pouch and gives a pass/fail result for each sample. If bacteria are present in the platelet sample collected, an increasing amount of oxygen is consumed through the metabolic activity and proliferation of the bacteria in the sample during incubation, resulting in a measurable decrease in oxygen content of the plasma as well as the air within the sample pouch. Any platelets that are sampled on Day 2 and have not been transfused by Day 5 are held until Day 7 and retesting using the same technology. The incubation period for Day 7 samples using both testing platforms is 24 hours. Samples that are flagged as positive on the BacT/ALERT or flagged as 'Fail' with eBDS require further investigation.

All components associated with a positive result are traced and quarantined. The BacT/ALERT bottles that are flagged as positive and the corresponding platelet dose and red cell components where available are sent to an accredited microbiology laboratory for gram stain, culture and identification. If the platelet dose is unavailable, the sample pouch containing the remaining 6ml of sample is sent for investigation. The same protocol is followed for the samples that result in a 'Fail' indicator on the eBDS. If the follow-up investigation is negative, the original result is considered to be a false positive.

To date, 3933 platelet pools and 1287 apheresis platelet doses have been sampled on Day 2 using BacT/ALERT. Of these, 1 confirmed positive and 7 false positives have been reported. 937 platelet concentrates have been sampled using eBDS and no positive results have been recorded.

Blood Bank Stocks Project Update

The NZBS Blood Bank Stocks Project is one of the initiatives arising from the NZBS/DHB consultative process and has initially focussed on the supply and stock levels of red cell components for each ABO Rh(D) blood group. Eventually the project aims to include other fresh blood components and ultimately stocks of manufactured plasma products to blood banks in New Zealand. Current supply arrangements and the means used to establish the maximum and minimum stock levels by a particular blood bank vary across the country and the project aims to establish agreed stock levels and supply arrangements.

A consultative document was distributed in November 2004 detailing a number of possible models for determining appropriate stock levels for individual blood banks. Two specific stock measures were identified. The Minimum Stock is the level at which emergency re-supply will be required. The majority of respondents expressed a preference for the minimum stock level model based on a formula using a combination of routine activity supplemented by a safety stock. The safety stock takes into account the variation in daily activity and the ease

of supply. The impact of this model is an increase to the total historical minimum stocks of 10%. The stock levels held by non-NZBS stocks is 30% more units compared to a 6% decrease in NZBS sites. The designated stock level is the level that NZBS aims to reach immediately following a routine delivery of blood. Two proposed models for designated stock level had a similar level of acceptance and there was general preference for the model providing stock levels close to (or higher) than those currently being used.

Pilot Study

Two blood banks within each of the four regions supplied by the NZBS processing sites have been selected as pilot sites for the Blood Bank Stock Project. This includes five DHB and one NZBS blood bank. As part of the implementation process NZBS will introduce a system to monitor and report the following performance parameters:

- Minimum and designated stock levels
- Optimum delivery cycles
- Expiry rate of red cells, total and group specific
- Number of red cell units returned to NZBS prior to expiry, total and group specific
- Number of urgent orders placed
- Percentage of orders that are filled in full
- Percentage of orders filled and delivered on time

Future Initiatives

Major differences exist between blood banks on the definition of fresh blood components with respect to age and the clinical indications for use. Guidelines are being prepared by NZBS to define the age and clinical use of fresh blood. Wide clinical consultation will be undertaken before this policy is finalised.

The current shelf life of red cell components is 35 days, the blood pack specifications support 42 days storage and this shelf life is already in place in Australia and the United States. Extending the shelf life to 42 days may improve the supply of group O red cells but will not allow NZBS to reduce current collection levels. It may however in the future reduce the rate at which collection requirements will increase.

Cryoprecipitate Audit

When treating patients with actual or potential bleeding, it is important to ensure that the patient has the ability to clot and that adequate amounts of fibrinogen are present. It is generally accepted that clot formation may be impaired or unstable if the fibrinogen concentration is less than 1.0 g/L. When this occurs the optimal replacement therapy is cryoprecipitate because of its high concentration of fibrinogen.

The cryoprecipitate component produced by NZBS is collected by apheresis from donors with high fibrinogen levels unlike many other transfusion services that prepare cryoprecipitate from unselected whole blood donations. NZBS cryoprecipitate contains an average of 1.4g fibrinogen (range 0.75–2.0g) in 80-120ml. The recommended dose is 1unit per 30kg bodyweight to achieve a fibrinogen increment of 1.0g/L.

Clinical Guidelines

The clinical indications for the use of cryoprecipitate developed by the Australian National Health and Medical Research Council (NHMRC)[1] and the Australian and New Zealand Society of Blood Transfusion (ANZSBT)[2] include:

- Disseminated Intravascular Coagulation: where there is a fibrinogen level <1.0g/L and clinical bleeding, cryoprecipitate may be indicated to maintain levels above 1.0g/L.
- Fibrinogen deficiency and dysfibrinogenaemia: where there is clinical bleeding in the event of an invasive procedure, trauma or DIC, cryoprecipitate may be indicated.
- Coagulation Factor Deficiencies: as an alternative product for the treatment of bleeding associated with Von Willebrand Disease, Haemophilia A and a Factor XIII deficiency.
- Bleeding associated with uraemia: cryoprecipitate may be useful to stop bleeding.

A dose of 1 unit of cryoprecipitate per 30kg body weight is expected to produce an increment of approximately 1g/L. The dose should however be adjusted depending upon the patient's pre transfusion fibrinogen level, the patient's response to cryoprecipitate and the nature of the bleeding.

The NZBS Audit

To assess appropriateness of transfusions of cryoprecipitate, an audit was conducted in 6 DHBs - Auckland, Counties/ Manukau, Waikato, Capital & Coast, Christchurch and Otago. Transfusion Nurses Specialists collected 30 data on episodes of transfusion of cryoprecipitate over a 10 week period. Data was also collected on patients who had fibrinogen levels less than 1.0g/L on coagulation screening and who did not receive cryoprecipitate (non-recipients). An episode was defined as each time Blood Bank issued one or more units of cryoprecipitate or, for the non-recipients, one fibrinogen level less than 1.0g/L per 24 hour period.

Data collection for each episode included patient demographics (date of birth, gender and weight); laboratory data (pre and post transfusion fibrinogen levels with dates and times, Hct, INR, PTT, platelet count and platelet function); transfusion data (date, time and number of units of cryoprecipitate transfused for this episode; other blood components transfused in the previous 12 hours); and clinical data (the patient's diagnosis, indication for the use of cryoprecipitate and location, rate of blood loss, and comorbidities).

Weight-adjusted doses and increments were calculated on patients with weights over 15kg. This excluded paediatric patients who may be transfused less than the entire unit. A dose was considered low if it was less than 0.5 units per 30 kilograms bodyweight, and a high dose was more than 1.5 u per 30 kg bodyweight. For increment calculations the pre and post transfusion fibrinogen tests had to be within 6 hours of the transfusion commencement. The increment was adjusted to reflect what the increment would have been if the recommended dose, 1 unit per 30 kilograms bodyweight, had been given.

Data was reviewed by two Transfusion Medicine Specialists. Key points used in the assessment were the rate of blood loss, as identified from general comments and the volumes of various blood components and other fluids infused, together with measurements of haemostatic data: platelet count, fibrinogen, APTT, PT, information on platelet function abnormalities where present, and the time interval between the tests and the request for cryoprecipitate.

Limitations

Specific limitations identified for this audit were the inclusion of retrospective episodes due to the slow acquisition of episodes; incomplete data sets for certain aspects of the analysis (especially patient weights and pre and post transfusion fibrinogen levels); the lack of review of thromboelastograph (TEG) results as an indicator of fibrinogen concentration; a level of assumption based on the information available in the assessment of appropriateness and the disproportionate contribution that single patients contributed in some instances. For example, a single patient in a Haematology ward received cryoprecipitate on 12 occasions, contributing 7% to all episodes. In addition this audit did not assess the clinical outcome other than the post-infusion fibrinogen concentration.

Results and Analysis

6 DHBs participated in the study. Two DHBs collected only prospective episodes and four included retrospective episodes. The longest retrospective audit performed was back to 20th November 2003. Of the 181 cryoprecipitate recipient episodes, 7 (3.9%) were used to make fibrin glue and 22 (12.2%) as part of paediatric cardiac bypass surgery protocol (all in Auckland) and were not analysed further.

The remaining 152 episodes, involving the administration of 316 units of cryoprecipitate, were assessed as non-elective transfusions in response to a coagulopathy. 26% of pretransfusion fibrinogen levels in recipients were more than 1.5g/L, compared with Schofield at al's report[4] of 43%. The mean pre-transfusion fibrinogen concentration was 1.3g/L but with wide confidence intervals and all DHBs showed overlapping confidence intervals. 18% (n=27) of all cryoprecipitate transfusion episodes were considered inappropriate by the two reviewing Transfusion Medicine Specialists. This compares with 24% reported by Pantanowitz et al[3] and 49% by Schofield et al[4]. However, Schofield defined an inappropriate transfusion dependent upon the patient's fibrinogen level, whereas this present audit took into consideration the overall clinical situation. It is generally accepted that cryoprecipitate is useful when the fibrinogen level is below 1.0g/L[5,6,7] and in the clinical situation where the patient is bleeding or is at risk of bleeding. The routine use of cryoprecipitate in the presence of hypofibrinogenaemia without risk factors is not considered good transfusion practice. The trend suggested from this audit is that a significant proportion of cryoprecipitate usage is inappropriate (18%). There was no significant variation across the participating DHBs.

A tendency to underdose patients was noted. With a recommended dose of one unit of cryoprecipitate per 30kg bodyweight, 24% of patients received less than half the recommended dose. There was no correlation between pre-transfusion fibrinogen levels and dose received. 8% of doses were more than 1.5 times the recommended dose with 3 evaluable episodes showing more than twice the recommended dose. Patients who were given a low dose initially showed a poorer response to transfusion despite an appropriate indication, requiring more than twice as many transfusions of cryoprecipitate than those patients given the right dose. The average increment was 1.2g/L for those episodes where the transfusion was appropriate and the dose was the recommended dose. Overall 53% of all recipient episodes were appropriate and involved use of the right dose. The lack of patient weights and fibrinogen levels made for small patient numbers and wide confidence intervals around the increment.

There were 134 episodes involving 86 patients who did not receive cryoprecipitate despite a fibrinogen less than 1.0g/L. The non-recipients showed a similar pattern of fibrinogen levels to the recipients, indicating that the fibrinogen level alone was not the reason the patients were not transfused. On review of these cases it was considered that 27% of these patients would have benefited from receiving cryoprecipitate and that there was significant variation between DHBs possibly reflecting differences in practice and policy.

In summary, this audit has shown good increments where cryoprecipitate is used correctly. However significant problems exist with transfusions at high fibrinogen levels, underdosing, inappropriate transfusions and inappropriately not transfusing patients.

Recommendations

Clinical education to clinical staff in relation to

- The correct dose of cryoprecipitate (1u per 30kg bodyweight) to obtain a fibrinogen increment of 1.0g/L
- The importance of checking fibrinogen levels before giving cryoprecipitate to ensure that the component is used appropriately
- Ensuring appropriate systems are available for monitoring fibrinogen levels when cryoprecipitate is being used in a massive transfusion setting (e.g. the use of TEG)

Blood Bank staff will in future ask for the patients weight before issuing cryoprecipitate.

1. Clinical Practice Guidelines on the use of Blood Components. NHMRC & ASBT 2001
2. ANZSBT guidelines
3. Pantanowitz L et al. Cryoprecipitate. Patterns of use. American Journal of Clinical Pathology. 2003; 119(6):874-81.
4. Schofield WN et al. Appropriateness of platelet, fresh frozen plasma and cryoprecipitate transfusion in New South Wales public hospitals. Medical Journal of Australia. 2003. 178(3):117-121.
5. Eddy VA et al. Hypothermia, Coagulopathy and Acidosis. Surgical Clinics of North America. 2000. 80(3):845-854.
6. Vaslef SN et al. Massive Transfusion Exceeding 50 units of Blood Products in Trauma Patients. The Journal of Trauma Injury, Infection and Critical Care. 2002. 53(2): 291-296.
7. Hardy JF et al & members of the Groupe d'Interet en Hemostase Periopertoire. Massive transfusion and coagulopathy: pathophysiology and implications for clinical management. Canadian Journal of Anesthetics. 2004. 51(4):293-310.