Universal leucodepletion: experience of implementation in Scotland

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Le Autorità sanitarie del Regno Unito hanno deciso che, a partire dal luglio 1998, tutti gli emocomponenti prodotti fossero sottoposti a leucodeplezione. Tale decisione era motivata dalla necessità di evitare il rischio (peraltro, soltanto ipotetico) della trasmissione, per via ematica, della variante della malattia di Creutzfeldt-Jakob (vCJD). L'articolo riporta la personale esperienza dell'Autore, nella sua qualità di responsabile del Servizio Trasfusionale Nazionale Scozzese, nella realizzazione di questo programma in Scozia. Vengono illustrati i metodi utilizzati, i problemi incontrati e gli importanti risultati raggiunti.

Key words: universal leucodepletion, filters, CJD

Parole chiave: leucodeplezione totale, filtri, malattia di Creutzfeldt-Jakob

Introduction

The intent to introduce universal leucodepletion in the UK was announced in July 1998, although the four Blood Transfusion Services involved had been warned to plan for this in November of the previous winter. The reason given was the avoidance of the hypothetical risk of variant Creutzfeldt-Jakob disease (vCJD) infectivity in blood, and the cost has been £80-£100M for the UK, and one tenth of that in Scotland which processes around 300,000 donations per year.

There is no evidence that vCJD is transmitted by blood or its components, but some may regard this as absence of evidence. In some animal models low levels of TSE (Trasmissible Spongiform Encephalopathy) infectivity have been reported in blood. This appears to be concentrated in the leucocyte fraction, but is also found in plasma. Immunohistology has demonstrated that, in contrast to classical CJD, vCJD patients have detectable levels of abnormal prion protein in their tonsils and appendix, suggesting a greater level of involvement of the immune system in vCJD. In addition, leucocytes are required for the development of peripherally transmitted disease in animal models of TSE. As the incubation period of TSE's is dependent on the infective dose, removing some infectivity would be expected to prolong the time to onset of clinical disease. Specific studies on vCJD are hampered by the fact that as of June 2000 there have only been 58 deaths due to vCJD, with a further 10 probable cases still alive (Figure 1).

Other countries have introduced leucodepletion to improve the storage properties of cellular components, to avoid non-haemolytic febrile transfusion reactions, and avoidance of alloimmunisation, platelet refractoriness, or viral infections. In the UK, however, the reason is avoidance of infection, even if theoretical, and hence requires a greater level of certitude in relation to consistency and level of leucodepletion.

The method used

In the absence of any evidence on whether vCJD infectivity is present in the blood, let alone its titre, we arbitrarily chose to set a target for leucodepletion of 5M residual leucocytes per unit in 99% of units with 95% confidence. This is approximately equivalent to the European standard of 1M leucocytes per unit in 90% of
units and has been achievable. To achieve this level of performance it was agreed early on that blood bags with integral filters would be used and that pre-storage filtration would be carried out in processing laboratories, rather than at the bedside. Concerns in this area related to the potential for leucocyte fragmentation, either as a result of normal cell breakdown on storage, cold storage prior to filtration or due to filtration itself. For this reason, our intent was to filter at room temperature as soon as possible after donation, although a minimum time after donation was required both to allow cooling to room temperature, aided by cooling trays, and to allow phagocytosis of any bacterial contamination. Selection of this route of processing necessitated introduction of overnight shift working so that donations could be processed and filtered at 20 °C within 24 hours of donation. During this time, we also reduced the number of processing sites from five to two, each processing around 150,000 donations per year. The longer transport distances resulting from this meant some units do not reach the processing laboratory until after 10 hours post donation. To assure ourselves of filter operability and yield, and that filtration did not result in leucocyte fragmentation or activation of plasma proteins, we undertook a detailed examination of available filters initially9, alongside the issuing of a tender based on the above specifications in July 1998. This resulted in selection of two preferred suppliers, Pall and NPBI, now Fresenius (Pall Medsep, Portsmouth, UK - Fresenius NPBI, W. Midlands, UK), in December 1998. There followed a phased staff training period, involving the manufacturer, and staged introduction of filtration of whole blood, then platelets and then red cell units. This order was chosen on the basis that greater volume products should be dealt with first, since we were acting to reduce a hypothetical risk of infection (Figure 2). This process was completed by September/October 1999 (Figure 3). During this period we have also instigated a number of clinical assessments to compare the safety and efficacy of leucodepleted products, as compared to unfiltered ones. These include studies on the frequency of side-effects, an evaluation of the rate of post-surgery, post-transfusion sepsis and observations on the increments in circulating cell counts after transfusion in selected patient groups. Data collection on these studies is nearing completion.

**Problems encountered**

As one would expect, a number of problems were encountered in the rapid introduction of universal leucodepletion. Most of these have been overcome.

Foremost among these were related to staffing. We closed three processing laboratories and expanded the remaining two. We recruited new staff and had to train them, relying for this partly on the manufacturers. We introduced, and hence had to validate and write, procedures for all the components we make. We introduced large scale leucocyte counting. We purchased bags and filters from manufacturers, who themselves were scaling up manufacture of these on an unprecedented scale. We introduced shift working arrangements, over and above existing extended day working, for processing and counting staff, both new and old. We put in place a transport system
to bring blood from donor sessions to the two processing sites. And we achieved this all on a strict timetable and budget, set at the beginning of the process. Currently we are reasonably content with the solution found for most of these, although the staffing structures, particularly at supervisory level, require fine tuning.

A number of bag and filter faults were discovered during the period of introduction. These have been dealt with in discussion with the manufacturers and using an audit process. New problems of this type do continue to appear. One problem faced, as an example, was the discovery that some of the filters used for room temperature filtration began to perform less well when blood was stored for longer than 10 hours after donation. For such units we therefore had to cool the donations to 4 °C and filter the next morning, most filters being more efficient at cold temperatures.

Since we were undertaking filtration to guard against infection, we felt that residual leucocytes should be assessed in representative samples of all units produced, prior to their release to the blood bank.

This required counting staff to work at night. In initial studies the residual cell content depended on the method used. To allow batch release, sample sizes depend on whether absolute counts of residual leucocytes can be determined so that a normal distribution can be assessed. We chose to standardise on a single flow cytometric method rather than to use Nageotte or microfluorimetry (Imagn®) methodology.

In terms of the extent of leucodepletion achieved during the implementation process, we have evidence from our ongoing quality assurance that compliance is improving as we become more familiar with leucofiltration (Figure 4). We are meeting the specification for residual leucocyte content in all products, but do see occasional failures in less than 1% of units. A concern is whether the method used to deal with batch release of products, using statistical process control, is valid. Most approaches assume that the distribution of residual leucocytes fits a log normal distribution and it is not yet clear that this can be proven, particularly for leucocyte counting methods that have limited sensitivity.

While not a significant problem in Scotland, there is now good evidence that sickle trait donations are often
Leucodeplezione totale in Scozia

**Figure 3:** timetable for introduction of universal leucodepletion in Scotland

**Figure 4:** declining rate of leucodepletion failures in Scotland. Number of failures (>5M leucocytes per unit or platelet pool) per month for whole blood units, red cell units and platelet pools (4 buffy coats)
Figure 5: preliminary evaluation of 4 whole blood filters. Use of surrogate markers (platelet β-thromboglobulin:BTG, neutrophil elastase and normal soluble prion protein:PrP), showing no increase over the filtration process. Expressed as a percentage of the pre-filtration value.

Figure 6: mean platelet content of concentrates prepared from 4 buffy coats. Mean content of platelet pools and percentage meeting the UK specification of >240 x 10^9 per pool (requirement 75%) for period November 1999 to February 2000.

Due to this, the English Service are considering introducing screening of donors for sickle trait. This

not adequately depleted by most currently available filters\textsuperscript{12}. 
concern is likely to be further emphasised in Mediterranean and African services.

Due to concerns over the possible effects of leucocyte fragmentation, a detailed study of the effects of filtration was undertaken.

Based on use of surrogate markers, including normal prion protein and soluble annexin V, we have found no evidence of cell lysis or fragmentation for units filtered at room temperature within 24 hours of donation (Figure 5). In collaboration with 3 other groups within the UK these studies are being extended to additional filters and processing modes, with an extended set of assays e.g. cell microvesicles.

To date, these have confirmed our original findings that no cell fragmentation is evident as a result of filtration, but that prolonged storage is associated with cell lysis. When filtering, some product is lost due to the volume of the filter itself. For red cell products this reduces the total dose of haemoglobin per donation by about 10%.

For platelets the volume loss is less.

With careful attention to processing, we have found it possible to still meet the UK specification for platelet products (pool of 4 prepared by the buffy coat method) of >240 x 10^9 perpool in 75% of pools (Figure 6). Currently we, as other services, provide a separate bank of cytomegalovirus (CMV) negative products for use in susceptible CMV seronegative immunocompromised patients.

This is currently achieved by screening for antibody to CMV in selected units. However, since CMV is carried in the leucocyte fractions, one could consider leucofiltration as yielding a CMV safe product, without the need to check CMV serological status, and hence save on the costs of serological screening.

Following discussion with clinical users of these products, this has not as yet been possible since there is a concern that in the leucodepletion process only a selection of units are assessed, whereas all relevant units are screened in the serological approach.

The fact that serological assays also have a false negative rate, and that some seronegative units do appear to transmit CMV may allow such steps to be taken in the future, but at present this is not accepted in the UK, or Canada. In the UK it is difficult to assess whether the introduction of leucodepletion has a cost-benefit since the theoretical risk of vCJD infection cannot be accurately quantified.

However, the other possible benefits of leucodepletion may be cost beneficial. Initial estimates of these (M Murphy, personal communication) indicate a wide range of costs from significant losses to major savings. We are trying to refine these estimates through the prospective clinical studies mentioned above.

Conclusions

The introduction of universal leucodepletion, to avoid the theoretical risk of vCJD infection, in Scotland has not been an easy process but has been achieved over a period of 14-15 months.

This has entailed huge changes in the way the Blood Transfusion Service is run, considerable cost and at least some clinical benefits. The longer term outcomes, side-effects and cost benefit of this are the subject of ongoing evaluation.

Abstract

In UK universal leucodepletion was introduced in July 1998.

The reason for this resolution was the avoidance of the hypothetical risk of variant Creutzfeldt-Jakob Disease (vCJD) infectivity in blood.

The paper reports the personal experience of the Author, as Research Director of the Scottish National Blood Transfusion Service, the method used, the problems encountered and the important results achieved.

References


