

Position Statement

Granulocyte Therapy

13 November 2008 (reconfirmed November 2009)

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Introduction

Granulocyte transfusions are requested by clinicians for use in patients with refractory infection or at high risk of developing severe infection (Strauss 2003). Most patients prescribed granulocyte transfusions are those with cancer related neutropenia, who are receiving myeloablative chemotherapy with or without haemopoietic stem cell rescue. Interest in the use of granulocytes remains high (Van Burik & Weisdorf, 2002; Price 2006), and requests for granulocyte components for transfusion have steadily increased in England and Wales during the last five years. This has been driven by publications describing transfusion in neutropenic patients both for *therapeutic* indications, when they have an infection refractory to antimicrobials (Hubel et al. 2002) and for secondary *prophylaxis*, in patients who have had severe bacterial or fungal infections previously but who require a further cycle of chemotherapy or haemopoietic stem cell rescue (Kerr et al. 2003, Oza et al., 2006). Recent studies with variable or promising, but overall inconclusive, results have been reported both in adults (Oza et al. 2006, Seidel et al, 2008) and children (Sachs et al., 2006).

Requests for use of granulocyte transfusions in other clinical groups of patients (e.g. neonates) are much less common, and will not be discussed further in this article (Baley et al., 1987; Wheeler et al., 1987). However similar broad principles of treatment apply, although in the case of neonates (or very small children), much higher doses of granulocytes can be provided per kg body weight.

Methods of collection in UK

In the UK, granulocytes for transfusion are produced by one of two means:

- by apheresis (from stimulated or unstimulated donors – see below), or
- as a component derived from whole blood donations.

The administration of Granulocyte Colony Stimulating Factor (G-CSF) and steroids to donors increases the circulating granulocyte count prior to apheresis, enabling greater yields of granulocytes to be collected for transfusion. The Table below summarises information on cell counts for the main sources of granulocytes.

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Properties of different granulocyte concentrates

(data provided by the UK National Blood Services: Rebecca Cardigan, Saber Bashir, Fred Goddard)

	Single buffy coat (n=21) (mean, SD)	10 buffy coats (dose typically transfused for adults)	Pooled granulocytes from whole blood, in development (n=13) (mean SD)	Unstimulated apheresis collection (n=20) (mean, SD)	Stimulated apheresis collection (n=5) (median, range)
Volume (ml)	59 (3)	590	250 (10)	276 (26)	299 (214-333)
Neutrophils ($10^{10}/U$)	0.105 (0.04)	1.05	0.88 (0.14)	0.54 (0.2)	6.37 (3.69 – 8.47)
Haematocrit (%)	45 (6)	45	21 (2)	23 (7)	9 (7-20)
Lymphocytes ($10^9/U$)	0.88 (0.41)	8.80	6.72 (0.75)	5.90 (1.38)	N/A
Monocytes ($10^9/U$)	0.18 (0.07)	1.80	1.22 (0.37)	0.95 (0.39)	N/A
Platelets ($10^9/U$)	75 (17)	750	344 (96)	111 (25)	160 (82 – 293)
Red cells ($10^{12}/U$)	0.27 (0.04)	2.70	0.57 (0.06)	0.71 (0.23)	0.3 (0.28 – 0.61)

Collections of granulocytes by apheresis in UK

The UK Blood Services have made a decision not to permit G-CSF and steroid administration to volunteer unrelated donors for the purpose of collecting granulocytes (Guidelines for UK Transfusion Services), in view of the paramount need to ensure absolute safety of volunteer donors (see below for details of specific although small risks). In some hospitals in UK, granulocyte collections are obtained from directed G-CSF and/or steroid stimulated donors who are 'family and friends' of patients. This process involves multiple steps, including:

- Identifying and selecting potential 'family and friends' of patients
- Checking suitability and eligibility in keeping with national requirements and guidelines for blood collection (including medical examination)

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- Counselling donors, and obtaining consent
- Microbiological screen testing (including e.g. HIV, as for any blood donation)
- Immunohaematological testing (e.g. for atypical red cell antibodies)
- Administering G-CSF and steroids to the donor
- Apheresis
- Post- collection processing (if appropriate)

Whilst on paper this appears feasible and even straightforward, there are a number of potentially significant constraints in this process which can (and do) limit provision of apheresis products on a regular and timely basis in the UK in response to all potential requests from hospitals:

- Hospitals managing granulocyte collections by apheresis now have a requirement for meeting 'blood establishment status', as a consequence of EU legislation, enacted in the UK as the Blood Safety & Quality Regulations 2005.
- There are often (major) resource limitations at already overstretched apheresis units, alongside, for example, pre-booked stem cell collections, yet requests for granulocytes are unpredictable
- Ensuring all volunteer 'family and friends' of patients are given time and adequate explanation of the (potential small) risks they are exposed to by both taking specific drugs (steroids and G-CSF) to mobilise granulocytes into the peripheral blood (see Ghodsi & Strauss 2001; Gutierrez-Delgado & Bensinger, 2001; Bennett et al., 2006; Goldman et al., 2006) and by undergoing an apheresis procedure.
- UK blood services do not recommend the collection of whole blood or other component donations from directed donors, for well established reasons of blood safety (Pink et al., 1994).
- The risks of delay in collecting and administering apheresis granulocytes for transfusion, given all the above steps (Hubel et al. 2002), which could be very important in cases of severe life-threatening infection (Sachs et al., 2006)
- Unexpected variation in collection yield – sometimes very low doses are obtained by apheresis (Strauss 2005)
- The use of hydroxyethyl starch or dextran to sediment red cells during processing (Poon & Wilson, 1980, Rock et al., 1984) may have deleterious effects on chemotactic and oxidative killing activity of neutrophils (Hofbauer et al., 1999, Jaeger et al., 2001), as well as having a risk of allergic reactions in recipients

There are also remote, but presumably finite, risks of developing life-threatening haematological malignancies even after short courses of G-CSF (Bennett et al., 2006; Goldman et al., 2006). *This very important issue of potential, albeit very low, risk to healthy donors needs to be considered alongside the uncertain benefits of granulocyte transfusions*

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to patients, as summarised in the section on the evidence base (see below).

Granulocytes derived from whole blood

The alternative source of granulocytes, derived from whole blood donations, has been available for many years and has some immediate advantages of availability, but the component has not been evaluated in any detail (Poon & Wilson, 1980, Rock et al., 1984). These donations are commonly described as “buffy coats” as they are derived from the buffy coat layer between red cells and plasma in centrifuged whole blood. The main disadvantage of this source of granulocytes is the lower yield, by comparison to apheresis collections. Risks of “buffy coats” granulocyte transfusion also include alloimmunisation and transfusion transmitted infection associated with multiple donor exposure, given that 10 buffy coats are typically transfused for an adult dose (Schiffer et al., 1979). Such risks would extend to vCJD. However, patients for whom granulocyte transfusions are considered are often acutely ill and unwell, with life-threatening infection, and these patients require extensive transfusion support with other blood components.

In England and Wales, there has been a significant increase in requests for the buffy coat granulocyte component over the last five years. As mentioned, usually 10 buffy coats are transfused to give a dose of approximately 1×10^{10} neutrophils for an adult. In addition to the low cell dose, the current buffy coats are also heavily contaminated with red cells and platelets, and repeated transfusion can result in polycythaemia necessitating venesection.

Evidence Base

There has been a general resurgence of interest in granulocyte transfusion therapy over the last decade largely as a consequence of using G-CSF and steroids to ‘prime’ donors for apheresis, which has permitted the collection of significantly greater yields of granulocytes for transfusion (Dale et al., 2000; Yeghen & Devereux, 2001; Hubel et al., 2001, Robinson & Marks 2004, Murphy et al., 2000). These higher yields for transfusion are considered clinically important and the transfusion of these components is associated with definite post-infusion increments and appropriate localisation in vivo (Adkins et al., 1997). However, the apheresis granulocyte component for transfusion has not to date been evaluated for efficacy in a large prospective randomised controlled trial, perhaps in part because of the major logistic difficulties required in the planning and design of such a trial which would require significant resources and hundreds of enrolled patients (Price et al., 2006). Indeed, a group in Europe very recently published a randomised controlled trial of granulocytes collected by apheresis from GCSF and steroid stimulated donors. For a number of methodological and logistic reasons however, this trial of therapeutic granulocytes failed to establish evidence of benefit (Seidel et al, 2008).

The exact role for granulocyte transfusions (whether derived from whole blood or collected by apheresis) therefore remains unclear. Potential efficacy including a dose dependent effect has been raised by systematic reviews/meta-analyses (Vamvakas et al. 1996; Vamvakas et al. 1997; Stanworth et al., 2004), and in animal studies. The existing literature is, perhaps not

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surprisingly, otherwise heavily dominated by case reports and small case series, with the significant attendant risk of publication bias. However, it should be acknowledged that anecdotal evidence of benefit in selected patients from physicians in the UK and abroad can be found, and that a number of very recent publications have again pointed to evidence of benefit, including one study based on biological randomisation - although this study was underpowered to detect an effect on mortality (Oza et al., 2006).

Current developments in UK Blood Transfusion Services: A better 'optimised' component of granulocytes derived from whole blood

Recent work in the National Blood Service Components Development Laboratory (CDL) has reported the characterisation of a purer pooled granulocyte component derived from whole blood donations. The method involved the addition of platelet additive solution but without the need for hydroxyethyl starch or dextran to sediment red cells during processing (Bashir et al., 2006). In addition to cell content, a range of *in vitro* tests for measures of neutrophil function were determined during storage (Bashir & Cardigan, 2003; Bashir et al., 2008). The volume and red cell contamination of this product is vastly reduced compared to standard buffy coats and is similar to an apheresis granulocyte collection. The results for pH, viability and neutrophil function indicated well maintained function during storage up to 24 hours and some measures of neutrophil function were preserved for longer (for comparison see Schwanke et al., 2005). There were no statistically significant differences when this optimised granulocyte component was compared to either the standard buffy coat or fresh whole blood. Therefore the method for producing a pooled granulocyte component derived from whole blood donations described above appears to provide granulocytes whose *in vitro* function is maintained for up to 24 hours of storage.

The component has advantages of ready availability for transfusion on a daily basis, and this may be clinically important given that there is some evidence that provision of granulocytes at early onset of severe infection may be critical (Sachs et al., 2006). In addition, by providing a standard adult component derived from twenty donations, a consistent daily cell dose of around 2×10^{10} cells may be transfused to patients, which is considered by many physicians a clinically 'meaningful' yield for transfusion.

At the time of writing 92 doses of optimised pooled granulocyte components made from 10 donations each have been transfused into patients with neutropenia and sepsis, as part of a safety trial. There have been no recorded adverse events other than one episode of fluid overload in a patient with a history of cardiac failure who received other blood components and fluids in addition to the optimised component. This dose does not produce a measurable increment in granulocyte count 12-18 hours post infusion but a larger dose as discussed above is now being given without the volume and haematocrit constraints associated with unprocessed standard buffy coats.

Compatibility testing

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Granulocyte components should be treated as whole blood when compatibility testing is performed. In view of the residual red cells still present in the final component granulocytes need to be ABO and RhD compatible with the recipient. If the recipient is eligible for electronic issue, crossmatching is not required. If not eligible for electronic issue for technical reasons in the absence of an antibody specificity, granulocytes should be crossmatched against recipient plasma by IAT technique (British Committee for Standards in Haematology, 2004).

If there is a specific antibody present, the guidance for red cell significance should be followed (Daniels et al. 2002). For some antigens it would be purely IAT crossmatch compatibility (e.g. Cw, Kp^a or M detectable at 22°C only); for other antigens, antigen negative donations crossmatched by IAT technique would be recommended (e.g. Rh, K, Jk, Fy). This is a cautious standard based upon the available components with the greatest volume of red cells from each individual donor. The risks posed by red cell incompatibility of the optimised component would be less than apheresis granulocytes or unprocessed standard buffy coats because in 250ml there will be less than 50ml of red cells i.e. less than 5ml from each donor.

If ABO compatible but non-identical granulocytes are used (e.g. O donor, A recipient) the plasma should not have high titres of anti-A and anti-B using the laboratory standards defined for platelets. The plasma used for resuspension of the optimised component should ideally be from a male contributor to the pool (to reduce risk of TRALI). The risk of immunological complications occurring as a result of donor derived antibodies is least for the optimised component as a substantial proportion of the suspending fluid is an additive solution rather than plasma.

It is advised that all patients receiving granulocyte transfusions are screened for HLA class I and II antibodies when granulocytes are requested. In the absence of transfusion reactions or previously identified refractoriness to platelet transfusion, the significance of the positive antibody screen is very unclear. The development of platelet or granulocyte refractoriness or severe transfusion reactions would prompt repeat screening for HLA, HPA and granulocyte antibodies. If available, antigen matched granulocytes would be preferable but logistically are rarely available (Elebute et al. 2004).

Summary

The issue of efficacy of granulocytes (either therapeutically for refractory infection or as secondary prophylaxis for high risk groups of patients with prior severe infection) is still very much an open question. Provision of granulocytes by apheresis collection from G-CSF and steroid stimulated donors remains the standard, but a number of logistic and other constraints currently limit wider provision in UK. An 'optimised' component derived from whole blood, described above, is currently being assessed in patients to establish safety, by evaluating the frequency and severity of adverse events. Any additional risks associated with high donor exposure for this component including alloimmunisation and vCJD would need to be considered in the context of the use of this component in very sick and immunosuppressed patients. Possible future clinical studies of this component to address

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efficacy in patients with neutropenia will need to evaluate how best to use the component alongside granulocytes for transfusion collected by apheresis from G-CSF/steroid stimulated donors. New studies to definitively address the issue of effectiveness are required.

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