CLINICAL GUIDELINES FOR THE USE OF GRANULOCYTE TRANSFUSIONS

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Clinical Guidelines for the use of Granulocyte Transfusions

Purpose

This document outlines:
1. The clinical indications for the use of granulocyte transfusions
2. The options in England and North Wales for the provision of granulocytes
3. A referral system for provision of granulocytes in England and North Wales

These guidelines are not meant to be prescriptive and the decision for each request for granulocyte transfusions should be made following detailed assessment of the clinical details, in conjunction with the referring Consultant (or their designated deputy).

Method

Recommendations are based on review of the literature and review of accepted current clinical practice. The definitions of the types of evidence and the grading of recommendations used in this document originate from the US Agency for Health Care Policy and Research and are provided in Appendix 1.

Consultation

NHSBT consultants

Status

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Summary

Granulocyte transfusions can be used as supportive therapy in patients with life-threatening neutropenia caused by bone marrow failure or in patients with neutrophil dysfunction. With the advent of potentially curative intensive chemotherapy regimens used alone or in combination with stem cell transplantation, there has been an increase in patients with fungal infections during periods of prolonged neutropenia and subsequently, a renewed interest in the use of granulocyte transfusions to support these patients. Despite the potential availability of this component, there is limited published literature on the in vivo efficacy of whole blood derived granulocyte concentrates. From October 2012 a pooled whole blood derived component became available. The NHSBT component portfolio name for this pooled component is “Granulocytes, Pooled, Buffy Coat Derived, in Platelet Additive Solution and Plasma, Irradiated”. This component should replace the old unpooled component, “leucocytes, Buffy Coat, Irradiated” except in exceptional circumstances. Adverse events such as febrile reactions, HLA alloimmunisation and transfusion related acute lung injury (TRALI) are well recognized complications following granulocyte transfusions. The use of granulocyte transfusions should, like all blood components, be limited to patients in whom the possible benefits outweigh the hazards. Granulocytes should always be irradiated as they contain a large number of white blood cells which can, like a bone marrow donation grow in the recipient and form an immune system or “graft”. This unmatched, unintended graft may cause Transfusion Associated Graft Versus Host Disease (TA-GVHD) if the transfused immune cells recognise the recipient or “host” as different and attack / reject the host’s liver, blood, gut and skin cells in the same way the immune system would attack an infection or un matched transplant. TA-GVHD is almost universally fatal.
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1. BACKGROUND

Functioning white blood cells (WBCs) are a vital component of the defence system against infection in humans. Neutrophils are a subtype of granulocytes and are the most numerous circulating WBC in healthy adults. Granulocytes in general and neutrophils in particular are crucial in protection against bacterial and fungal infection. A persisting reduction in neutrophil numbers is called neutropenia, the severity of which has been classified by the World Health Organisation [WHO 1999]. When the peripheral blood count is below a level of $0.5 \times 10^9$/litre there is an increased risk of severe infection (the normal neutrophil count ranges from $2.0$ to $7.5 \times 10^9$/litre in adults).

Neutropenia usually occurs as a result of impaired production of neutrophils and other blood cells in the bone marrow. Diseases infiltrating the bone marrow, such as leukaemias or drugs that are toxic to the bone marrow, such as chemotherapy are typical reversible causes of neutropenia. Even if the numbers of neutrophils are normal, patients may suffer from a similar inability to adequately fight infections if there is an impairment of the function of their neutrophils. Some people are born with such disorders, which may be suspected from family history or by demonstrating the inability of neutrophils to function normally on laboratory testing [Kuijpers et al 1999].

Despite the use of specific and appropriate antibiotic and antifungal drugs, infection in patients with neutropenia can be associated with hospital admission, organ damage, and a significant number of deaths. Clinical experience and data from animal studies suggest that control of infection in these patients requires recovery of bone marrow neutrophil production [Dale et al 1976]. The first documented attempt to reverse neutropenia using granulocyte transfusions was during the 1930s [Strumia 1934]. Twenty years later, Brecher and colleagues gave granulocyte transfusions to neutropenic dogs, in which they showed that the transfused cells migrated to the areas of infection [Brecher et al 1953].

There are a number of different methods for collecting granulocytes for transfusion in humans. When donated blood is being separated into its main components for transfusion there is a layer between the red cells and plasma called the “buffy coat”. The buffy coat contains a high concentration of white cells and platelets. Buffy coats that are not used to make platelet concentrates are discarded as waste but a small proportion are transfused as a granulocyte containing component. Apheresis (from the Greek ‘to take away’) was later developed and utilized for increased efficiency; this technique removes specific blood cells or fluid from the donor or patient whilst the cells or fluids that do not need to be removed are returned to the donor. Although apheresis involves machinery, it does allow selective collection of a bigger dose of granulocytes than would be found in whole blood with the added advantage for the donor of minimal red cell loss. Granulocytes collected in this way were transfused into patients with severe neutropenia that was not responsive to antibiotics [Freireich et al 1964].
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More recent studies have suggested that the efficacy of granulocyte transfusions in neutropenic patients is proportional to the dose of granulocytes transfused. Doses of at least $1 \times 10^{10}$ granulocytes per transfusion appear to be required to treat or prevent infection [Estcourt et al 2015 and 2016]. There are a number of technical problems that make it difficult to collect adequate granulocyte doses for transfusion. Granulocytes are difficult to separate from other blood cells even if this has been facilitated by commercially available long-chain starch solutions (sedimenting agents) such as hetastarch and pentastarch. Normal donors also do not have very high levels of circulating granulocytes in the peripheral blood and as a result can donate doses of granulocytes that are only likely to be sufficient for very small children.

In the early 1990s growth factors that stimulate the bone marrow to produce more WBCs (particularly granulocytes) were developed for therapeutic use. These drugs allowed high peripheral blood white cell counts to be achieved in healthy donors. The most commonly used growth factor is granulocyte colony stimulating factor (G-CSF). Steroids can also increase the white cell count, but alone they are not as effective as G-CSF. The use of a single injection of G-CSF alone or combined with a single oral dose of steroids has enabled the collection of significantly greater yields of granulocytes by apheresis. Using this method, doses of granulocytes in excess of $5 \times 10^{10}$ cells can be produced for larger children and adults [Hubel et al 2001 and 2002]. The exposure of a healthy volunteer donor to any form of medication with potential side effects does however present ethical and safety issues [Gutierrez-Delgado & Bensinger 2001, Bennett et al 2006, Goldman et al 2006].

The ability to collect greater numbers of granulocytes has been a major factor influencing the rekindling of interest in the potential role of granulocyte transfusions as additional therapy for patients with neutropenia and established infections [Hubel et al 2002, Peters et al 1999, Dale and Liles 2000, Price et al 2000]. Studies with promising but overall inconclusive results have been reported both in adults and children [Oza et al 2006, Sachs et al 2006, Seidel et al 2008, Price et al 2015]. Blood Centres in England now regularly provide granulocytes derived from whole blood donations (Leucocytes, Buffy Coat, Irradiated) for transfusion to children and adults. A small number of donations are collected from relatives and friends of patients following the administration of G-CSF and the steroid Dexamethasone for transfusion to children or adults [Hubel et al 2002, Kerr et al 2003]. Adverse events such as febrile reactions, occasional severe pulmonary reactions and HLA (human leucocyte antigen) alloimmunisation are well recognized complications in the recipients of granulocyte transfusions. Although there is evidence that donated granulocytes are functional [Bashir and Cardigan 2003, Bashir et al 2008], published controlled trials have reported conflicting results of clinical effect. A number of these issues have been raised in systematic reviews [Estcourt et al 2015 and 2016, Vamvakas et al 1996 and 1997].
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2. CLINICAL INDICATIONS FOR GRANULOCYTE TRANSFUSIONS

2.1 Therapeutic granulocyte transfusions may be indicated for patients with severe neutropenia who fulfil all of the following criteria:

2.1.1 Severe neutropenia, defined as ANC <0.5 x 10^9/L [WHO 1999] due to congenital or acquired bone marrow failure syndromes.

2.1.2 Receiving active treatment in an attempt to achieve disease remission.

2.1.3 Proven or highly probable fungal or bacterial infection that is unresponsive to appropriate antimicrobial therapy as demonstrated by visible spreading lesions on skin, mucosa or radiological examination [Ascioglu et al 2002].

2.1.4 In whom neutrophil recovery is expected (ANC>0.5x10^9/L) in the near future and / or in whom definitive therapy of curative potential is planned.

2.2 Therapeutic granulocyte transfusions may also be indicated for patients with a known congenital disorder of neutrophil function [Kuijpers et al 1999] regardless of neutrophil count with proven or highly probable fungal or bacterial infection unresponsive to appropriate antimicrobial therapy, demonstrated by visible spreading lesions on skin, mucosa or radiological examination.

2.3 Granulocyte transfusion should not be issued for therapeutic use in:

2.3.1 Patients with bone marrow failure where neutrophil recovery is not anticipated to recur spontaneously and no further active treatment is planned.

2.3.2 Sepsis in the absence of either neutropenia or known neutrophil dysfunction.

2.3.3 Pyrexia of unknown origin (PUO).

2.4 Requests for granulocyte transfusions should be referred to the duty NHSBT consultant.

3. SOURCE OF GRANULOCYTES

3.1 Leucocytes, Buffy Coat, Irradiated: This component MUST be irradiated for all patients: This component will only be used if the component in 3.2 below, (Granulocytes, Pooled, Buffy Coat Derived, in Platelet Additive Solution and Plasma, Irradiated) is not available

3.1.1 Each pack of Leucocytes, Buffy Coat, Irradiated is approximately 50ml in volume, has a haematocrit of 45%, contains 1-2 x10^9 white cells, 90x10^9 platelets and 9.5g of haemoglobin.

3.1.2 These are indicated for children and for adults.

3.1.3 Clinicians should be warned of the haematocrit of leucocytes, buffy coat, irradiated, which will result in a significant rise in the patients’ haematocrit reducing the need for top-up red cell transfusions. Venesection may be needed if given daily to patients who are not heavily red cell dependent. Similarly, ten packs are equivalent to 2.5 adult therapeutic doses of platelets and additional platelet transfusions are therefore less likely to be required.

3.1.4 A dose of ten packs for adults and 10 -20ml / kg for children less than 50kg (to a maximum of 10 packs) is suggested.
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3.2 Granulocytes, Pooled, Buffy Coat Derived, in Platelet Additive Solution and Plasma, Irradiated. This component MUST be irradiated for all patients.

3.2.1 The NHSBT Components Development Laboratory (CDL) has reported good in-vitro functionality of a purer pooled granulocyte component derived from whole blood donations [Bashir et al 2008]. The method involved the addition of platelet additive solution but without the need for hydroxyethyl starch or dextran to sediment red cells during processing. A clinical study undertaken in the UK has demonstrated acceptable in-vivo safety of this component [Massey et al 2012].

3.2.2 As 10 donations are pooled into a final volume of 200-250ml, adults may receive up to two packs or 20 donations. Each pack contains approximately $1 \times 10^{10}$ granulocytes.

3.2.3 Children less than 30Kg should receive 10-20ml/Kg to a maximum of two packs.

3.2.4 As the haematocrit is <20% venesection is unlikely to be required but red cell transfusion requirements may be diminished.

3.2.5 Each pack contains 2.5 adult therapeutic doses of platelets so platelet transfusion requirements will be significantly diminished if not abrogated.

3.2.6 Only small numbers of whole blood donations are collected on Saturdays and Sundays and hence it is very unlikely that Granulocytes, Pooled, Buffy Coat Derived, in Platelet Additive Solution and Plasma, Irradiated or Leucocytes, Buffy Coat, Irradiated will be available on Sundays or Mondays.

3.3 Unstimulated apheresis donations providing an average of $5 \times 10^6$ granulocytes/bag.

3.3.1 These are no longer collected in the UK and are therefore not available.

3.4 Granulocytes Apheresis, Irradiated: Stimulated donations from relatives and friends. This component MUST be irradiated for all patients:

3.4.1 These contain considerably higher numbers of granulocytes, (averaging $6 \times 10^{10}$) leading to more sustained increments.

3.4.2 Donations may only be required 2 or 3 times per week.

3.4.3 The use of GCSF and steroids for stimulation of granulocyte donations from volunteer unrelated donors is not currently permitted in the UK. NHSBT does not administer GCSF or steroids to donors and only provides a service for the collection of granulocytes following donor selection (including weight, blood group, CMV status if appropriate and venous access), counselling and priming at the referral centre.

3.4.4 Stimulated donations should only be used in the context of an approved clinical protocol ensuring informed consent and safe donor selection.

4. GRANULOCYTE STORAGE, RELEASE AND TRANSPORTATION

4.1 Granulocytes are stored at $22+2^\circ\text{C}$ ideally without agitation. If granulocytes are agitated in error this does not preclude their transfusion as there is limited evidence that agitation affects them functionally [McCullough 1980, Sasakawa & Miyamoto 1987].
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4.2 Granulocytes MUST be irradiated to prevent transfusion associated graft versus host disease [Treleaven et al 2011, Bashir and Cardigan 2003].

4.3 All testing for mandatory microbiological markers must be completed before granulocytes are issued for transfusion with the exception of HCV PCR and HTLV testing.

4.4 In view of the residual red cell and plasma content, granulocyte preparations should fulfil the compatibility requirements outlined in section 6.1:

4.5 Granulocytes should be infused as soon as possible after collection. The times of dispatch and infusion should be recorded.

4.6 The product has a 24 hour expiry shelf-life.

5. DOSE ADMINISTRATION & COURSE OF GRANULOCYTE TRANSFUSIONS

5.1 There is currently no consensus on the specified effective dose required. However, larger numbers of cells transfused result in higher increments (in the absence of allo-immunisation).

5.2 Granulocytes should be transfused through a standard red cell giving set. The whole dose should be infused over 1-2 hours. Other than the screen filter present in a red cell giving set no further filter should be used.

5.3 Infusions should be given until one of the following events:

- Clear evidence of endogenous recovery, based on neutrophil count occurs.
- Resolution of infection occurs.
- Clinical deterioration despite a minimum of three days of transfusions.
- Severe reactions to granulocyte transfusions.
- Granulocytes are manufactured specifically on a named patient basis so changes in clinical condition of patient e.g. recovery or death, meaning they are no longer needed should be relayed to NHSBT urgently to allow resources to be appropriately reallocated.

6. COMPATIBILITY TESTING

6.1 In view of the residual red cell and plasma content, granulocyte preparations should fulfil the following compatibility requirements:

a) ABO compatible with the recipient’s plasma and crossmatch compatible by immediate spin (for ABO incompatibility) in those not eligible for electronic issue.

b) ABO compatible (reverse) for ABO antibodies in the donor against recipient antigens i.e low titre for anti-A or B if group O granulocytes are provided for A, B or AB recipients.

c) RhD negative granulocytes should ideally be provided for RhD negative females of childbearing potential who have not formed immune anti-D.

d) More extensive matching or compatibility testing in the presence of red cell antibodies or to prevent their formation is not required. ie IAT crossmatch and attempts to provide granulocytes that are negative for antigens other than ABO and RhD are not required.
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6.2 CMV negative products should only be issued for CMV negative recipients at risk of CMV disease. (See section 7 for potential substitutions).

6.3 HLA compatible apheresis granulocytes should be considered for alloimmunised patients who fulfil criteria 6.3.1 or 6.3.2. Suitable donors who are HLA matched are however unlikely to be available:

6.3.1 Have previously documented refractoriness to platelet transfusions due to HLA antibodies (i.e. responsiveness to HLA matched platelets but refractoriness to random platelets). A trial of unmatched components should be undertaken however in the absence of criterion 6.3.2 in view of the extremely limited component availability.

6.3.2 Who have had severe transfusion reactions as a result of such antibodies (e.g. hypotension, or hypoxia / TRALI).

Neither “Leucocytes, Buffy Coat, Irradiated” nor “Granulocytes, Pooled, Buffy Coat Derived, in Platelet Additive Solution and Plasma, Irradiated”. can be HLA selected.

6.4 It is recommended that the following investigations should be requested on a pre-granulocyte transfusion sample taken from the recipient:

a) HLA typing
b) Anti-HLA class I and II antibody screening

6.5 Antibody screening as defined in 6.4 should be repeated if:

a) Platelet transfusion refractoriness occurs
b) Severe reactions occur (hypoxia / TRALI and/or hypotension)

6.6 HNA antibody screening should also be undertaken if:

a) Severe reactions occur (TRALI and/or hypotension)

6.7 The presence of antibodies alone, in the absence of platelet or granulocyte refractoriness, is not an indication for HLA matched products in view of the logistical difficulties in obtaining such donations and the lack of evidence of benefit from avoiding the implicated antigen in this situation.

6.8 Careful monitoring for refractoriness and reactions such as TRALI should be undertaken both in the presence and absence of incidentally identified antibodies.

7. POTENTIAL SUBSTITUTION STRATEGIES

Alternative options for supplying granulocyte components may be considered for patients where identical blood group donations cannot be obtained, or if the patient cannot tolerate large volumes of fluid.

7.1 Volume:

7.1.1 Granulocytes, Pooled, Buffy Coat Derived, in Platelet Additive Solution and Plasma, Irradiated: One pack for adults or 10ml/Kg for children under 30Kg may be used if the recipient has difficulty tolerating larger volumes or if larger volumes are not available.

7.1.2 Leucocytes, Buffy Coat, Irradiated: Similarly, less than ten donations or less than 10ml/Kg may be transfused for adults or children respectively. These components may also need to be used instead of Granulocytes, Pooled, Buffy Coat Derived, in Platelet Additive Solution and Plasma, Irradiated if the delay in availability of exact multiples of ten donations manufactured into pools leads to a greater risk than providing ten or less unpooled donations.

7.2 ABO blood group compatibility (see table 1 for hierarchy of donation selection):
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7.2.1 Residual red cells in the pack: As stated in section 6 compatibility rules for ABO provision of red cells MUST be complied with for all patients as the haematocrit for all available components is high enough to cause an acute haemolytic transfusion reaction as a result of ABO incompatibility.

7.2.2 Plasma compatibility: If giving donations that may contain ABO lysins against the recipient (the greatest risk being a group O donor to group A recipient) then the component must be negative for high titres of anti-A and / or anti-B as appropriate (HT neg). Only the donor of the resuspending plasma strictly needs to be HT neg for Granulocytes, Pooled, Buffy Coat Derived, in Platelet Additive Solution and Plasma, Irradiated.

Table 1: Hierarchy for selection of granulocytes by ABO blood group

<table>
<thead>
<tr>
<th>Recipient group</th>
<th>1st choice donation</th>
<th>2nd choice donation</th>
<th>3rd choice donation</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>O</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>A</td>
<td>A</td>
<td>O HT neg</td>
<td>N/A</td>
</tr>
<tr>
<td>B</td>
<td>B</td>
<td>O HT neg</td>
<td>N/A</td>
</tr>
<tr>
<td>AB</td>
<td>AB</td>
<td>A (or B) HT neg</td>
<td>O HT neg</td>
</tr>
</tbody>
</table>

7.3. RhD

7.3.1. Recipients with preformed anti-D antibodies,
  First choice RhD negative
  Second choice RhD positive (anti-D prophylaxis is not indicated)
  The risk of haemolysis is low for RhD as a result of the small volumes of red cells present in Granulocytes, Pooled, Buffy Coat Derived, in Platelet Additive Solution and Plasma, Irradiated and in Granulocytes Apheresis, Irradiated. See section 7.4.

7.3.2 RhD negative recipients who do not have preformed anti-D
  a) Females of child bearing potential
     First choice: RhD negative donations.
     Second Choice: RhD positive donations. Consideration would need to be given to giving anti-D prophylaxis. If using D-GAM intramuscularly (or subcutaneously – D-GAM MUST NOT be given intravenously) 125IU/ml per ml of donor red cells infused. If using Rhophylac intramuscularly or intravenously 100IU/ml of RhD positive red cells transfused. To calculate the number of red cells transfused utilise this equation:.
     Volume of donor red cells transfused = (Hct of pack(s)) x (average volume of the pack(s)) x (number of packs transfused).
  b) Males and females who do not have child bearing potential
     First choice RhD negative
     Second choice RhD positive (anti-D prophylaxis is not indicated)

7.4. Other red cell antibodies

There is no need to provide granulocytes from donors who are matched for red cell antigens other than ABO and RhD as outlined in sections 7.1-3 if the recipient has antibodies or to prevent their formation. Acute intravascular haemolysis leading to significant symptoms is unlikely for antibodies other than ABO as a result of the small volumes present in Granulocytes, Pooled, Buffy Coat Derived, in Platelet Additive Solution and Plasma, Irradiated and in Granulocytes Apheresis, Irradiated. More rapid turnover of the red cells present in a granulocyte pack is not an issue as unlike red cell concentrates transfused for anaemia the “contaminating” red cells are not an intentional therapy. Attempts to provide more extensive matching, will lead to delay and potentially to the supply of less optimal transfusion support (eg the increased volume, red cell content and female plasma content of individual buffy coats).

7.5 Cytomegalovirus (CMV)
CMV IgG negative granulocytes should ideally be provided for recipients who are at risk of CMV disease (infants, pregnant women, CMV negative recipients of CMV negative allogeneic bone marrow transplants)

As for red cell antigen negative donations, the risk of failure to supply and morbidity / mortality from bacterial or fungal infection would need to be balanced against a risk of subsequent CMV disease. Discussion between an NHSBT consultant and the consultant looking after the patient would be required if there were inadequate supplies to support the issue of CMV negative components to a patient in the above at risk groups

8. REQUESTING PROCEDURE (SEE FLOW CHART ON PAGE 11)

Requests for granulocyte transfusions should be made in the same manner as other blood components using the Online Blood Ordering System (OBOS). In view of the logistics required to manufacture and supply doses such requests will need to be referred to the duty NHSBT consultant.

8.1 Take a detailed clinical history from the referring clinician to define indications for the request.

8.2 If clear indications for granulocyte transfusion are presented, agree with the referring clinician to provide granulocytes if operationally possible. Advise the referring clinician that their hospital transfusion laboratory should order the granulocytes from NHSBT Hospital Services on the Online Blood Ordering System (OBOS).

8.3 Liaise with the NHSBT Hospital Services department to determine if and when the provision of the component(s) is possible.

8.4 Report back to the referring clinicians on availability of the component(s) and agree start date and duration.

8.5 If a referral is received but there is no clear indication for granulocytes, discuss with referring Consultant or designated deputy.
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PROCEDURE FOR MANAGING REQUESTS FOR GRANULOCYTE CONCENTRATES

A HOSPITAL REQUESTS GRANULOCYTES FOR TRANSFUSION

REFER TO THE DUTY CONSULTANT FOR PATIENTS DURING THE DAY AND OUT OF HOURS

THE DUTY CONSULTANT takes a detailed clinical history from the referring clinician to define indications for request.

Does the case fall into category A or B

A. Satisfies all criteria 1-4
1. Severe neutropenia, defined as ANC < 0.5 \( \times 10^9 \)/L (WHO 1999) due to congenital or acquired bone marrow failure syndromes.
2. Under active treatment in an attempt to achieve disease remission.
3. Proven or highly probable fungal or bacterial infection that is unresponsive to appropriate antimicrobial therapy as demonstrated by visible spreading lesions on skin, mucosa or radiological examination.
4. Neutrophil recovery (to ANC > 0.5 \( \times 10^9 \)/L) is anticipated

B. Satisfies criteria a and b
a) A congenital disorder of neutrophil function regardless of neutrophil count with
b) proven or highly probable fungal or bacterial infection unresponsive to appropriate antimicrobial therapy, demonstrated by visible spreading lesions on skin, mucosa or radiological examination

Agree to provide granulocytes if operationally possible. Advise the referring clinician to place an order with NHSBT Hospital Services via their Hospital Transfusion Laboratory on the Online Blood Ordering System (OBOS).

Choose appropriate granulocyte component

1. Granulocytes, Pooled, Buffy Coat Derived, in Platelet Additive Solution and Plasma, Irradiated: 2 packs (adult) 10-20ml/kg (child)
2. Leucocytes, Buffy Coat, Irradiated only if option 1 is not available (10 packs for an adult 10-20ml/kg for a child)

Discuss with referring Consultant or designated deputy.

Liaise with NHSBT Hospital Services (and Therapeutic Apheresis Services if necessary). Report back to the referring clinicians on the availability of component and agree start date and duration. Request an HLA type and antibody screen.

Yes

No

Granulocytes not provided
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REFERENCES


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Sasakawa S and Miyamoto M Studies on granulocyte preservation III. Effect of agitation on granulocyte concentrates. Transfusion 1987. 27(2): 165-6


