Granulocytes; Buffy Coat; Irradiated products; Platelets Additive Solutions (PAS); When to use them and what is the evidence?

Dr. Aseem K Tiwari
Associate Director, Transfusion Medicine,
Medanta-The Medicity Hospital, Gurgaon

Acknowledgements

- Chiara Cugno, Sara Deola, Perla Filippini, David F. Stroncek and Sergio Rutella. Granulocyte transfusions in children and adults with hematological malignancies: benefits and controversies. *J Transl Med (2015) 13:362*

- Jennie Treleaven, Andrew Gennery, Judith Marsh, Derek Norfolk, Lizanne Page, Anne Parker,* Frank Saran, Jim Thurston and David Webb. Guidelines on the use of irradiated blood components prepared by the British Committee for Standards in Haematology blood transfusion task force. British Journal of Haematology, 152, 35–51

Outline

- Preamble: How are children different from adults?
- Granulocyte Transfusions
  - Indications
  - Mobilization, dose (minimum and optimum)
  - Buffy coat and apheresis, storage
  - Modifications, cross-match and administration
  - Evidence in various indications
  - Cocharne reviews
- Irradiation of prodcuts
  - TA-GvHD, Indications of irradiation
  - Dose; Components that need to be irradiated
  - Labeling and documentation

How are children different from adults?

- Adaptive processes as oxygen delivery decreases with anemia - LIMITED
  - Increased oxygen extraction
  - Increased heart rate and stroke volume
  - Preferential perfusion of head and heart at the expense of splanchnic perfusion
- Hb decline during first few weeks. “physiologic anemia of infancy”/“physiologic anemia of prematurity
- Small Blood Volume
  - Have loss to frequent samplings (Iatrogenic)

How are children different?

- Transfusion volumes and rates for children should be carefully calculated and prescribed in mL, not component units, to minimize dosing errors and reduce the risk of circulatory overload
- In comparison to adult practice there is a relative lack of high-quality research to inform evidence-based guidelines
- Higher incidence of serious adverse events related to transfusion have been reported in children (including identification errors)
- Children transfused in fetal or neonatal life have the longest potential lifespan in which to develop late adverse effects of transfusion

Granulocyte Transfusions

Indications

- Neonates with sepsis
- Patients with chronic granulomatous disease
- Stem cell transplant recipients during pancytopenic phase
- Patients with hematologic malignancies and low neutrophil counts due to chemotherapy

- *Proven or highly probable bacterial or fungal infection*
- *No response to appropriate antimicrobial therapy*
- *Absolute neutropenia (<500 granulocytes/microliter)*
- *A reasonable expectation that the patient will begin producing granulocytes soon*

Mobilization: *G-CSF/G-CSF+ Dexa*

Single-dose G-CSF (5–10 µg/kg) - Neutrophil count increased 6.2- to 7.4-fold over baseline values*

Combination of G-CSF and dexamethasone - 20 donors received oral dexamethasone (8 mg) plus a placebo injection, subcutaneous G-CSF (5 µg/kg) plus placebo capsules, or G-CSF plus dexamethasone. The administration of **G-CSF plus dexamethasone produced the greatest yields and was not associated with increased toxicity as compared with G-CSF alone****

* McCullough J, Clay M, Herr G, Smith J, Stroncek D. Effects of gCSF on potential normal granulocyte donors. Transfusion. 1999;39:1136–40.
** Stroncek DF, Yau YY, Oblitas J, Leitman SF. Administration of G-CSF plus dexamethasone produces greater granulocyte concentrate yields while causing no more donor toxicity than G-CSF alone. Transfusion. 2001;41:1037–44.

Mobilization: *G-CSF+ Dexa*

Prospective study, 52 healthy unrelated volunteers were treated with a single s/c injection of glycosylated G-CSF, lenograstim, at a median dose of 3.1 µg/ kg plus dexamethasone (8 mg orally) or with a median dose of 11.8 µg/kg of G-CSF lenograstim without dexamethasone (n = 23). Mobilization kinetics and leukapheresis yields were similar in the low-dose compared with the high-dose G-CSF group. Donor adverse reactions were of greater clinical significance in donors given high-dose G-CSF alone. The **combination of glycosylated G-CSF and dexamethasone allowed a significant reduction of G-CSF dose and enhanced the tolerability of the mobilization regimen to the donors***

* Heuft HG, Goudeva L, Sel S, Blasczyk R. Equivalent mobilization and collection of granulocytes for transfusion after administration of glycosylated G-CSF (3 microg/kg) plus dexamethasone versus glycosylated G-CSF (12 microg/kg) alone. Transfusion. 2002;42:928–34.

Mobilization: *Sequential Collection*

Sequential granulocyte collections from a single donor given G-CSF daily. This approach has been prospectively evaluated in 76 healthy donors, who were allowed a **maximum of five consecutive donations**. This mobilization schedule translated into a continuing increase of white blood cells and neutrophils, leading to better collection yields. The side effects related to repeat administrations of G-CSF were tolerable, not exceeding WHO grade II status. Bone pain, headache, arthralgia, and myalgia were commonly observed (24 % of the donors), but were transient and responsive to paracetamol*

* Worel N, Kurz M, Peters C, Hocker P. Serial granulocytapheresis under daily administration of rHuG-CSF: effects on peripheral blood counts, collection efficiency, and yield. Transfusion. 2001;41:390–5.

Harvest: *Dose!*

Standard dose is **1.0 x 10^{10}** (that number is the minimum requirement in at least 75% of collections, according to *AABB Standards*) {Unstimulated}*

Desirable dose: Yield of **2.0-4.0 x 10^{10}** granulocytes or more *{Stimulated}*

- *G-CSF is not FDA-approved for use in stimulating donors, so donors should have a formal informed consent prior to undergoing stimulation*
- *UK does not permit G-CSF or steroid stimulation of granulocyte donors that are not family or friends of the patient*

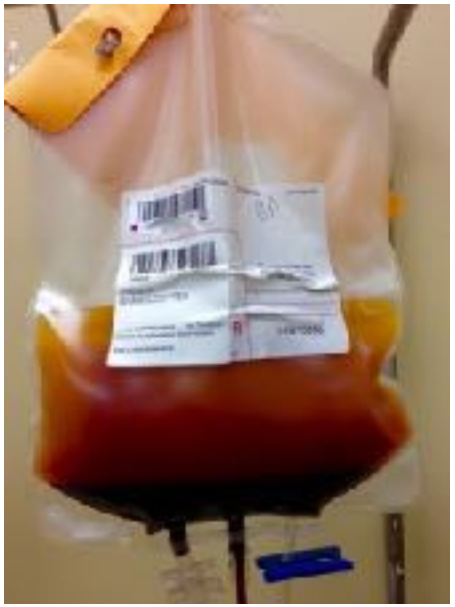
* Circular of Information for the Use of Human Blood and Blood – AABB; https://www.aabb.org/tm/coi/Documents/coi1113.pdf

Harvest: *Buffy-coat pools*

Standard adult granulocyte component can be derived from 10-20 whole blood donations, providing a daily dose of approximately 1-2 × 10^{10} granulocytes. The adverse events in recipients of granulocytes prepared with this approach appear to be comparable to those of recipients of other granulocyte components*

* Massey E, Harding K, Kahan BC, Llewelyn C, Wynn R, Moppett J, Robinson SP, Green A, Lucas G, Sadani D, et al. The granulocytes in neutropenia 1 (GIN 1) study: a safety study of granulocytes collected from whole blood and stored in additive solution and plasma. Transfus Med. 2012;22:277–84..

Harvest: *Physical look!*



Units typically contain between 30 and 50 mL RBC

Storage

High cell counts achieved in granulocyte concentrates may reduce nutrients and lower pH, resulting into neutrophil death. The production of pyrogenic cytokines may also be increased. According to current standards, granulocyte storage should be limited to 24 h. After 2 days of storage in RPMI-1640 medium at 4 °C, only 2–7 % of the granulocytes remain viable*. Infusible solutions to be used in place of autologous plasma have been designed and tested with the aim of improving granulocyte storage. For instance, lactated Ringer's solution or Plasma-Lyte A supplemented with buffers and albumin hold promise as effective and licensable solutions for granulocyte storage **

* Chun H, Cipolone K, Procter J, Stroncek DF. Granulocyte storage and antigen stability. Transfusion. 1999;39:983–90.
** Lightfoot T, Gallelli J, Matsuo K, Kwon SW, Leitman SF, Stroncek DF. Evaluation of solutions for the storage of granulocyte colony-stimulating factor-mobilized granulocyte concentrates. Vox Sang. 2001;80:106–11.

Crosss-match, Irradiation and "NO LEUCODEPLETION"

There are enough RBCs (>2 ml) in each granulocyte product, the donor must be ABO compatible with the recipient, and the unit must be crossmatch-compatible with the recipient*

If the patient has developed anti-HLA antibodies then the donor should be HLA matched or at least HLA compatible with the patient's antibodies

Irradiated

No Leucodepletion

* AABB standards

Evidence – *Children with Neutropenia*

A study assessed feasibility, safety and efficacy of early-onset G-CSF mobilized GTX in an open, single-center, prospective phase II trial in immune-compromised children with neutropenia and severe infections, who failed to respond to broad-spectrum antibiotics . The study utilized granulocytes collected from community donors. Some patients also received G-CSF/GMCSF. GTXs were well tolerated, without any pulmonary transfusion reactions. 25/27 patients cleared their initial infection. All 6 patients with invasive aspergillosis showed clinical and radiological improvement.

Remarkable response rate was probably due to the early initiation of GTX, i.e., after a median infection of 6 days (range 3–18 days), compared with 8 days/12 days in other studies

* Sachs UJ, Reiter A, Walter T, Bein G, Woessmann W. Safety and efficacy of therapeutic early onset granulocyte transfusions in pediatric patients with neutropenia and severe infections. Transfusion. 2006;46:1909–14.

Evidence – *Children with Neutropenia*

In another study of 35 children with high-risk febrile neutropenia or with granulocyte function defects, GTX were given for 3 consecutive days. The mean granulocyte content per concentrate was 2.74×10^{10}. Infection-related survival and overall survival rates were 82 and 77 %, respectively, at day 30*

A 59 % overall survival rate was obtained in a cohort of 32 children, with particularly favorable results in bacterial infections (8/11 patients with documented bacterial infection survived) and fungal infection (4/6 patients with documented fungal invasive infection survived)**

* Atay D, Ozturk G, Akcay A, Yanasik M, Anak S, Devecioglu O. Effect and safety of granulocyte transfusions in pediatric patients with febrile neutropenia or defective granulocyte functions. J Pediatr Hematol Oncol. 2011;33:e220–5.

**Grigull L, Beilken A, Schmid H, Kirschner P, Sykora KW, Linderkamp C, Donnerstag F, Goudeva L, Heuft HG, Welte K. Secondary prophylaxis of invasive fungal infections with combination antifungal therapy and G-CSF-mobilized granulocyte transfusions in three children with hematological malignancies. Support Care Cancer. 2006;14:783–6.

Evidence – *Children with Neutropenia*

Seidel et al. suggested that a tight schedule with daily transfusions of at least 1.4×10^8 granulocytes/kg likely contributed better clinical outcomes. This minimum recommended dose was derived from a Cochrane meta-analysis*. They also reported the effect of daily GTX over at least 5 days containing a minimum of 3×10^8/kg neutrophils per concentrate was able to generate a stable ANC increment, to shorten the duration of neutropenia, and to support the control of infections in neutropenic patients with high-risk infections**

* Stanworth SJ, Massey E, Hyde C, Brunskill S, Lucas G, Navarrete C, Marks DI. Granulocyte transfusions for treating infections in patients with neutropenia or neutrophil dysfunction. Cochrane Database Syst Rev. 2005;(3):CD005339.

**Seidel MG, Minkov M, Witt V, Matthes-Martin S, Potschger U, Worel N, Leitner G, Stary J, Gadner H, Peters C. Granulocyte transfusions in children and young adults: does the dose matter? J Pediatr Hematol Oncol. 2009;31:166–72.

Evidence - *CGD*

Granulocyte transfusion therapy has been used in three patients with chronic granulomatous disease (CGD) and disseminated invasive aspergillosis. Healthy donors were mobilized with 450 μg G-CSF and dexamethasone approximately 12 h before collection. Patients received between 0.4 and 3.0 × 10^9/kg granulocytes. Two out of three patients survived the infectious episode*

* Ikinciogullari A, Dogu F, Solaz N, Reisli I, Kemahli S, Cin S, Babacan E. Granulocyte transfusions in children with chronic granulomatous disease and invasive aspergillosis. Ther Apher Dial. 2005;9:137–41.

Evidence

Patients with hematologic malignancies and low neutrophil counts due to chemotherapy

Granulocyte transfusions from family volunteers were used prior to allogeneic HSCT in three children with poorly controlled bacterial or fungal infections. No transfusion-related reactions and no flares of the infection were observed.

All HSCT procedures were successful*

* Sharon RF, Bierings M, Vrielink H, Versluys B, Boelens JJ. Pre-emptive granulocyte transfusions enable allogeneic hematopoietic stem cell transplantation in pediatric patients with chronic infections. Bone Marrow Transplant. 2006;37:331–

Evidence – *Adverse Events*

Concern for potentially serious pulmonary complications is one of the major limiting factors for the routine use of GTX. Some studies of GTX recipients have documented acute pulmonary transfusion reactions with shortness of breath, dyspnea, hypoxemia, and lung edema. In a Cochrane meta-analysis, adverse events occurred in 15 % of the transfusions that had been collected by apheresis

No reactions occurred in **pre-medicated patients** receiving granulocytes collected by apheresis*

* Stanworth SJ, Massey E, Hyde C, Brunskill S, Lucas G, Navarrete C, Marks DI. Granulocyte transfusions for treating infections in patients with neutropenia or neutrophil dysfunction. Cochrane Database Syst Rev. 2005;(3):CD005339.

Take-Home Message

Although randomized controlled trials are not available in children yet, the current evidence supports the early use of GTX, especially for patients with bacterial infections.

However, patients should be closely monitored for adverse pulmonary transfusion reactions.

Evidence – Cochrane I

Ten randomized clinical trials were identified that assessed the safety and effectiveness of prophylactic transfusions* Eight trials were undertaken in the US, one in Spain and one in the UK. All the studies but one were published between 1978 and 1987. Donors were given either steroids or no form of medication. G-CSF was used in only one trial, published in 2006. Although the summary results for mortality, mortality due to infection and data on episodes of infection failed to reach statistical significance, there were consistent trends in favor of the intervention. When the trials collecting $<1 \times 10^{10}$ granulocytes were excluded, the relative risk ratio was significantly in favor of the intervention.*

* Massey E, Paulus U, Doree C, Stanworth S. Granulocyte transfusions for preventing infections in patients with neutropenia or neutrophil dysfunction. Cochrane Database Syst Rev. 2015;6:CD005341. doi: 10.1002/14651858.CD005341.pub3.

Evidence- Cochrane II

11 trials eligible involving 653 participants. Ten studies included only adults, and two studies included children and adults. Overall, the quality of the evidence was judged to be very low or low across different outcomes according to GRADE methodology. All-cause mortality was reported for nine studies (609 participants) and mortality due to infection was reported for seven studies (398 participants). There was no difference in all-cause mortality measured over 30 days between patients receiving prophylactic granulocyte transfusions and those that did not. Similarly, mortality due to infection over 30 days was not different in patients receiving granulocyte transfusions and in those that did not. In the low-dose granulocyte group ($<1.0 \times 10^{10}$ granulocytes/day), the number of patients with infection was similar in the two patient groups.

* Metzendorf MI, Schulz M, Braun V. All information is not equal: using the literature databases PubMed and the Cochrane Library for identifying the evidence on granulocyte transfusion therapy. Transfus Med Hemother. 2014;41:364–74.

Evidence- Cochrane II

However, the number of patients with infection was lower among recipients of intermediate doses of granulocytes ($1.0–4.0 \times 10^{10}$/day). Also, the number of patients with bacteremia and fungemia was lower among recipients of prophylactic granulocyte transfusions. This systematic review concluded that there is low-grade evidence that prophylactic granulocyte transfusions decrease the risk of bacteremia or fungemia. Similarly, there is low-grade evidence that the effect of prophylactic granulocyte transfusions is dose-dependent, with doses of at least 1.0×10^{10}/day being more effective at decreasing the risk of infection. Collectively, there is insufficient evidence to determine any difference in mortality rates due to infection, all-cause mortality, or serious adverse events.

* Estcourt LJ, Stanworth S, Doree C, Blanco P, Hopewell S, Trivella M, Massey E. Granulocyte transfusions for preventing infections in people with neutropenia or neutrophil dysfunction. Cochrane Database Syst Rev. 2015;6:CD005341.

Evidence- Cochrane III

Eight randomized trials, published between 1975 and 1984. 8 in US, one in Canada; one in Switzerland and multicenter European study. Overall, 149 patients were available for analysis in intervention arm. In these trials no granulocytes were collected after administration of G-CSF and/or steroids. Method of granulocyte procurement differed, being filtration leukapheresis in 3 studies, discontinuous flow centrifugation in 2 and continuous flow in remaining 3. Evidence from eight randomized clinical trials (RCTs) was considered to be inconclusive to support or refute use of granulocyte transfusions for treatment of severe infections in neutropenic patients. Although statistical heterogeneity and clinical diversity of 8 studies may have affected clinical outcome, there may be a survival benefit for patients administered >1 × 10^{10} granulocytes.

* Stanworth SJ, Massey E, Hyde C, Brunskill S, Lucas G, Navarrete C, Marks DI. Granulocyte transfusions for treating infections in patients with neutropenia or neutrophil dysfunction. Cochrane Database Syst Rev. 2005;(3):CD005339.

Take-Home Message

Granulocyte transfusions decrease the risk of bacteremia or fungemia

Doses of at least 1.0×10^{10}/day is more effective at decreasing the risk of infection

There may be a survival benefit for patients administered $>1 \times 10^{10}$ granulocytes

Irradiated Components

Transfusion Associated -GvHD

- TA-GvHD is a potential complication of transfusion of any blood component containing viable T lymphocytes when there is disparity in the histocompatibility antigens between donor and recipient
- TA-GvHD is characterized by profound marrow hypoplasia and mortality in excess of 90%
- There is a particular risk of TA-GvHD when the donor and patient share an HLA haplotype, as occurs within families

Irradiated Blood Components: *Indications!*

- All blood for intrauterine transfusion (IUT) should be irradiated

- Blood for neonatal exchange transfusion (ET) must be irradiated if there has been a previous IUT or if the donation comes from a first- or second-degree relative. For other neonatal ET cases, irradiation is recommended. Blood should be transfused within 24 h of irradiation and, in any case, by 5 d or less from collection
- It is not necessary to irradiate red cells for routine 'top-up' transfusions of premature or term infants unless either there has been a previous IUT, in which case irradiated components should be administered until 6 months after the expected delivery date (40 weeks gestation),

Treleavenet al. Guidelines on the use of irradiated blood components prepared by the British Committee for Standards in Haematology blood transfusion task force. British Journal of Haematology. 2010; 152:35–51

Prevention: *Dose*

- The minimum dose achieved in the irradiation volume should be 25 Gy, with no part receiving more than 50 Gy [BCSH Guidelines 2010*]
- The American Association of Blood Banks (AABB) recommends a dose of 25 Gy to the central area of the component with no portion receiving <15 Gy (AABB 2014**)

*Treleaven et al. Guidelines on the use of irradiated blood components prepared by the British Committee for Standards in Haematology blood transfusion task force. British Journal of Haematology. 2010; 152:35–51

** *Larry J. Dumont, Mona Papari, Colleen A. Aronson, Deborah F. Dumont.* Whole-Blood Collection and Component Processing In: Mark K. Fung, Brenda J. Grossman, Christopher D. Hillyer, Connie M. Westhoff, editors. Technical Manual, AABB. 18th edition. Bethesda, MD. 2014

Components: *Irradiation*

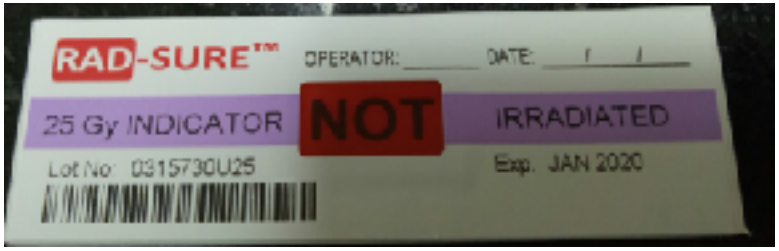
- Red cells may be irradiated at any time up to 14 d after collection, and thereafter stored for a further 14 d from irradiation. Where the patient is at particular risk from hyperkalemia, e.g. intrauterine or neonatal exchange transfusion, it is recommended that red cells be transfused within 24 h of irradiation or that the cells are washed
- Platelets can be irradiated at any stage during storage and can thereafter be stored up to their normal shelf life after collection
- All granulocytes should be irradiated before issue and transfused ASAP
- It is not necessary to irradiate FFP, cryoprecipitate, fractionated plasma or cryopreserved red cells after de-glycerolization.

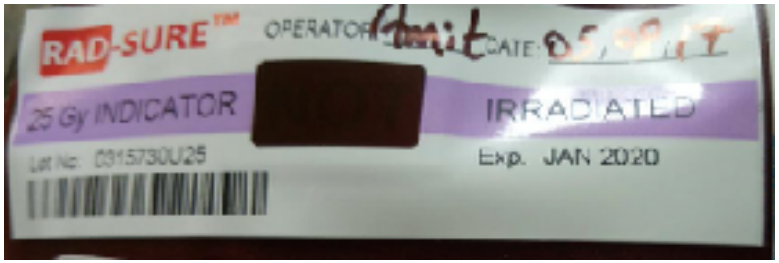
Treleaven et al. Guidelines on the use of irradiated blood components prepared by the British Committee for Standards in Haematology blood transfusion task force. British Journal of Haematology. 2010; 152:35–51

Labeling and Documentation

- Irradiated components not used for the intended recipient can safely be returned to stock to be used for recipients who do not require irradiated components with appropriate reduction in shelf-life

- All irradiated units should be labeled as such, using an approved bar code label. Each unit should be monitored using a radiation-sensitive device, and the result should be permanently recorded, manually or by computer.





Treleaven et al. Guidelines on the use of irradiated blood components prepared by the British Committee for Standards in Haematology blood transfusion task force. British Journal of Haematology. 2010; 152:35–51

Summary

Overall, granulocyte transfusions remain an important therapeutic modality in patients with difficult-to-treat opportunistic infections, especially as a bridge towards spontaneous recovery of neutrophil counts in patients who achieve remission of their underlying disease

There is a risk of TA-GVHD in several pediatric situations like IUT/ET and components receiving 25 G irradiation at the center can prevent this disease which is fatal otherwise


Thank You