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


Outline

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• Irradiation of products
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  – Dose; Components that need to be irradiated
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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**

** 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*

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.

Harvest: Dose!

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

Desirable dose: Yield of $2.0-4.0 \times 10^{10}$ 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

Harvest: *Buffy-coat pools*

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

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**

Cross-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.

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**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.*

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 $2.74 \times 10^{10}$. 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).

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Evidence - *Children with Neutropenia*

Seidel et al. suggested that a tight schedule with daily transfusions of at least $1.4 \times 10^8$ 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 $3 \times 10^8$/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**.


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 × 10⁹/kg granulocytes. Two out of three patients survived the infectious episode.

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.*

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.
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.*

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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 × 10^{10} granulocytes/day), the number of patients with infection was similar in the two patient groups.

However, the number of patients with infection was lower among recipients of intermediate doses of granulocytes (1.0–4.0 × 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.

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 \( >1 \times 10^{10} \) granulocytes.

Granulocyte transfusions decrease the risk of bacteremia or fungemia

Doses of at least $1.0 \times 10^{10}$/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 days 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).

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**)


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.

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.

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.
Based on available evidence, institution-specific protocols should screen for high-risk patients, as blood conservation interventions are likely to be most productive for this high-risk subset. Available evidence-based blood conservation techniques include (1) drugs that increase preoperative blood volume (eg, erythropoietin) or decrease postoperative bleeding (eg, antifibrinolytics), (2) devices that conserve blood (eg, intraoperative blood salvage and blood sparing interventions), (3) interventions that protect the patient's own blood from the stress of operation (eg, autologous predonation and normovolemic hemodilution), (4) consensus, institution-specific blood transfusion algorithms supplemented with point-of-care testing, and most importantly, (5) a multimodality approach to blood conservation combining all of the above.