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Granulocyte transfusions in neutropaenic children: A systematic review of the literature

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ABSTRACT

Background: Granulocyte transfusions (GTX) have been used for decades in paediatric neutropaenic patients, but uncertainty remains regarding their effectiveness. We reviewed all the paediatric data available on GTX, to gain a insight in to the indications for use, favourable effects and side effects in patients and donors.

Methods: A comprehensive search was done in MEDLINE, EMBASE, LILACS and CENTRAL (1966 until 2006). All studies including children (1–18 years) who received GTX were included.

Results: A total of 66 observational studies were included:Seven using prophylactic and 59 therapeutic GTX. Of the therapeutic studies 55 reported a proven sepsis caused by Gram-negative bacteria (34%) or fungal disease (48%) as the indication for GTX. Concerning effectiveness 70% survival was reported, but no controlled studies were identified. Side effects were mentioned in 27 studies including mild respiratory symptoms, allergic reactions and infection related complications (CMV). Side effects in the donor were mainly flu-like illness.

Discussion: In this first review covering 30 years of experience on the use of GTX in children, we found no randomised evidence showing a positive benefit risk ratio. The available case reports and cohort studies alert us as to the potential benefits and harms of the use of GTX in neutropaenic children and provides the basis for a well designed trial in children.

1. Introduction

Improvement has been made over the past 30 years in cure rates for paediatric oncology patients. This is now estimated around 70%.[1] With the intensification of chemotherapy, the need for adequate supportive care is of utmost importance in maintaining this survival rate. As therapy becomes more intense, infectious complications play a major role. Neutropenia, defined as a neutrophil count of less than 500 cells/μL, is one of the most frequent side effects of childhood cancer treatment. Already in 1966, Bodey et al. showed that the risk of infections increased rapidly if the neutrophil count dropped below 500 cells/μL and a neutrophil count of less than 100 cells/μL increased the chance of severe infections.[2] Nowadays, of all neutropaenic patients with fever, 12–17% will have a proven bacteraemia or fungaemia.[3] Morbidity due to infections in paediatric oncology patients approaches 30%, and mortality approaches 1%.[4]

Although antibacterials are generally effective, there is a subgroup of patients who do not respond and whose clinical condition deteriorates. For control of infections, granulocytes are crucial. Granulocyte transfusions (GTX) seem a logical and

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potentially effective solution to prevent or overcome longstanding neutropenia and its associated morbidity. GTX have been given to patients as long as 70 years ago.[5] Because of the adverse effects, especially pulmonary reactions and the lack of proven efficacy, GTX almost disappeared from clinical practice for several years. Due to improvement in technology, the interest in GTX has been revived. Three aspects are important, the dose of granulocytes obtained by leukapheresis, the efficiency of the collection procedure, and the donor's neutrophil count.

Granulocytes are now routinely collected by continuous flow centrifugation leukapheresis, and for acceleration of red cell sedimentation, increasing the efficiency of separation a starch and citrated anticoagulant are added.[6] With the availability of recombinant granulocyte-colony-stimulating factor (G-CSF) combined with corticosteroids (oral dexamethasone) higher mean granulocyte yields can be achieved in healthy donors. The granulocyte yield increased 2–10 times compared with the granulocyte yield of controls.[7,8] In addition, after preparation of the donor with G-CSF, the granulocytes showed a better yield and a prolonged half-life of the cells transfused into the recipient,[9] potentially reducing the number of transfusions needed. Side effects were reduced by introducing pre-collection screening of the donor for viral infections (hepatitis, CMV, HIV, EBV). In some critical cases, HLA-cross matching of the donor and the recipient resulted in less sensitisation for other blood products or granulocyte alloimmunisation and reduced the complications of graft-versus-host disease (GVHD).[10,11]

Two meta-analyses were performed in the nineties,[12,13] retrieving studies on GTX published between 1970 and 1994. Both studies concluded that GTX were effective for treatment of severe infection if there was a low survival rate of the controls (<40%), if an adequate number of granulocytes was transfused ($>10\times10^9$), and if compatibility of granulocytes was assessed prior to transfusion. However, all the GTX studies performed previously differ in many aspects: the variety of patients included, indications for use, supportive care measures, granulocyte collection procedures and transfusion methods. This makes it very difficult to interpret the data.

Two Cochrane reviews have recently been published.[14,15] In the review of Mohan et al.[14] 4 RCT's were analysed including 44 neonates. Three trials using a small number of patients randomised patients to GTX or no transfusion and one study randomised to GTX or immunoglobulin. Metaanalysis was possible but showed a high percentage of heterogeneity between the trials. The results showed no evidence for use of GTX in septic neonates. All studies transfused a relatively low dose with a mean of $0.3–1.0\times10^9$/kg. In these studies, 4% pulmonary complications were recorded. Stanworth et al.[15] included 8 RCT's; 4 trials included children but the paediatric data were not presented separately. In this review, all but the neonatal RCT's were considered with a total of 310 patient episodes. The relative risk (RR) for mortality extracted from 6 studies was 0.64, but if only studies were included which administered granulocyte counts $>10\times10^9$, mortality was significantly reduced (RR = 0.37).

To gain an insight into the indications for use, the possible favourable effects of GTX in children and the Side effects for both patient and donor, we reviewed all published paediatric data available on its use.

2. Patients and methods

2.1. Identification of studies

All relevant literature was searched for, regardless of language or publication status. An internet search was done using the search terms; neutropenia, neutrophil, granulocyte and transfusions. Our search was not limited to children aged 1 year to 18 years, as it was realised that many adult studies included children in their data. If paediatric data within larger studies could be separately analysed they were included in our review. The databases searched were the Cochrane Central Register of Controlled Studies (CENTRAL), published in The Cochrane Library (Issue 4, 2006) MEDLINE (1966 to August 2006), EMBASE (1980 to August 2006), LILACS (1982 to August 2006) and The Web of Science (until August 2006).

2.2. Inclusion/exclusion criteria

We included randomised and quasi-randomised controlled studies, phase II trials, cohort, case–control studies, case reports and data from abstracts that included children aged 1 year to 18 years with a malignancy or immunological disorder, neutropenia and evidence of infection (proven bacteraemia, proven fungal sepsis or clinical strong suspicion) or children having an increased chance for infection. Studies using GTX from all sources of granulocytes and using all different methods of collection both for therapeutic or prophylactic purpose were included. We excluded studies giving GTX to neonates, as this was covered in a separate Cochrane review.[14]

2.3. Data extraction and outcome measures

Two reviewers (MvdW, TK) independently abstracted the following data: type of study, year of publication, characteristics of the patients, including age and type of malignancy, the number of patients with bacteraemia, the number of patients with fungaemia and the number of patients with a strong clinical suspicion of infection. The number of days of neutropenia (granulocytes $\leqslant$500 cells/μL), the mean number of granulocytes transfused, the response of the infectious episode (survival or death) were collected. Side effects in the patient such as TRALI (transfusion related acute lung injury), the insidious onset of pulmonary insufficiency manifested by severe dyspnoea, hypoxia and radiographical evidence of pulmonary oedema with normal cardiac function,[16] platelet or erythrocyte sensitisation, allergic reaction to granulocyte concentrates, and transmission of infections (HIV, hepatitis B or C virus CMV, EBV) were recorded. Data recorded on the donor were stimulation of the donor using G-CSF, corticosteroids, or both, and side effects seen in the donor. The method of collection of granulocytes was documented. Disagreements with regard to the correct data to be extracted were resolved between the two reviewers by discussion.

2.4. Data analysis

The data were summarised in a descriptive analysis.

3. Results

3.1. Identification of studies

Our literature search revealed 468 eligible studies (Fig. 1). Of those, 349 studies were not included because these were studies on growth factors including only adult data and studies on transfusions other than GTX. A total of 119 articles were retrieved. Of these, 53 did not fulfil the inclusion criteria because these combined both paediatric and adult cohorts and the data pertaining to the paediatric population were not clear. Therefore, 66 studies were included. Of these, 38 were case reports, 23 cohort studies, 1 randomised trial not controlled (prophylactic GTX), 2 abstracts, and 2 phase I/II trials (see Tables 1–5).

3.2. Study characteristics

We included 52 retrospective studies, and 14 prospective studies. There were 7 studies using prophylactic GTX. Of these, 4 were retrospective and 3 prospective. There were 59 studies using therapeutic GTX. Of these, 48 were retrospective and 11 prospective.

Forty-four studies included oncology patients (66.7%), 16 studies included patients with a granulocyte dysfunction (i.e. chronic granulomatous disease (CGD)) (24.3%) and 6

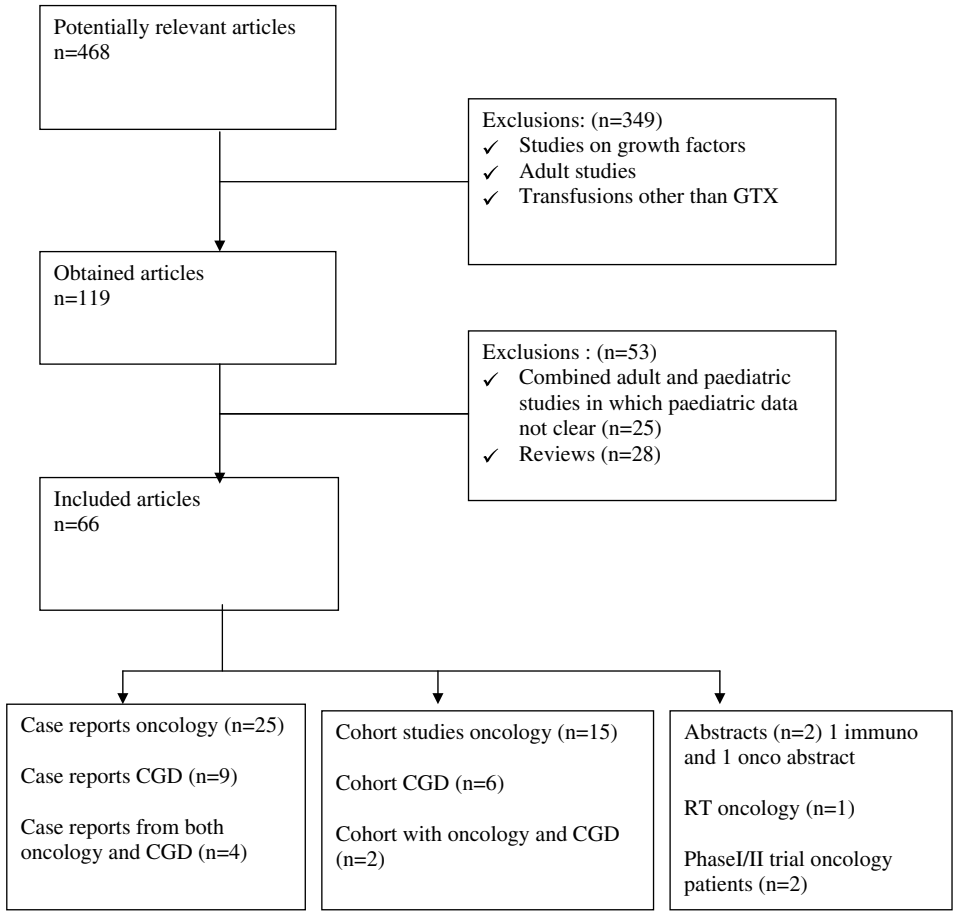

Fig. 1 – Breakdown of articles as found in the search.

Table 1 – Reports <1984 use of granulocyte transfusions in immunologic disorders

Study	Patients (#)	Organism	Mean # GTX (granulocytes)	Donor	Side effects	Outcome
Ippolito et al.[26]	CGD (2)	Fungal	13	Not stimulated		1 Survival, 1 death
Pedersen et al.[27]	CGD (1)	PCP	12 (3.8×10^9 total)	Not stimulated	Sensibilisation	1 Survival
Chusid et al.[28]	CGD (1)	Bacterial (Gram-negative)	2	Steroids		1 Survival
Cohen et al.[29]	CGD (8)	Fungal	8	Not stimulated	Transmission of infection	6 Survival, 2 death
Yomtovian et al.[30]	CGD (1)	Bacterial (Gram-positive)	13 (9×10^9 total)	Not stimulated		1 Survival

CGD = chronic granulomatous disease, PCP = pneumocystis carinii pneumonia.

Table 2 – Reports <1984 use of granulocyte transfusions in oncological disorders

Study	Patients (#)	Organism	Mean # GTX (granulocytes)	Donor	Side effects	Outcome
Pole et al.[31]	Haematological (30)	Bacterial (Gram-positive, $n = 12$; Gram-negative, $n = 10$) fungal ($n = 2$)	20×10^9 + HES, 7×10^9 – HES	Not stimulated	Pulmonary (3)	25 Survival, 5 death
Schmitz-Valkenberg et al.[32]	Haematological (1)	Bacterial	2 (23×10^9 total)	Not stimulated		1 Survival
Alavi et al.[33]	Haematological (10)	Bacterial (Gram-negative, $n = 4$)	3–18 ($33 \times 10^9/m^2/d$)	Steroids	Allergic reactions (3); fever (1)	5 Survival, 1 death
Salfner et al.[32]	Haematological (1)	Pneumonia	2 (65×10^9)	Steroids	Allergic reaction (1)	1 Death
Rosen et al.[34]	Haematological (1)	Bacterial (Gram-positive, $n = 1$; Gram-negative, $n = 1$)	4	Steroids		1 Death
Berkman et al.[35]	Haematological (28)	Bacterial	($21 \pm 1.3 \times 10^9/m^2/d$)	Not stimulated		25 Survival, 3 death
Fernandez et al.[36]	Haematological (7)	Bacterial ($n = 5$) fungal ($n = 2$)	Range 6–25 ($1–5 \times 10^9/m^2/d$)	Steroids		5 Survival, 2 death
Pflieger et al.[37]	Haematological (1)	Bacterial (Gram-negative)	21 ($63 \times 10^9/m^2/d$)	Not stimulated		1 Death
Estes et al.[38]	Haematological (1)	Fungal	1 (60×10^9 total)	Not stimulated		1 Death
Ritchey et al.[39]	Haematological (5)	Bacterial (Gram-positive, $n = 3$; Gram-negative, $n = 1$)	7 ($4–20 \times 10^9$ total)	Not stimulated		5 Survival
Dana et al.[40]	Haematological (1)	Fungal	1 (13×10^9 total)		Dyspnoea during GTX	1 Death
Pflieger et al.[41]	BMT (15)	Bacterial (Gram-negative, $n = 5$) fungal ($n = 1$)	5 ($47 \times 10^9/m^2/d$)	Steroids		14 Survival, 1 death
Graubner et al.[42]	Haematological (10)	Bacterial Gram-negative ($n = 3$)	7, range 6–12... (21×10^9 total)	Steroids		7 Survival, 3 death
Kulkarni et al.[43]	Haematological (1)	Fungal ($n = 1$)	5			1 survival
Winton et al.[44]	Haematological (20)		(16×10^9 total)	Steroids		14 Survival, 6 death
Schmidmeier et al.[45]	Lymphoma (1)		($10–15 \times 10^9$ total)	Not stimulated	Allergic reaction ($n = 2$)	1 Death

Table 3 – Reports >1984 use of granulocyte transfusions in immune disorders

Study	Patients (#)	Organism	Mean # GTX (granulocytes)	Donor	Side effects	Outcome
Fanconi et al.[46]	CGD (2)	Bacterial (Gram-positive, $n = 1$) fungal ($n = 1$)	5 (4×10^9 total)	Not stimulated		2 Survival
Depalma et al.[47]	CGD (1)		46 (16×10^9 total)	steroids		1 Survival
Drakos et al.[48]	Aplastic anaemia (1)	fungal ($n = 1$)		Not stimulated		1 Death
Lekstrom-Himes et al.[49]	CGD (1)	Bacterial (Gram-positive)	8		Allergic reaction	1 Survival
Stroncek et al.[50]	CGD (11)		8 (20×10^9 total)	Steroids and G-CSF	Pulmonary (7) & allergic reaction (11)	
Van Planta et al.[51]	CGD (1)	Bacterial (Gram-positive)	8 (49×10^9 total)	G-CSF		1 Survival
Ozsahin et al.[52]	CGD (1)	Bacterial (Gram-negative) & fungal	4 (30×10^9 total)	G-CSF		1 Survival
Van't Hek et al.[53]	CGD (1)	Fungal	5			1 Survival
Girmenia et al.[54]	Aplastic anaemia (1)	Fungal	39 (2×10^9/kg/d) (50×10^9/m^2/d)	G-CSF		1 death
Notarangelo et al.[55]	CGD (1)	Fungal	7	?	Headache, fever	1 Survival
Ikinciogullari et al.[56]	CGD (3)	fungal	8 (0.4–3 $\times 10^9$/kg/d)	steroids and G-CSF		2 Survival, 1 death

CGD = chronic granulomatous disease, GCSF = granulocyte colony stimulating factor.

Table 4 – Reports >1984 use of granulocyte transfusions in combined immune and oncology disorders

Study	Patients (#)	Organisms	Mean # GTX (granulocytes)	Donor	Side effects	Outcome
Saarinen et al.[57]	Haematological (9), SAA (1)	Bacterial (Gram-positive, $n = 4$; Gram-negative, $n = 3$) fungal ($n = 3$)	38 (59 $\times 10^9$ total) (0.1–1 $\times 10^9$/kg/d)	G-CSF	None	10 Survival
Parco et al.[58]	Haematological (3) thalassaemia (1)	Bacterial (Gram-negative, $n = 2$) fungal ($n = 1$)	8 (40×10^9 total)	G-CSF		3 Survival
De Montalembert[59]	BMT (41), other (4)		4 (0.9×10^9/kg/d)	Steroids	Pulmonary (1)	26 Survival, ? death
Grigull et al.[60]	Haematological (3) SAA (1)	Fungal ($n = 2$)	7.5 (50×10^9 total) (1.5×10^9/kg/d)	G-CSF	Pulmonary (1)	2 Survival, 2 death
Lemau de Talance et al.[61]	Haematological (4) CGD (7)	Fungal ($n = 8$)	8 (30×10^9 total)	Steroids and G-CSF		10 Survival, 1 death
Sharon et al.[62]	CGD (1) Blackfan-Diamond (1) ALL (1)	Bacterial (Gram-positive; $n = 1$) fungal ($n = 2$)	9 (5×10^{10}/m^2/d)	Steroids and G-CSF	GvHD	3 Survival

BMT = bone marrow transplant, CGD = chronic granulomatous disease, GCSF = granulocyte colony stimulating factor, SAA = severe aplastic anaemia.

Table 5 – Reports >1984 use of granulocyte transfusions in oncology disorders

Study	Patients (#)	Organisms	Mean # GTX (granulocytes)	Donor	Side effects	Outcome
Yamada et al.[63]	haematological (7)	?	3, range 2–5 (13.6 ± $5 \times 10^9/m^2/d$)	?	40% Unexpected fever, no serious complications	?
Sciorelli et al.[64]	Haematological (30)	?	3 (19×10^9 total)	Steroids		27 Survival 3 death
Dutcher et al.[65]	Haematological (1)	Fungal ($n = 1$)	4 (25×10^9 total)	Steroids		1 Survival
Engelhard et al.[66]	Haematological (2)	Bacterial (Gram-negative, $n = 2$)	3.5 (0.5×10^9/kg/d)	Not stimulated		2 Survival
Barrios et al.[67]	BMT (1)	Fungal ($n = 1$)				1 Survival
Angel et al.[68]	Haematological (16)	Bacterial (Gram-negative, $n = 16$)	2			11 Survival 5 death
Duncan et al.[69]	Haematological (2)	Bacterial (Gram-negative, $n = 3$)				2 Survival
Morrison et al.[70]	BMT (1)	Fungal ($n = 1$)	3			1 Survival
Bhatia et al.[71]	BMT (50)	Fungal ($n = 50$)	14, Range 4–20... (10×10^9 total)	Steroids and G-CSF	Pulmonary	17 Survival 33 death
Rabodonirina[72]	Haematological (6)	Fungal ($n = 6$)				4 Survival 2 death
Murphy et al.[73]	Haematological (1)	Bacterial (Gram-negative, $n = 1$)		G-CSF	None	1 Survival
De Silvestro et al.[74]	Haematological (5)		8 (47×10^9 total)			4 Survival 1 death
Bielorai et al.[75]	Haematological (1)	Bacterial (Gram-positive, $n = 1$)	(48–68 $\times 10^9$ total)	G-CSF		1 Survival
Price et al.[8]	BMT (4)	Bacterial (Gram-negative, $n = 1$) fungal ($n = 4$)	12	Steroids and G-CSF	Chills (37%), temp rise (32%) and itching (11%)	1 Survival 3 death
Lee et al.[76]	Haematological (2)	Bacterial (Gram-negative, $n = 2$)	2 (66×10^9 total)	Steroids and G-CSF	Pulmonary	1 Survival 1 death
Johnston et al.[77]	Haematological (4)	Bacterial (Gram-negative, $n = 3$) fungal ($n = 1$)	5	Steroids and G-CSF		3 Survival 1 death
Illerhaus et al.[78]	Haematological (3)	Bacterial (Gram-negative, $n = 1$) fungal ($n = 1$)	5 (26×10^9 total)	G-CSF	Infection (CMV)	2 Survival 1 death
Vij et al.[79]	BMT (2)		2	G-CSF	Infection (CMV)	
Cesaro et al.[80]	Haematological (13)	Bacterial (Gram-positive, $n = 5$; Gram-negative, $n = 8$) fungal ($n = 4$)	4 (52×10^9 total)	G-CSF	Pulmonary	7 Survival 6 death
Lin et al.[81]	Haematological (1)	Bacterial (Gram-negative, $n = 1$)	10 (32×10^9 total)	G-CSF		1 Survival
Lee et al.[82]	Haematological (32)	Bacterial (Gram-positive, $n = 12$; Gram-negative, $n = 18$) fungal ($n = 15$)	4 (76–92 $\times 10^9$ total)	Steroids and G-CSF	Pulmonary	10 Survival 1 death
Yoshihara et al.[83]	Haematological (2)	Bacterial ($n = 2$)	4 (0.7–2.1 $\times 10^9$/kg/d)	Steroids and G-CSF		2 Survival
Meyer-Koenig et al.[84]	Haematological (1)	Bacterial (Gram-negative) & fungal	3	G-CSF	CMV	1 death
Ansari et al.[85]	Haematological (1)	Fungal ($n = 1$)	?	Steroids and G-CSF		1 Survival
Grigull et al.[86]	Haematological (3)	Fungal ($n = 3$)	9, range 6–15	G-CSF	Fever & chills after 5 GTX	3 Survival
Grigull et al.[87]	Haematological (32)	Bacterial (Gram-positive, $n = 6$; Gram-negative, $n = 3$) fungal ($n = 6$)	3, range 1–19 (2×10^9/kg/d)	Steroids and G-CSF	Small % fever, resp distress, hypotension and erythema	13 Death (8 because of infection)
Kikuta et al.[88]	Haematological (13)	Bacterial (Gram-positive, $n = 3$; Gram-negative, $n = 8$) fungal ($n = 2$)	2, range 1–4 (0.6×10^9/kg/d)	G-CSF	Transient hypoxia ($n = 2$)	9 Survival, 2 death
Sachs et al.[89]	Haematological (26) aplastic anaemia (1)	Bacterial (Gram-positive, $n = 2$; Gram-negative, $n = 3$) fungal ($n = 7$)	2 (0.8×10^9/kg/d)	Steroids and G-CSF	Minor transfusion reactions (2)	25 Survival

CMV = cytomegalovirus.

Table 6 – Number of patients recorded with infection

	Therapeutic GTX-onco 37 studies	Therapeutic GTX-imm. 13 studies	Therapeutic GTX combined 5 studies	Total therapeutic GRX	Prophylactic GTX-onco 3 studies	Prophylactic GTX-imm 1 study	Prophylactic GTX-combined 0 studies	Total prophylactic GTX
Gram positive sepsis	44 Patients	5 Patients	4 Patients	53 Patients	0 Patients	0 Patients	0 Patients	0 Patients
Gram negative sepsis	97 Patients	1 Patient	5 Patients	103 Patients	4 Patients	1 Patient	0 Patients	5 Patients
Fungal	108 Patients	19 Patients	14 Patients	141 Patients	3 Patients	1 Patient	0 Patients	4 Patients

studies combined these patients (9%). In the 44 oncology studies and the 6 combined studies, a total of 456 patients were included of whom the majority were haematological patients ($n = 340$; 74.5%), and all other patients were allogeneic bone marrow transplant (BMT) patients ($n = 116$; 25.4%). In the 16 studies with CGD patients and in the 6 combined studies 54 patients were included.

Fifty-nine studies reported the exact age of the included patients (mean age of 8.9 years, range 2–17 years), and 7 studies reported age as <18 years.

3.3. *Infections*

In the 59 studies using GTX therapeutically, 55 studies reported a proven sepsis. There were 53 patients (18%) who received granulocytes because of a Gram-positive bacteraemia, 103 patients (34%) received GTX because of a Gram-negative bacteraemia and 141 patients (48%) received this for proven fungal disease. In the 7 studies using prophylactic granulocyte transfusions 4 studies reported a proven sepsis, 5 patients had a previous Gram-negative bacteraemia, and 4 patients had previous fungal disease. The GTX were used as secondary prophylaxis.

The numbers of oncology and immunology patients reported with a proven sepsis is reported in Table 6. Gram negative infections and fungal infections were mainly recorded a as cause for intervening with GTX; however Gram positive infections were also recorded. All these studies reported that the patient's condition did not improve on adequate antibiotic treatment and adequate supportive care management. These Gram positive infections were for example *Staph. aureus* hepatic abcesses leading to a sepsis, or *Streptococci* infections leading to an ARDS. In all described oncology patients with a proven sepsis the mean duration of granulocytopaenia was 13 days (range: 2–30 days).

3.4. *Granulocyte transfusions*

In the studies using therapeutic GTX, a mean number of 8.7 granulocyte concentrates were administered (range: 1–46). In the studies using prophylactic GTX the mean number was 4.8 (range: 2–8). Selecting only the paediatric oncology patients receiving therapeutic GTX, a mean of 5.4 transfusions were administered (range: 1–21).

The mean number of granulocytes transfused in all therapeutic GTX studies was 32×10^9/L ($2–82 \times 10^9$/L), which is a mean of 2×10^9/kg/day (range: $0.5–7.0 \times 10^9$/kg/day). The mean number of granulocytes transfused as prophylactic treatment was 33×10^9/L ($22–46 \times 10^9$/L) which is a mean of 1.3×10^9/kg/day (range: $0.9–1.8 \times 10^9$/kg/day).

3.5. *Donor*

Data on the donor were reported in 51 (45 therapeutic studies on GTX and 6 prophylactic use of GTX) out of 66 studies. Out of 45 studies using therapeutic GTX 20 (44.4%) reported the use of related donors, 24 (53.6%) used unrelated donors, and one trial used both. Of these donors 23.4% were stimulated with G-CSF, 19.2% stimulated with steroids, 34% were not

stimulated and 23.4% were stimulated with both corticosteroids and G-CSF.

There was a significant difference in the yield of granulocytes if the donors were not stimulated or if they were stimulated with both G-CSF and steroids (mean: $18.5 \pm 16 \times 10^9$/L *versus* $58 \pm 26 \times 10^9$/L, respectively; $p = 0.003$). The difference between G-CSF stimulated only and steroid-stimulated only was not significant (mean: $42 \pm 13 \times 10^9$/L *versus* $30 \pm 17 \times 10^9$/L, respectively; $p = 0.132$).

In the studies using prophylactic GTX, data from the donor were known in 6 out of 7 studies. Three studies used related donors, and three studies used unrelated donors. Of these 60% were stimulated with G-CSF and 40% were stimulated with steroids without any significant difference in yield.

3.6. Graft handling

To increase the granulocyte yield various methods have been used. The use of 6% hydroxyethyl starch (HES) infused continuously during the granulocyte collection was reported in 31 studies out of 66 (67.3%). The procedures used to collect granulocytes have been done with the IBM 2997 cell separator (10%), the Cell Separator 3000 (42%), the COBE cell separator (26%), and a continuous flow cell separator (22%). This was reported in 41 out of 66 included studies. To decrease the risk of Side effects all leucocyte concentrates were irradiated before administration to the patient; that this was actually done was reported in 19 of the included studies.

3.7. Clinical outcome

In patients receiving therapeutic GTX, the outcome was recorded in 57 included studies, which includes 11 prospective studies and 46 retrospective studies. In the 31 case reports, 81 patients survived and 53 patients died. In the 21 cohort studies altogether 168 patients survived and 50 died. Of the reported patients 70% showed survival and 30% of patients died.

There was no significant difference in the granulocyte number infused between the patients who survived ($21 \pm 15 \times 10^9$/L) and the patients who died ($20 \pm 10 \times 10^9$/L). However, if one calculated the number of deaths in the group of which the donors were stimulated with G-CSF and steroids compared to no stimulation, 50% less deaths were seen in the stimulated group.There were 43 deaths in the non-stimulated group and 20 deaths reported in the stimulated group.

3.8. Side effects

Pulmonary complications in the recipient were reported in 7 of the included studies. This ranged from mild symptoms to more severe respiratory symptoms, TRALI was not reported. This was mostly seen before 1974 when graft handling was essentially different. GVHD was seen in 3 case studies. In these 3 studies from the 197's the transfused granulocytes were *not* irradiated. In 9 studies allergic reactions were recorded; all these resolved without complications. In 3 studies transmission of infection was reported mainly as CMV infection. Side effects in the donor were mentioned only in a few studies. Flu-like symptoms were mentioned in 3 studies in donors who were stimulated with G-CSF.

4. Discussion

In this review all paediatric data on the use of GTX were recorded. In the time period between 1976 and 2006 this was reported in 510 paediatric patients. All these patients were treated to the best available knowledge on GTX at that time. Yet, no RCT was performed exclusively in children >1 year of age, and it is therefore difficult to estimate the efficacy of GTX in neutropaenic paediatric patients with severe infections. Reported indications for GTX use in this group of patients show that more than 80% of patients received GTX for a proven Gram-negative or fungal infection. The mortality and morbidity for these infections is much higher than for Gram-positive organisms, and, in addition, an increase is seen in recent years in the occurrence of Gram-negative infections.[17–19]

The mean duration of neutropaenia in these described patients was 12.3 days which corresponds with the high risk criteria for febrile neutropenia where >10 days is defined as prolonged neutropaenia.[20] From all data reviewed on paediatric patients GTX might be beneficial in severe neutropaenia of long duration who experience a Gram-negative or fungal sepsis. In these reported paediatric patients 70% of patients survived and 30% of patients died. This does not however reflect the efficacy as positive results could be reported more than negative results. In the recently published Cochrane review on 8 RCTs mainly in adult patients [15] the RR for mortality was only significantly reduced (RR 0.37, 95%CI 0.17–0.82) when studies were considered in which adequate numbers of granulocytes were transfused ($>10 \times 10^9$). None of these RCT's looked at exactly the same outcome, so the authors concluded that these results should be interpreted with caution.

In all the described studies of our review, it was shown that donors stimulated with a combination of corticosteroids and G-CSF had the highest granulocyte yield and in the group of patients receiving granulocytes of prestimulated donors fewer deaths were recorded. This suggests that patients benefit more from donor-prestimulated than unstimulated neutrophils even when receiving equal numbers of cells. This can be best explained by an inherent advantage of G-CSF and/or dexamethasone to the cellular activities of donor neutrophils, such as survival, motility or killing capacity, some of which have been well documented.[21,22]

Concerning Side effects in the patient, the severe pulmonary complications that were seen in the past were not reported in all the case reports and cohort studies described after introduction of novel leukopheresis methods. There were mild pulmonary signs and symptoms that all resolved without late effects. Other complications were GVHD and allergic reactions. After irradiating all granulocyte products the risk of GVHD seems irrelevant. Side effects in the donor were only minimally reported, mainly consisting of flu-like symptoms in the G-CSF stimulated donors. No long term follow-up has been reported of these donors. Safety concerns on administration of G-CSF therapy to healthy donors has been reported. Bennett et al.[23] describe 5 cases (out of 738 donors)

who developed a haematologic malignancy years after G-CSF therapy, a causal relationship cannot be demonstrated but it does stress the need for long term follow-up.

There are no RCT's for the use of GTX in paediatrics. Our review covers 30 years of observational experience and reflects the 'best available evidence'. Weaknesses of our analysis are the retrospective, uncontrolled, non-randomised, non-blinded intervention, the non-blinded outcome assessments, and lack of adequate follow-up. Selection, performance, detection and attrition bias could all play a role and a simple conclusion on the efficacy of the use of GTX in neutropaenic children can not be drawn.

Several authors[24,25] have emphasised the potential importance of observational evidence. It alerts us as to the potential harms or benefits of a treatment. Given the results of this review, there remains doubt on the efficacy of the use of GTX in reducing mortality without increasing morbidity in paediatric neutropaenic patients.

We have learned from this review in children that there should be a clear clinical indication for the use of GTX and that granulocytes should be collected under optimal, donor-prestimulated circumstances.

In conclusion, paediatric neutropaenic patients with a fungal infection or bacterial infection form a target group of patients who could benefit from GTX. We are as yet uncertain on the exact timing and the exact dose of GTX, and are definitely in need of standardised indications and protocols. We recommend that GTX should not be given outside the realm of a well-designed clinical trial.

Conflict of interest statement

None declared.

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