Eighteen Years Experience of Granulocyte Donations—Acceptable Donor Safety?

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Background: Granulocyte transfusions are given to patients with life-threatening infections, refractory to treatment. The donors are stimulated with corticosteroids ± granulocyte colony stimulating factor (G-CSF). However, data regarding the donors’ safety is sparse. The objective was therefore to evaluate short- and long-term adverse events (AE) in G-CSF stimulated donors.

Study design and methods: All consecutive granulocyte donors from 1994 to 2012 were identified through our registry. From the donation records, the number of aphereses, stimulation therapy, AE, blood values post donation, and recent status were evaluated.

Results: One hundred fifty-four volunteer donors were mobilized for 359 collections. Age at first granulocyte donation was 43 years (median; range 19–64 years). Follow-up was 60 months (median; range 0–229 months). The dose of G-CSF per collection was 3.8 ug/kg body weight (median; range 1.6–6.0 ug/kg). Sedimentation agent was HES. Short-term AE were mild. Blood values 4 weeks post donation with minor reductions/elevations mostly resolved in later donations. Fourteen donors were excluded from the registry due to hypertension (4), diabetes (2), atrial flutter (1), breast carcinoma (1), urethral carcinoma in situ (1), MGUS (1), thrombosis (1), anaphylaxis (1), primary biliary cirrhosis (1), and unknown (1). Three donors are deceased due to diabetes, acute myocardial infarction, and unknown cause. All excluded/deceased donors except one were excluded/died at least 6 months after first granulocyte donation. Conclusion: No serious short-term AE were observed. Due to the variability of diagnoses among excluded/deceased donors, we propose that it is less likely that granulocyte donations have a causative impact on these donors’ exclusion or death. J. Clin. Apheresis 30:265–272, 2015. © 2014 Wiley Periodicals, Inc.

Key words: granulocyte donation; donor; safety; follow-up

INTRODUCTION

Granulocyte transfusions (GTX) are given to neutropenic patients with life-threatening infections, refractory to antimicrobial treatment [1–4]. Data regarding the clinical efficacy of GTX has been derived mostly from case reports and smaller uncontrolled series, with donors stimulated with corticosteroids only [1].

Corticosteroid stimulation gives doses of approximately 20 × 10⁹ granulocytes per unit [5]. A dose–response relationship between total granulocyte dose and clearance of infection was proposed in the beginning of the 1990s, and granulocyte colony stimulating factor (G-CSF) was introduced as a mobilizer of granulocytes to the peripheral blood [6]. However, more recent reviews of GTXs, including G-CSF-stimulated with higher doses of granulocytes, could not draw any firm conclusions regarding the effect [7–9]. All authors concluded that more studies are necessary, and we eagerly await the results of the randomized Resolving Infections in Neutropenia with Granulocytes (RING)-study [10].

There have been, and still are, concerns about giving healthy donors treatment with corticosteroids and G-CSF. Some centers use related donors only [11,12], and others do not use G-CSF [13]. Immediate events related to G-CSF such as fatigue and pain are well known, and usually mild and reversible [14,15]. Serious adverse events (SAE) such as cardiovascular/thromboembolic events, splenic rupture, and pulmonary symptoms are reported in stem cell donors, where the G-CSF is dosed differently (by weight) and given in a series [16,17]. G-CSF given to stem cell donors has also been discussed as having a potential impact on the developing of hematological malignancies [17–20]. Data from registries in Europe and in USA does not support this issue [16,21], although Hölig et al. reported an increased incidence in Hodgkin lymphoma in donors as compared to...
the natural incidence in the German population [22]. The sample size of that study was however too small to allow any firm conclusions other than the importance of a prolonged and thorough follow-up. Quillen et al. reported in 2009 a ten-year follow-up of 83 granulocyte donors, and concluded the donation procedure to be safe [23]. The donors in that study received a combination of corticosteroids and G-CSF.

Long-term follow-up of granulocyte donors is sparse [14,23], although the procedure might have a long-term impact. In the recommendations from the World Marrow Donor Association (WMDA) Ethical and Clinical Working groups, granulocyte donors should be offered a long-term follow-up program [24].

The sedimentation agent hydroxyethyl starch (HES) has been reported to give mild AE such as dermatologic symptoms and insignificant laboratory alterations, but also SAE such as anaphylactoid reactions, severe pruritus, shock, and disseminated intravascular coagulation [25]. Significant blood levels of HES persist from weeks to years [26,27]. Also the side-effects of corticosteroids (diabetic decompensation, osteoporosis, cataracts/glaucoma, mood disorders among others) are well known [28,29].

The hypothesis that granulocyte donation is safe is difficult to answer today because of the limited information available [14,23]. We therefore carried out this retrospective study to evaluate possible AE in stimulated granulocyte donors.

**MATERIALS AND METHODS**

**Donors and Protocol**

All consecutive granulocyte donors during the period 1994–2012 were identified through the blood donor registry and retrospectively included in the study. These donors were already donors of platelets, and they were chosen to donate granulocytes due to their negative cytomegalovirus (CMV)-status and their compatibility with the recipients’ ABO- and Rhesus blood group. The day before donation, the donors answered a health questionnaire and were examined by the transfusion medicine physician. Laboratory tests were analyzed. The donors
Laboratory Analyses Before Donation

Blood tests: Hemoglobin (Hb), white blood cell (WBC) counts, platelets, sodium, potassium, albumin, calcium, creatinine, CMV, HBsAg, anti-HCV, anti-HIV I/II, syphilis screen IgG and IgM were evaluated before the administration of corticosteroids and G-CSF. Since 2005, these analyses are supplemented with uric acid, alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), alkaline phosphatase (ALP), and glucose.

Collection Procedures

All collections were performed via peripheral venous access with the continuous flow cell separator Spectra (COBE, Terumo BCT). The WBC/PMN program, version 4.7, 5.1, 6.0, or 7.0, was used. Six to seven liters of whole blood was processed.

A mixture of 30 mL of 46.7% trisodium citrate and 500 mL sedimentation solution HES was used. Due to difficulties obtaining HES, different solutions have been used (Fig. 1). The donors were given tablets calcium gluconate/carbonate 1–2 g orally, calcium gluconate 9 mg/mL 5 mL intravenously when needed or an intravenous infusion with a rate of 10–30 mL/h.

Acute Collections

When the need for granulocytes was immediate, the collection was performed the same day as the donor was asked for donation. Neither orally corticosteroids nor G-CSF could therefore be given the day before this collection. Instead, hydrocortisone 100 mg was given intravenously 15 min before the collection. As these donors were exposed to corticosteroids and HES, they were not excluded from the study although they did not receive G-CSF. (During the years 1994–2008 all donors received hydrocortisone intravenously, not only the acute collections, Fig. 1.)

Follow-up

Since November 1997, a check-up was offered 4 weeks post collection: Hb, WBC counts, platelets, sodium, potassium, calcium, glucose, and creatinine were evaluated. Beginning in the year 2005, uric acid, ALAT, ASAT, ALP were additionally tested. The intention was to analyze glucose in fasting donors. However, some donors arrived non-fasting, for them we used a slightly higher acceptable value for them when evaluating their results.

The donors have continued to donate platelets or other blood components after the granulocyte donations. Before every donation, the donors fill out a health questionnaire, which is evaluated by a registered nurse. Some of the questions include “Do you regularly see a doctor? Why? Do you use pharmaceuticals? Have you since the last blood component donation been examined or treated for an illness? Have you since the last blood component donation consulted a doctor?” When the answer is yes, the nurse consults the written rules for deferral or discusses with the transfusion medicine physician if a temporary or permanent deferral is necessary. The reason of deferral is documented in the donation records. Before platelet donation, Hb, WBC counts, and platelets are analyzed.

Evaluation

From the donation records, the number of aphereses, stimulation therapy, yield, blood values post donation, AE, and recent status were evaluated. No ophthalmic examination was done. All granulocyte donors, also those who were stimulated but not collected (n =12) were followed as below:

Healthy donors at follow-up: Observation time was calculated from the first granulocyte collection to the latest blood component collection, where the donor was evaluated healthy. Excluded donors: Observation time was calculated from the first granulocyte collection to the date of the contact with the donor, where the illness was reported. If necessary, medical charts were evaluated. Deceased donors: Death certificate including cause of death was reviewed.

Statistics

The Mann–Whitney U-test was used to evaluate differences between groups (Statistica). P < 0.05 was considered significant. The correlation coefficient analysis (Pearson’s r) was used to evaluate correlations (Excel).
RESULTS

Donors

One hundred fifty-four unrelated volunteer donors (136 M/18 F) were mobilized for 359 collections (median 2.3 collections/donor). Age at first granulocyte donation was 43 years (median; range 19–64 years). One hundred thirteen out of 154 donors (73%) donated two consecutive days. Many donors have donated granulocytes subsequently (Fig. 2). The dose G-CSF/apheresis was 3.8 ug/kg body weight (median; range 1.6–6.0 ug/kg).

Collections

Of 359 collections, nine collections were cancelled due to changes in the patients’ conditions, three were cancelled due to donor conditions (1 feeling ill, 1 increased glucose, 1 decreased Hb, Fig. 3). Three hundred forty-seven collections were done with a median yield of $40 \times 10^9$ granulocytes/unit (Table I).

Follow-Up

Median follow-up was 60 months (range 0–229 months). Seventy-seven donors (50%) had an observation time of ≥5 years, 34 donors (22%) ≥10 years.

Short-Term Adverse Events

Immediate AE related to HES, corticosteroids, or G-CSF were all well known from the literature (pain, headache, insomnia, fatigue) and of mild intensity. However, one donor refused apheresis because of feeling ill related to G-CSF and maybe corticosteroids. No SAE was observed in relation to the apheresis.

Laboratory Data at Follow-Up Post Donation

One hundred and twelve of the 137 (82%) donors donating from November 1997 accepted the offered follow-up visit and were evaluated regarding laboratory data four weeks post donation. From 2005, 88/99 (89%) donors were also checked with liver tests and uric acid. Only minor reductions or elevations close to the reference level were seen. Reduced Hb ($n=5$), reduced ($n=6$) or increased ($n=4$) WBC counts, reduced ($n=1$) or increased ($n=6$) platelets were normalized at later analyses with the exception of two donors, that still have occasionally slightly increased platelet values, and three donors, who continue to have moderate reduced white cell counts. As they all have been healthy, these donors have continued to donate platelets. Three of four donors with increased creatinine levels at four weeks follow-up have normalized the values and still donate platelets regularly. The fourth donor is deceased (acute myocardial infarction), see below. Two of four donors with increased glucose have diabetes mellitus, their glucose values were increased already at granulocyte donation.

Four donors had increased ALAT levels. Of these, one donor with increased ASAT and ALP was diagnosed as having primary biliary cirrhosis, the remaining continue to donate platelets.

Sodium, potassium, calcium, and uric acid were all normal in all donors at follow-up.

<table>
<thead>
<tr>
<th>Stimulation therapy</th>
<th>$n$ collections</th>
<th>$\times 10^9$ per unit median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corticosteroids</td>
<td>51</td>
<td>8 (3–51)</td>
</tr>
<tr>
<td>Corticosteroids and G-CSF</td>
<td>255</td>
<td>44 (7–115)</td>
</tr>
<tr>
<td>Missing values of yield</td>
<td>41</td>
<td>—</td>
</tr>
<tr>
<td>All donations</td>
<td>347</td>
<td>40 (3–115)</td>
</tr>
</tbody>
</table>

Fig. 2. Number of donors and collections during the years 1994–2012.

Fig. 3. Donors, stimulation therapy and collections during the years 1994–2012.
Exclusion From Blood Donation

Fourteen donors were excluded from the registry and further donations of blood components (Table II). Age at first granulocyte donation for the excluded donors was 50 years (median; interval 29–59), while corresponding figures were 42 years (median; interval 19–64) for the non-excluded donors (not significant). Time from first donation to exclusion was 37 months (median; range 1–198). No correlation was seen between the dose of G-CSF and the duration to exclusion ($r = 0.08$). One of the excluded donors, and two not previously excluded, are deceased (Table III). None of the donors with corticosteroids only were excluded/deceased. Sixteen donors are thus excluded or dead.

### TABLE II. Excluded Donors From the Registry of Blood Donors

<table>
<thead>
<tr>
<th>Diagnosis/cause of exclusion</th>
<th>$n$ donors excluded</th>
<th>$n$ granulocyte collections/donor</th>
<th>Time between first granulocyte collection and exclusion (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td>4</td>
<td>2</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>2</td>
<td>3</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Also hypertension</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>1 atrial flutter</td>
<td>2</td>
<td>198</td>
</tr>
<tr>
<td>Heart disorder</td>
<td>1</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Malignancy</td>
<td>2</td>
<td>2</td>
<td>68</td>
</tr>
<tr>
<td>1 breast carcinoma</td>
<td>2</td>
<td>2</td>
<td>36</td>
</tr>
<tr>
<td>1 urethral carcinoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>in situ</td>
<td>MGUS$^a$</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Mesenteric vein thrombosis</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Anaphylaxis due to wasp</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Primary biliary cirrhosis</td>
<td></td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>14</td>
<td></td>
</tr>
</tbody>
</table>

$^a$MGUS = monoclonal gammopathy of undetermined significance.

### TABLE III. Deceased Donors

<table>
<thead>
<tr>
<th>Donor</th>
<th>Cause of exclusion (when applicable)</th>
<th>Cause of death</th>
<th>Age at death (years)</th>
<th>Time from first granulocyte collection to death (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Mesenteric vein thrombosis (mutation in factor V)</td>
<td>Diabetes mellitus</td>
<td>67</td>
<td>166</td>
</tr>
<tr>
<td>2.</td>
<td>Not excluded, healthy at last contact</td>
<td>Not possible to determine</td>
<td>70</td>
<td>154</td>
</tr>
<tr>
<td>3.</td>
<td>Not excluded, healthy at last contact</td>
<td>Acute myocardial infarction</td>
<td>43</td>
<td>32</td>
</tr>
</tbody>
</table>

DISCUSSION

This follow-up study of granulocyte donors adds information of events and laboratory variables to the sparse documentation that exists today. Despite limitations due to the retrospective nature of this study, we estimate that our results confirm previous conclusions that G-CSF/dexamethasone stimulation of donors has an acceptable safety profile [14,23].

In a two-year follow-up study by Bux et al. of 183 granulocyte donors, no SAE were noted [14]. However, in a longer follow-up of median 10 years, seven events (8.4%) were observed among 83 granulocyte donors: 2 lymphoma, 1 lung cancer, 1 melanoma, 1 deep venous thrombosis, 2 coronary artery disease (CAD) [23]. However, both malignancies and CAD were also observed in the control group of platelet donors, and the conclusion was that G-CSF and dexamethasone stimulated granulocyte apheresis was not associated with long-term AE. A myocardial infarction (AMI) during granulocyte donation has been reported from the same group [31]. This donor appeared to have experienced, but not reported new-onset angina prior to donation.

Sixteen of the 154 donors (10%) in the present study were excluded from further donations of blood components/deceased. None of those were excluded/deceased of events during the granulocyte donation. All except one was excluded/died at least 6 months after the first granulocyte donation, most of them after a period of many years post granulocyte donation. None of them had donated granulocytes for more than three collections and none of them had been given G-CSF in doses higher than in other reports. The median age at first granulocyte donation for excluded donors was 50 years. It could therefore be reasonable to assume that diagnoses such as diabetes, hypertension, and atrial flutter appear without any causative link to the donation procedure. However, three deceased donors out of 154 is worth attention, especially as donors have been shown to have a 30 percent lower mortality than the background population as reported in the SCANDAT study [32].

The two deceased donors, who were healthy at last contact with the nurses, were reported dead when
checking up the follow-up dates in the donation records for this study. According to the definition for follow-up of the study, those should be evaluated as healthy (see Evaluation above). However, we find this information of potential importance. The man, where the autopsy showed AMI, was 43-years old at death. That year, a total of 17 men aged 40–44 died of AMI in Sweden [33]. However, we have no information on granulocyte donations in those 16 other men. Activation of coagulation factors that could favor the developing of thrombotic events has been shown in granulocyte donors receiving G-CSF [34]. Even if a possible correlation between granulocyte donation and death in AMI many months later seems unlikely, it supports the view that continued, careful, long-term surveillance of this donor group is warranted.

The possible long-term risks of G-CSF include an increased risk for malignancies and hematological disorders. Although the occurrence of those disorders in previous stem cell donors has been reported, there is currently no solid evidence for an increased cancer risk in this donor group [16]. In the present study, one 53-aged woman with breast carcinoma, one man with urethral carcinoma in situ, and one with MGUS were documented. These figures are in line with the report of Quillen et al. [23]. In Sweden, the age-specific incidence of breast carcinoma in women aged 50–54 is 754/100,000, and the cumulative risk to develop breast carcinoma is 10% among women <75 years, and 3% for women <55 years [35]. Breast carcinoma is thus the most common cancer type among women, and identifying a statistical relevant correlation between diagnosis and granulocyte donation requires a huge number of donors. During a long follow-up period, it is likely to expect a low frequency of serious diagnoses in donors, who once started as healthy persons, in line with the general incidence of these diseases. However, it is interesting that breast carcinoma was one of the cancer that was observed as having an increased relative risk in the SCANDAT study [32].

Most granulocyte donors have, as in our study, experienced the plasma expander HES. A recent meta-analysis of HES in resuscitation practice in patients with sepsis showed an increased incidence of kidney injury and 90-day mortality in HES-treated patients compared to those with crystalloid treatment [36]. Our healthy donor cohort experienced mild and reversible AE, comparable with others [37], but no kidney injury, and no mortality within 90 days from exposure to HES. Still, we find that these findings are worth attention, for both donors and recipients of HES-produced components. With the use of corticosteroids and G-CSF-stimulated granulocytes, the need for HES might be less than before the G-CSF era. Since January 2014, we no longer use HES as a sedimentation agent at our center.

Also AE due to corticosteroids were mild. The two donors that were excluded due to diabetes had increased blood values of glucose already simultaneously with the donation process. A limitation of the study is that no ophthalmic examination was done. This is a retrospective study, and eye examination was not included in our follow-up routine.

All abnormal blood values were small alterations from the reference level. However, three donors continued to donate platelets with a stable slightly decreased WBC counts. Two of these three had values at the lower level already before the granulocyte apheresis and all are frequent platelet donors with 12–32 platelet donations after the granulocyte donations. This observation is in line with a study by Quillen et al. that described a progressive decrease in pre absolute neutrophil count after increasing number of granulocyte donations [31]. However, that study is not directly comparable with our series. For example, the donors had a large number of granulocyte collections/donor, while our three donors above had one or two granulocyte collections only. But, as in the present series, the donors in the study by Quillen et al. had donated platelets in between granulocyte donations. Of interest, in our registry of platelet donors, there are some donors also with repeatedly slightly decreased WBC counts who have not donated granulocytes, which is in line with other reports [38]. Thus, the decreased WBC counts in our study might not be related to the granulocyte collections, but may be linked to the platelet collections or be regarded as within these donors’ normal range.

An increased platelet number in frequent platelet donors has previously been reported [38,39]. The platelet donations could be the cause of the two frequent donors’ high number post donation in these series rather than the few granulocyte donations. These donors are not blood donors and have acceptable values in Hb, so a reactive thrombocytosis due to iron deficiency seems less likely. An individual range above the reference level might also contribute, because one of the two donors had elevated platelets already before start of granulocyte apheresis.

In this retrospective series of consecutive granulocyte donors over a period of 18 years, the type of sedimentation agent and the dosage of corticosteroids and G-CSF have not been exactly identical during the years. However, we consider that the changes are small and have less impact when evaluating AE. The group of donors seems to be representative for granulocyte donors with respect to the yield of G-CSF-stimulated donors [8]. The donors where corticosteroids only were the stimulating agent (n = 13) might seem to have a smaller yield than expected [5]. Some of these donors had a low yield, and due to the low number of donors, this might have a big impact. A limitation is the lack
of longer follow-up in healthy donors who moved abroad or terminated blood component donation for other reasons than illness. Their follow-up is often short and an underreporting of events might be assumed.

To summarize, due to the variability of diagnoses among excluded/deceased donors, it seems unlikely that long-term SAE have a causative impact. The causes of the serious diagnoses in the present study could as well be part of “normal aging,” within the expected incidence in the general population. However, we believe that this study contributes to the sparse documentation of follow-up of granulocyte donors.

CONCLUSION

We confirm the findings of previous investigators that G-CSF/dexamethasone stimulation appears to be safe [14,23]. Still, it is of great importance, that all institutions that handle granulocyte donations include all donors in long-term follow-up programs, which also is recommended by the WMDA [24], and encourage participation in clinical studies when applicable.

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