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The determinants of granulocyte yield in 1198 granulocyte concentrates collected from unrelated volunteer donors mobilized with dexamethasone and granulocyte–colony-stimulating factor: a 13-year experience

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Abstract

BACKGROUND—The combination of granulocyte–colony-stimulating factor (G-CSF [filgrastim]) and dexamethasone (G-CSF/dex) is an effective granulocyte mobilization regimen, but the variables that affect donor neutrophil response and granulocyte collection yield are not well characterized.

STUDY DESIGN AND METHODS—A computerized database containing records of 1198 granulocyte collections from 137 unrelated volunteer apheresis donors during a 13-year period was retrospectively analyzed. Donors were categorized by age, sex, and cumulative number of granulocyte donations. Complete blood counts at baseline and after G-CSF/dex stimulation were recorded. The outcome variables include the pre-procedure absolute neutrophil count (preANC), which reflects G-CSF/dex stimulation, and the granulocyte product yield per liter processed (BagGranYield/L).

RESULTS—Higher baseline ANC and platelet (PLT) counts were significantly associated with higher preANC while a larger number of prior granulocytapheresis procedures was associated with lower preANC. Total filgrastim dose (used in weight-based dosing) did not significantly impact preANC or the granulocyte yield; weight-based dosing at 5 μg per kg and a uniform 480-μg dose produced equivalent preANC. PreANC and weight were the key determinants of granulocyte yield (BagGranYield/L).

CONCLUSION—Apheresis donors with higher baseline PLT counts and ANCs have higher ANCs after G-CSF/dex stimulation; donor age, weight, and sex do not have a significant impact. A uniform G-CSF dose of 480 μg is as effective as weight-based dosing at 5 μg per kg. Donor ANC monitoring should be considered after serial granulocytapheresis procedures.

Before the mid-1990s, granulocyte transfusion therapy was limited by collection techniques that yielded a cell dose that was often marginal. With the introduction of recombinant human granulocyte–colony-stimulating factor (G-CSF), it was discovered that a single subcutaneous administration of G-CSF in normal donors followed by leukapheresis 12 to 16 hours later harvested three times the number of functionally normal neutrophils compared with corticosteroid pretreatment;[1] the addition of corticosteroids resulted in even higher donor neutrophil counts.[2]

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Previous work by our group[3,4] and others[5,6] using sub-cutaneous G-CSF at 5 μg per kg 18 hours before and 8 mg of oral dexamethasone 12 hours before granulocytapheresis showed that the mean granulocyte yield ranges from 5.5×10^{10} to 7.2×10^{10}. The donor's neutrophil count at the time of leukapheresis is a key determinant of the granulocyte yield with any stimulation regimen; it ranges from 5.6×10^9 per L with dexamethasone alone to 13.6×10^9 per L with G-CSF alone to 28.9×10^9 per L with combined G-CSF and dexamethasone (G-CSF/dex).[3]

The purpose of this study was to explore the donor and procedural characteristics that affect neutrophil response to G-CSF/dex and granulocyte collection yield. Donor demographic and laboratory variables and the specifics of G-CSF dosing are some of the key variables that we considered.

MATERIALS AND METHODS

Donors

A total of 137 unrelated volunteer apheresis donors were recruited to undergo G-CSF/dex stimulation for granulocyte collection between October 1994 and November 2007. All donors met standard criteria for allogeneic blood donation, had donated PLTs by apheresis in the past, and had signed consent for participation in the granulocyte program. They underwent a thorough history and physical examination by a transfusion medicine physician before the first granulocyte mobilization and were specifically questioned about diabetes, hypertension, and a history of angina or untreated coronary heart disease before each subsequent granulocyte mobilization. These donors contributed 1198 granulocyte concentrates during the study period. The minimum interval between successive granulocyte donations was generally 1 month; exceptions were made if the donor was an HLA match for an alloimmunized recipient. At each donation visit, donor demographic information including sex, age, height, and weight were collected. Body mass index (BMI) was calculated as

$$\mathrm{BMI} = \mathrm{weight\ (kg)} / (\mathrm{height\ (m)})^2.$$

The donors received a single subcutaneous injection of filgrastim (Amgen, Thousand Oaks, CA) 12 to 18 hours before leukapheresis and took 8 mg of dexamethasone orally 12 hours before leukapheresis. The dose of G-CSF was 5 μg per kg until July 2005 and 480 μg as a flat dose thereafter. G-CSF was administered by the nursing staff of the apheresis center.

Granulocyte collection

Granulocyte concentrates were collected with a blood cell separator (CS3000 Plus, Fenwal, Deerfield, IL) using a granulocyte separation chamber and an interface offset of 45. Seven liters of whole blood was targeted to be processed with trisodium citrate anticoagulant (Citra Anticoagulants, Braintree, MA) at a ratio of 1:10–1:13; whole blood flow rates ranged from 50 to 55 mL per minute. The actual volume processed (VolProc) was recorded for each procedure. Six percent hydroxyethyl starch (HES; Hespan, Braun Medical, Irvine, CA) was used as a red blood cell (RBC)-sedimenting agent (500 mL HES to 30 mL trisodium citrate).

Laboratory testing

Complete blood counts (CBCs) were performed on the donors on the day of granulocyte collection: the preprocedure white blood cell (WBC) count with an automated differential generates the preprocedure absolute neutrophil count (preANC); postprocedure CBC/ANC (postANC) was obtained to calculate collection efficiency. Although CBCs were not

performed immediately before G-CSF administration, the donor's most recent unstimulated CBC, drawn just before a plateletpheresis donation within the prior 12 months, was recorded for each granulocyte collection procedure; these baseline variables included WBC count, ANC, PLT count, and hemoglobin level.

CBCs were performed on the granulocyte concentrates; the granulocyte concentrate dose ("BagGranYield") was obtained from the BagANC multiplied by the volume of the component. Granulocyte collection efficiency (GCE, %) was calculated according to the formula

$$\mathrm{GCE}=\mathrm{BagGranYield}\ \left(10^9\right)\div\left(\mathrm{MeanANC}\left(\times 10^9/\mathrm{L}\right)\times\mathrm{NetVolProc}\ (\mathrm{L})\right)\times 100$$

where

$$\mathrm{MeanANC}=(\mathrm{PreANC}+\mathrm{PostANC})/2$$
$$\mathrm{NetVolProc}=VolProc-Anticoaguant.$$

Outcome variables

The two outcome variables studied were the donor's preANC, reflecting the effects of G-CSF/dex stimulation, and the BagGranYield per liter processed. Collections where the minimal processing volume of 5 L was not reached due to donor discomfort or venous access issues were not evaluated for the BagGranYield per L analysis.We postulated that the preANC would be the major determinant of granulocyte yield, but that other variables might play a role as well.

Data analysis

Standard data analysis and graphics were performed with a spreadsheet application (Excel, Microsoft Corp., Seattle, WA). Data are provided as mean ± standard deviation (SD) unless otherwise noted. Comparisons of continuous variables between groups were performed with a two-tailed, nonpaired t test. Multivariate analyses were performed using stepwise forward logistic regression, based on variables that reached significance in univariate analysis (JMP, SAS Institute, Cary, NC).

RESULTS

Donor demographics and adverse events

A total of 137 donors underwent 1198 leukapheresis collections after G-CSF/dex stimulation, a mean of 8.7 ± 8.6 collections per donor (median, 5; range, 1–41). The mean interdonation interval was 198 days (median, 74; range, 14–3312), for the donors who donated granulocytes more than once. Approximately 12 percent of procedures (146/1198) were performed within 30 days of another granulocytapheresis, generally in support of an HLA-alloimmunized recipient; the minimum acceptable interval was 14 days. Since many of the granulocyte donors also donated PLTs, the cytapheresis interval (between any two cytapheresis procedures) was also calculated: the mean cytapheresis interval was 67 days. Ninety-six percent of the donors were white. There were almost twice as many males as females (90 vs. 47). For the male donors, the age was 52.04 ± 9.51 years (range, 27–77), and the weight was 89.01 ± 12.77 kg. For the female donors, the age was 47.63 ± 9.27 years (range, 20–68), and the weight was 74.64 ± 13.75 kg. The male donors contributed to 883 collections (mean, 9.8 collections per donor), while the female donors contributed to 315 collections (mean, 6.7 collections per donor).

There was one serious adverse event: a 62-year-old male who had undergone granulocyte collection eight times in the prior 4 years (interdonation intervals always greater than 30 days) experienced chest pain during the procedure and had electrocardiographic changes consistent with myocardial ischemia. In retrospect, he had been experiencing new-onset angina with exertion but did not mention this in the donor interview. He was documented to have a non-ST elevation myocardial infarction. He had a family history of coronary artery disease. He underwent coronary revascularization and has been doing well since.

Donor blood counts and granulocyte concentrate yields

The donors' blood counts are shown in Table 1. No donor was deferred permanently for persistently low PLT counts or absolute lymphocyte counts. The mean granulocyte concentrate yield (BagGranYield) was $7.26 \times 10^{10} \pm 1.98 \times 10^{10}$ (range, 1.64×10^{10}–14.92×10^{10}); there was no sex difference (data not shown). The BagGranYield per liter processed was $1.10 \times 10^{10} \pm 0.30 \times 10^{10}$ (range, 0.32×10^{10}–2.15×10^{10}) cells per liter processed. The VolProc was 6.61 ± 0.40 L (range, 2.26–7.34). The granulocyte collection efficiency was 55.8 ± 9.3 percent.

We compared donor blood counts and granulocyte yields for the two G-CSF dosing regimens: dosing by weight (956 procedures) and a uniform dose (242 procedures). The mean and median filgrastim doses in the weight-based group were 427 and 450 μg, respectively (range 300–660 μg), leading us to choose 480 μg as the uniform dose for safe, economical, and convenient dosing. There was no significant difference in the donor preANC between the two groups (27.71×10^9/L vs. 27.46×10^9/L) or in the BagGranYield per L processed (both 1.10×10^{10}). Within the weight-based dosing group, there was no significant association between total filgrastim dose and preANC (data not shown).

Association of preprocedure ANC and baseline PLT count, baseline ANC, age, and number of prior granulocyte donations

Donors with higher baseline PLT counts had significantly higher ANCs after G-CSF/dex stimulation (Fig. 1). This positive correlation was true for both male donors and female donors. Donors with higher baseline ANC also had higher preANC; for each increase in the baseline ANC of 1×10^9 per L, the preANC increased by approximately 3×10^9 per L (Fig. 2). Donor age was not significantly associated with preANC for the first procedure (median age, 47 years; range, 27–67 years). Repeat leukapheresis procedures were associated with lower baseline ANCs: we examined the change in baseline ANCs between the first and last granulocyte donations for 112 donors who donated more than once: the first baseline ANC was $3.650 \times 10^9 \pm 1.440 \times 10^9$ and L and the last baseline ANC was $3.160 \times 10^9 \pm 0.997 \times 10^9$ per L ($p < 0.001$). We examined the change in preANC (between the preANC for the first procedure and the preANC for the last procedure) grouping donors by donation frequency: for donors who underwent 2 to 10 granulocyte collections (n = 54), the preANC decrease was on the order of 1×10^9 per L; for donors who underwent more than 20 granulocyte collections (n = 25), the preANC decrease was on the order of 6×10^9 per L (Fig. 3). Since age may confound the effect of multiple donations, we examined the change in preANC for the first two donations over time (Fig. 4); there was a modest trend toward a lower preANC over time reflecting the pure effect of age, although it did not reach significance.

Regression analysis of factors affecting preprocedure ANC and granulocyte yield

In univariate logistic regression analysis of preANC, higher baseline PLT counts and baseline ANCs were associated with higher preANCs, while male sex, older age, shorter cytapheresis interval (which includes both PLT and granulocyte donations), higher total filgrastim dose (for donors dosed by weight), and a larger number of prior G-CSF–

stimulated granulocyte donations were associated with lower preANC. The interval between granulocyte donations and the total number of cytapheresis donations (PLTs and granulocytes) were not associated with preANC. In the multivariate stepwise regression analysis ($R^2 = 0.495$), higher baseline ANC and baseline PLT were significantly associated with higher preANC while a larger number of prior G-CSF–stimulated granulocytapheresis procedures and older age were associated with lower preANC; the effect of age was modest compared to the baseline ANC and PLT. Factors such as sex, filgrastim dose, and cytapheresis interval were no longer significantly associated with preANC, after adjustment for the other factors.

In univariate logistic regression analysis of BagGranYield per L processed, higher preANC, preprocedure PLT count, preprocedure hematocrit (Hct), baseline ANC, baseline PLT, baseline Hct, filgrastim dose, and higher weight or BMI were associated with higher BagGranYield per L processed. In the multivariate stepwise regression analysis ($R^2 = 0.744$), preANC and weight were the key determinants of granulocyte yield. The association between donor weight and BagGranYield per L processed is shown in Fig. 5; the association is weak ($r = 0.22$) and possibly explained by the effects of a larger blood volume on cell separation characteristics. Filgrastim dose and the other hematologic variables were no longer significantly associated with the granulocyte yield.

DISCUSSION

We have shown in a large cohort of granulocyte collections in volunteer apheresis donors mobilized with G-CSF/dex that there is a striking correlation between the donors' baseline (unstimulated) neutrophil and PLT counts and the neutrophil count after G-CSF/dex stimulation. Baseline blood counts were not obtained immediately before G-CSF/dex administration; this would have been preferable, but was not practical in a routine donor setting. The strong association between the baseline PLT count and the poststimulation ANC was somewhat unexpected, but is consistent with findings from our group that baseline PLT count was a significant predictor of peak CD34 cell mobilization after G-CSF administration.[7] There is evidence than thrombopoietin impacts the number of hematopoietic stem cells and progenitors of all myeloid lineages.[8,9] This implies that a vigorous steady-state equilibrium for thrombopoiesis, as reflected in the baseline PLT count, may be a surrogate for the neutrophil response to G-CSF stimulation. The association between baseline PLT count and ANC response to G-CSF implies that apheresis donors with a high PLT count would be the optimal subjects to recruit for granulocyte donation. Although the baseline ANC also predicts the ANC after G-CSF/dex stimulation, it may not be a variable that is routinely monitored in plateletpheresis donors.

Our donors range widely in age, including many who were older than 55 years of age. Specifically, 35 percent (420/1198) of donations were made by donors age 55 or greater at the time of donation. There was a modest although not significant trend for a lower preANC with increasing age. Older donors who tolerate and respond well to the stimulation regimen constitute a valuable pool of donors for granulocytapheresis, which takes more time than plateletpheresis and is often scheduled on short notice. A recent study examined collections from 329 granulocyte donors (related and unrelated); this was a younger population overall (median age, 33 years), but age was also not found to be a significant predictor of granulocyte yield.[10] The mobilization regimen and the granulocytapheresis procedure involving HES were well tolerated by both younger and older donors in our study. The one donor who experienced a myocardial infarction during the procedure had been experiencing unrecognized crescendo angina in the few weeks preceding the donation.

A progressive decrease in preANC was seen after increasing numbers of granulocyte donations. We observed the same phenomenon in repeat PLT donors who were not specifically selected to donate on the basis of sustainably high PLT counts; they experienced a decline in PLT count with increasing numbers of plateletpheresis donations.[11] However, the interdonation interval was longer for granulocyte donations than plateletpheresis donations. Our granulocyte donors could have donated PLTs on many occasions in between granulocyte donations, but there was no particular effect of successive plateletpheresis procedures on baseline (unstimulated) donor neutrophil counts in our study (data not shown). Computerized systems that track serial ANCs and automatically generate medical referral would be useful in a frequent granulocytapheresis program. Adoption of a preANC cutoff such as 15×10^9 per L could be considered to qualify the donor for future granulocytapheresis donations.

The effect of different filgrastim doses in granulocyte mobilization was reported by Liles and coworkers,[12] who compared filgrastim 450 μg versus 600 μg, combined with 8 mg of dexamethasone. These two filgrastim doses produced equivalent ANC responses. Heuft and coworkers[13] found a dose response for lenograstim (glycosylated G-CSF) up to 6 μg per kg. In our study, total filgrastim dose (used in weight-based dosing) did not significantly impact preANC or the granulocyte yield; there also was no change in preANC after a change from weight-based dosing to a uniform 480-μg dose; a vial-based flat dose is economical, convenient, and less prone to error. This finding was in contrast to our experience with a 5-day course of filgrastim in the mobilization of CD34+ peripheral blood stem cells, where total filgrastim dose was a significant predictor of peak blood CD34+ cell count.[7] Granulocyte mobilization, unlike stem cell mobilization, does not require upstream responses in the marrow progenitor cell niche and perhaps as such is less dependent on filgrastim dose. Neutrophilia after single-dose G-CSF/dex is attributed to a shift of neutrophils from the marrow storage pool into the peripheral blood; it has been estimated that approximately 75 percent of the mature neutrophils in the marrow are mobilized with this stimulation regimen.[14]

Our study has several limitations. First, our apheresis donor population is mostly white, so we are not able to study the effects of ethnicity on granulocyte mobilization. Second, our donor population is older than other series and our findings may not be generalizable to younger adults. Third, this granulocytapheresis series reflects multiple donations from a relatively small group of community apheresis donors, so our data points are not independent observations. On the other hand, the effect of prior filgrastim-stimulated granulocyte collections on subsequent preANCs would not have been evident if we had confined our study to first-time granulocyte donors only. Finally, we did not look at the donors' baseline RBC sedimentation rate, which has been shown to correlate positively with granulocyte collection efficiency and collection yields in dexamethasone-stimulated donors.[15]

In conclusion, we have shown that apheresis donors with higher baseline PLT count and ANC have a higher ANC response to G-CSF and dexamethasone stimulation; donor age, weight, and sex do not have a significant impact on the ANC after stimulation. A uniform dose of 480 μg is as effective as weight-based dosing at 5 μg per kg. Donor ANC monitoring should be considered after serial granulocytapheresis procedures.

ABBREVIATIONS

ANC(s)	absolute neutrophil count(s)
BagGranYield	granulocyte product yield

BMI	body mass index
CBC(s)	complete blood count(s)
G-CSF/dex	granulocyte–colony-stimulating factor and dexamethasone
postANC	postprocedure absolute neutrophil count
preANC	preprocedure absolute neutrophil count
VolProc	volume processed.

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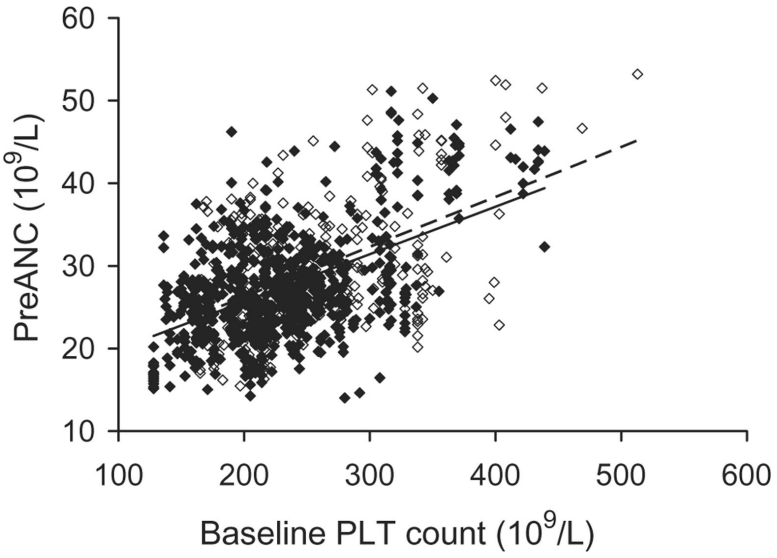

Fig. 1.

Association of baseline PLT count with preANC after G-CSF/dex stimulation. (◇) Female: $R^2 = 0.2260$, $p < 0.0001$; (◆) male: $R^2 = 0.2682$, $p < 0.0001$.

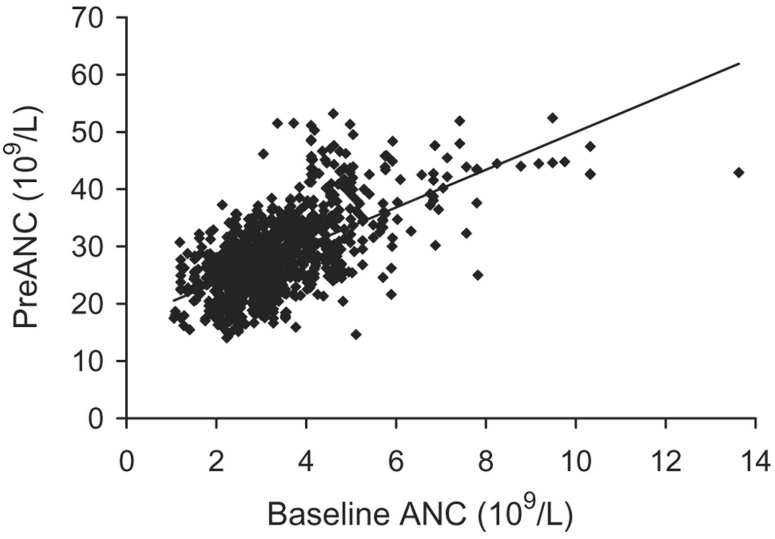

Fig. 2.

Association of baseline ANC with preANC after G-CSF/dex stimulation. $R^2 = 0.3726$, $p < 0.0001$.

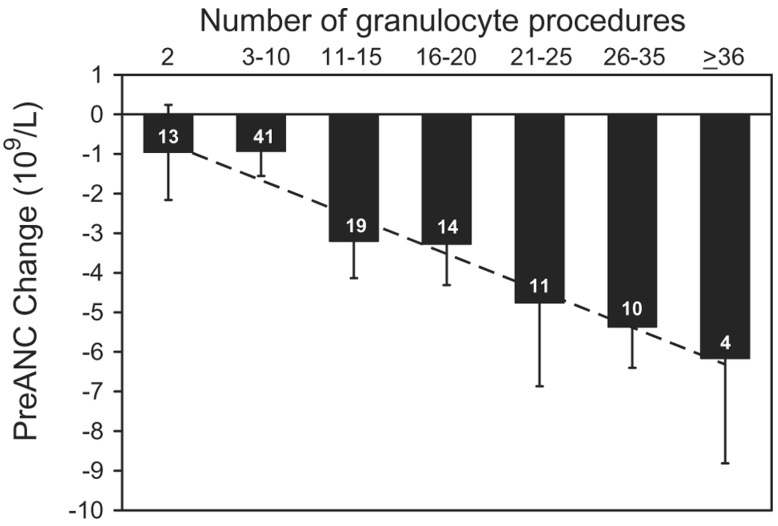

Fig. 3.

Effect of donation frequency on the mean change in preANC from the first to the last granulocyte donation, in the 112 donors who donated granulocytes more than once. Numbers in the bars represent the number of donors in each category of donation frequency.

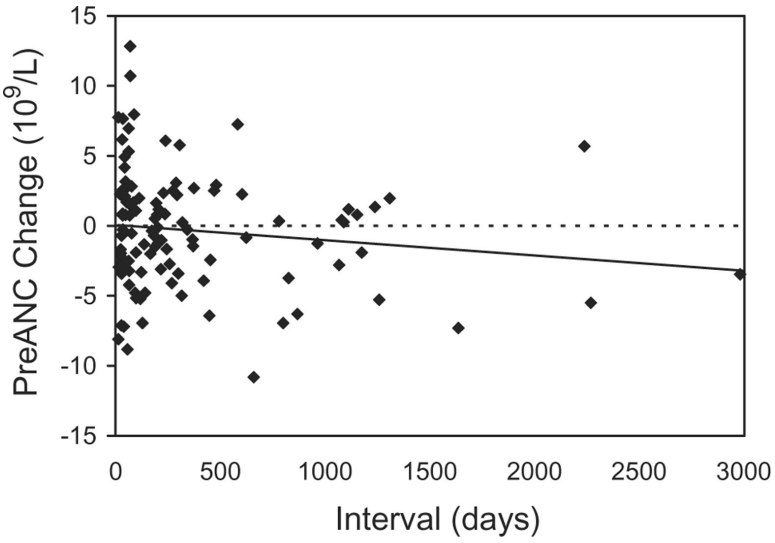

Fig. 4.

The effect of interdonation interval on the change in preANC for the first two granulocyte donations, in the 112 donors who donated granulocytes more than once.

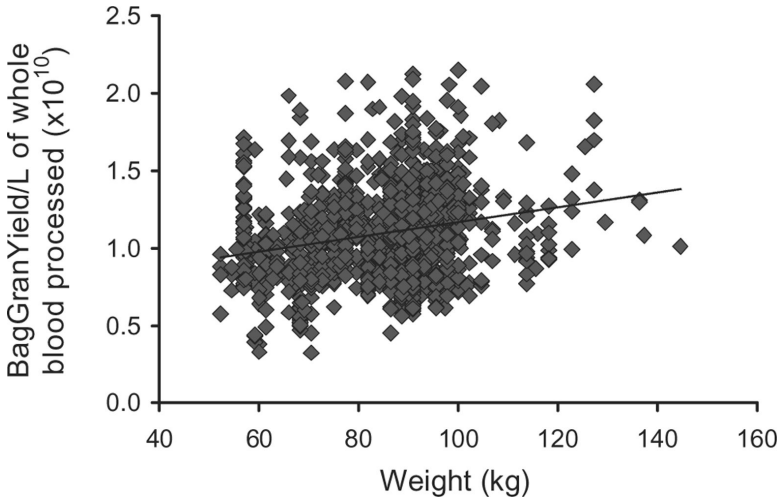

Fig. 5.

Association between BagGranYield per L of whole blood processed and donor weight. R^2 = 0.0514, p = 0.001.

TABLE 1

Granulocyte donors' baseline and preapheresis PLT count and preANC

Characteristic	All donations* (×10⁹/L)	Range (×10⁹/L)	Females* (×10⁹/L)	Males* (×10⁹/L)	Sex comparison (p value)
Baseline ANC (n = 1038)	3.320 (1.300)	1.060–13.630	3.450 (1.280)	3.220 (1.320)	0.009
Baseline PLT count (n = 985)	237,000 (61,000)	128,000–513,000	253,000 (62,000)	224,000 (61,000)	<0.001
PreANC (n = 1178)	27.660 (7.033)	11.370–53.190	29.370 (7.668)	27.050 (6.686)	<0.001
Preapheresis PLT count (n = 1184)	255,000 (67,000)	127,000–574,000	277,000 (64,000)	247,000 (66,000)	<0.001

*Data are reported as mean (SD).