Clinical and Ultrasonic Evaluation of Spleen Size during Peripheral Blood Progenitor Cell Mobilization by Filgrastim: Results of an Open-Label Trial in Normal Donors

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Rare reports of splenic rupture have been associated with filgrastim treatment during peripheral blood progenitor cell (PBPC) mobilization in allogeneic donors. We performed a prospective study of spleen volume change in 309 normal donors who received filgrastim according to local institutional practices. Splenic assessments consisted of ultrasonography and clinical examination at baseline and on the first day of leukapheresis in 304 donors. Of these, 90 donors were also examined 2 and 4 days after the first leukapheresis and 7 days after the last leukapheresis. Median spleen volume increased 1.47-fold (range: 0.63 to 2.60) on the first leukapheresis day and declined to near pretreatment levels at 7 days after last leukapheresis. Nine percent of donors had ≥2-fold increase in splenic volume. Spleen palpability did not correlate with change in spleen volume. No donors experienced a splenic rupture. There was no correlation between change in spleen volume and filgrastim dosage, number of doses/day, peak absolute neutrophil count (ANC), CD34+ yield, or donor baseline weight. Most donors experienced ≥1 adverse event, with 6 donors reporting serious adverse events. We conclude that the increase in splenic volume during PBPC mobilization in donors was transient, and that filgrastim was well tolerated in this study. This trial was registered at www.ClinicalTrials.gov as NCT00115128.

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KEY WORDS: Stem cell, Mobilization, Spenomegaly, Spleen size

INTRODUCTION

Mobilized peripheral blood progenitor cells (PBPC) are frequently used in allogeneic stem cell (SC) transplantation for hematologic disorders [1]. From 2002 to 2006, approximately 72% of allogeneic SC transplants performed in adults in the United States used PBPC, outpacing bone marrow (BN) as the most frequent source of donor hematopoietic cells [2].

With the advent of filgrastim (recombinant methionyl human granulocyte-colony stimulating factor [G-CSF], r-metHuG-CSF) over 17 years ago [3], nearly 7 million patients have been treated. Besides its use in PBPC mobilization for autologous or allogeneic transplantation, filgrastim holds indications in the additional settings of BM transplantation, chemotherapy-induced neutropenia, and severe chronic neutropenia. Retrospective analyses, both in the United States and...
worldwide, have provided assurance regarding the safety of filgrastim use in PBPC mobilization [4]. In a retrospective analysis from the International Bone Marrow Transplant Registry (IBMTR) and the European Blood and Marrow Transplant Group (EBMT), the incidence of serious complications was infrequent, occurring in 1% of 1488 donors receiving G-CSF for PBPC mobilization, with a safety profile that was comparable to BM harvesting. Within this experience, rare case reports of splenic rupture following G-CSF treatment have been reported [5-18]. Reported estimates of the incidence of splenic rupture vary in the literature from 0.08% [7,19] to approximately 1% [20].

Previous studies have reported changes in splenic size during PBPC mobilization with G-CSF, but did not take into account changes in splenic volume. The changes in size ranged from a 1.1-fold increase in maximal splenic length and width [21], to an increase in splenic length of 13.8% [22] and an estimated volume increase of 122% [23].

The purpose of the current study was to accurately assess the change in spleen volume in allogeneic PBPC donors during filgrastim administration. In contrast to other studies where the splenic length with or without the width dimension was used to demonstrate size changes, this study estimated splenic volume by ultrasonic measurement of the spleen in 3 dimensions. The primary endpoint was fold change from baseline in spleen volume in postbaseline measurements during mobilization as measured by ultrasound. The secondary endpoints of splenic palpation, hematologic values, and filgrastim dosing (ie, dose, frequency, and schedule) were correlated with spleen volume. Safety was measured by reports of adverse events.

**MATERIALS AND METHODS**

**Donor Recruitment**

Related and unrelated donors were eligible for PBPC donation according to local institutional criteria, plus the following protocol-specific criteria: age ≥18 years, no history of splenectomy, no known previous treatment with G-CSF or granulocyte macrophage colony-stimulating factor (GM-CSF), and no previous PBPC mobilization.

The study was approved by the institutional review board of each participating institution. Written informed consent was obtained from all participants.

**Investigational Product and Administration**

This was an open-label, single-arm study. Donors were administered filgrastim (NEUPOGEN®, Amgen Inc, Thousand Oaks, CA) by subcutaneous injection at a dosage, frequency, and schedule consistent with local institutional practice or National Marrow Donor Program guidelines.

**Assessments**

Baseline splenic ultrasound and palpation assessments were performed on day 1, before the first dose of filgrastim was administered. Splenic assessments were repeated on the day of the first leukapheresis, 2 days and 4 days after the first leukapheresis, and 7 days after the last leukapheresis (Figure 1). On the days of leukapheresis, donors, who were nonfasting, underwent spleen assessment before apheresis. Ninety donors completed the study according to this schedule. After an interim analysis, the protocol was amended to reduce the number of splenic assessments to facilitate accrual. Thereafter, the remaining 219 donors were evaluated only at baseline and the first day of leukapheresis (Figure 1). Complete blood counts (CBCs) and CD34+ cell yield (per kilogram [kg] of donor weight) were determined on the same day as ultrasound assessment. Adverse events were followed through 7 days after the last leukapheresis for all donors.

Splenic measurement was performed by a certified sonographer. Although a 3.5-MHz broadband curvilinear probe was the minimum recommended, the highest frequency transducer that allowed visualization was used. If able to visualize, the subject was examined while supine; otherwise, a right lateral decubitus approach was used in a longitudinal (coronal) axis of the spleen. A posterolateral approach was recommended, with the probe footprint aligned along an intercostal space. Measurements were made without inspiration unless the spleen was masked by the lung and then a deep inspiration was used to minimize and eliminate morphologic masking and variation during respiration.

Splenic measurements (transverse, longitudinal, and diagonal; Figure 2) were made on ultrasonographic images. The longitudinal diameter was defined as the greatest dimension of the spleen in a longitudinal image through the hilum; the transverse diameter was defined as the greatest dimension in a transverse image through the hilum; and the diagonal diameter (thickness) was determined in the transverse image through the hilum as the distance from the hilum to the outer convex surface, approximately perpendicular to the transverse diameter [24]. The splenic volume was estimated by multiplying the product of the longitudinal, transverse, and diagonal diameters (measured in centimeters [cm]) by a factor of 0.523, to approximate the volume of an ellipse [24]. Fold change was calculated as the ratio of volume at measurement to baseline volume.

Physical palpation and ultrasonography of the spleen were performed on the same day in no prespecified order. Clinicians performing splenic palpation were blinded to that day’s ultrasonographic results,
and sonographers were blinded to that day’s palpation result to reduce bias. If necessary for medical care, the clinician was permitted to review that day’s ultrasonographic results after the physical exam and palpation were completed and results recorded.

Statistical Analysis

Statistical analyses were descriptive in nature. Continuous variables were summarized by mean, standard deviation, median, first and third quartiles, and extreme values. Categoric variables were summarized with respect to frequency and percentage of each category.

The primary endpoint was the fold change from baseline in spleen volume in postbaseline measurements during mobilization as measured by ultrasonography. The Spearman Rank correlation coefficient was used to examine the relationship between fold change in spleen volume and secondary endpoints, including spleen palpability, hematologic values, filgrastim dosage, frequency, and schedule. Linear regression analysis was used to investigate the relationship between fold change in spleen size and age. The number and percentage of donors with adverse events were also summarized.

The clinical hypothesis was that the spleen may increase dimensionally and volumetrically with filgrastim administration and PBPC mobilization without serious or significant clinical sequelae. The sample size of approximately 300 donors provided 95% probability of observing at least 1 occurrence of an adverse event that would be expected to occur in 1.25% or more of donors receiving filgrastim. Incidence rates in this study were estimated with a standard deviation of <3%.

RESULTS

Subject Characteristics

Three-hundred nine allogeneic PBPC donors (related or unrelated) were enrolled and received at least 1 filgrastim dose at 14 clinical sites between November 2003 and May 2007. Donors were men and women ranging from 18 to 74 years of age, and were from several racial backgrounds (Table 1). The median baseline
spleen volume was 249.9 cm$^3$ (range: 42-1077). The minimum spleen volume was observed in a 44-year-old female donor weighing 65.5 kg and the largest spleen volume was observed in a 23-year-old male donor weighing 155 kg. A total of 306 donors underwent leukapheresis, and CD34$^+$ data were available for 305 donors. Ninety-eight percent (303/309) of the donors completed the study, and 97% completed filgrastim treatment.

Filgrastim Dosage and Administration

The median daily filgrastim dosage was 10.2 µg/kg, with a range of 5.0 µg/kg to 31.9 µg/kg. Sixty percent of donors received an average daily dosage of filgrastim between 9 µg/kg and 11 µg/kg, with 6% of donors receiving a dose of 9 µg/kg or less and 10% receiving a dose over 16 µg/kg. Twenty-six percent of donors received at least 1 split (morning and evening) filgrastim dose; the remainder received all once-daily doses. The maximum filgrastim dosage was 6.99 µg/kg/day and for donors receiving a dose of 9 µg/kg/day in a split dose over 16 µg/kg, with 6% of donors receiving an average daily dosage of filgrastim between 9 µg/kg and 11 µg/kg, with 6% of donors receiving a dose of 9 µg/kg or less and 10% receiving a dose over 16 µg/kg. Twenty-six percent of donors received at least 1 split (morning and evening) filgrastim dose; the remainder received all once-daily doses. The median duration of filgrastim dosing was 5 days (range: 1-8 days).

Spleen Assessment

The primary endpoint, median fold change in spleen volume from baseline, was 1.47-fold (range: 0.63-2.60) from baseline to the first day of leukapheresis, followed by a progressive decrease, returning to 0.63-2.60) from baseline to the first day of leukapheresis (Figure 3). Twenty-six of 305 (9%) donors evaluated had a 2-fold increase in spleen volume. Median fold changes in spleen volume were similar for donors receiving filgrastim once daily filgrastim or twice daily filgrastim doses above or below 10 µg/kg per dose, and the spleen was palpable at exams on days 9 and 10. Twelve of 17 palpable spleens after baseline which was at or below the median fold change (1.47) had corresponding fold changes between 1.0 and 1.5, and 10. Twenty of 17 palpable spleens after baseline which was at or below the median fold change (1.47) had a palpable spleen after baseline. The only characteristic that was identified that predicted a ≥2-fold change in spleen volume was older age, ranging from a median change (minimum, maximum) at the first leukapheresis of 1.35 (0.63, 2.41) in donors from 18 to 29 years to 1.52 (0.73, 2.41) in donors 60 years of age or older. The linear regression analysis indicated age was a significant predictor for fold change in spleen size from baseline at the first leukapheresis ($P = .0449$).

The maximum fold change in any donor was 2.9, which occurred on the second day of leukapheresis (study day 6) in a 63-year-old white woman with a history of multiple medical problems. She had received filgrastim 6.99 µg/kg/day in a split dose for 5 days.

The spleen was palpable in 19 of 861 exams (2.2%), including 2 baseline assessments, representing 14 donors. There was no relationship between splenic palpability and increase in spleen volume. Although 26 donors (9%) had a ≥2-fold increase in spleen volume, only 1 of these donors had a palpable spleen postbaseline. This donor had a 2.9-fold change in spleen volume, and the spleen was palpable at exams on days 9 and 10. Twelve of 17 palpable spleens after baseline had corresponding fold changes between 1.0 and 1.5, which was at or below the median fold change (1.47) for the population. Tenderness or guarding on physical examination was reported by 8 donors, 2 of whom also exhibited a palpable spleen.

Relationship between Volume Change and Other Characteristics

Hematologic and leukapheresis results are presented in Table 2. Peak absolute neutrophil count (ANC) during mobilization was not significantly correlated with splenic volume fold change (Spearman Rank coefficient = 0.06-0.17 [minimum to maximum observed across leukapheresis time points, respectively]). Neither CD34$^+$ cell yield (per kg donor weight) nor

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**Table 1. Donor Demographics and Baseline Characteristics**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects, N</td>
<td>309</td>
</tr>
<tr>
<td>Median age, years (range)</td>
<td>44.0 (18, 74)</td>
</tr>
<tr>
<td>Sex, male/female, N</td>
<td>173/136</td>
</tr>
<tr>
<td>Race, N (%)</td>
<td></td>
</tr>
<tr>
<td>African American or black</td>
<td>20 (6)</td>
</tr>
<tr>
<td>Caucasian or white</td>
<td>265 (86)</td>
</tr>
<tr>
<td>Other</td>
<td>24 (8)</td>
</tr>
<tr>
<td>Median baseline weight, kg (range)</td>
<td>85.1 (42, 217)</td>
</tr>
<tr>
<td>Median baseline height, cm (range)</td>
<td>171.5 (87, 198)</td>
</tr>
<tr>
<td>Median baseline spleen volume, cm$^3$ (range)$^a$</td>
<td>249.9 (42, 1077)</td>
</tr>
<tr>
<td>Spleen palpability at baseline, N (%)</td>
<td>304 (98)</td>
</tr>
<tr>
<td>Nonpalpable</td>
<td>304 (98)</td>
</tr>
<tr>
<td>Palpable</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Missing data</td>
<td>3 (1)</td>
</tr>
</tbody>
</table>

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$^a$Spleen volume $= (\text{longitudinal length} \times \text{transverse length} \times \text{diagonal length}) \times 0.523.$

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**Figure 3.** Distributions of spleen volume fold change on leukapheresis day 1, leukapheresis day 1 + 2 days, leukapheresis day 1 + 4 days, and the last day of leukapheresis + 7 days. Solid line indicates a fold change = 1. Dashed line indicates median.
baseline body weight was significantly correlated with splenic volume change (Spearman Rank coefficient = -0.06-0.05 and -0.12-0.06, respectively). Although there was a wide range of filgrastim dosages administered, and daily frequency (once per day or in split or 2 doses per day), these variables were not significantly correlated with fold change in spleen volume (Spearman Rank coefficient = -0.07-0.06 and -0.10-0.05, respectively).

**Adverse Events**

Of the 309 donors enrolled, 292 (94%) had at least 1 adverse event. The most common adverse events were headache (44%) and bone pain (36%), both of which were generally considered related to filgrastim (42% and 36%, respectively). Most adverse events were considered to be mild or moderate in intensity and no donors experienced adverse events that were considered life-threatening or fatal. Severe adverse events experienced by more than 1 donor are listed in Table 3. Six donors (2%) experienced serious adverse events, which led to study withdrawal for 3 donors (Table 3). None of the donors with ≥2-fold change in spleen volume experienced serious adverse events, withdrew from the study prematurely, or discontinued filgrastim early. Upper abdominal pain (all mild to moderate severity) was reported by 6 donors.

**DISCUSSION**

This is the largest study designed to assess changes in spleen size during PBPC mobilization with filgrastim in normal donors who were scheduled for PBPC mobilization and collection. The change in spleen volume was accurately measured by 3-dimensional ultrasonography following filgrastim administration in “normal” donors of PBPC. To be able to review spleen size dynamics across the range of real-world practices, filgrastim was administered according to institutional standard practices, National Marrow Donor Program (NMDP), or other similar guidelines.

The results of this study suggest that increases in spleen volume following filgrastim mobilization were transient, peaked on the first day of leukapheresis (median fold change 1.47), and returned to near baseline levels by 7 days after the last leukapheresis (median fold change 1.08). Changes in spleen volume did not correlate with filgrastim dose level or frequency, or with total CD34+ yield, ANC, or baseline weight. Median fold change from baseline tended to be largest for the oldest donors (≥60 years); this is hypothesized to be related to the spleen’s increased role in myelopoiesis or increased intrasplenic accumulation of circulating granulocytes and myeloid precursors in the spleens of older donors undergoing mobilization. Preclinical models have suggested decreasing hematopoietic stem cell homing after mobilization with aging [25, 26], which could result in splenic accumulation of progenitor and stem cells.

No donors experienced splenic rupture in this study. However, the incidence of splenic rupture may be too rare to be detected in a study of 309 individuals. This study was powered to detect an event that occurred with a frequency of 1.25% or more, but the risk is probably much lower. Between February 1997 and November 2007, 7669 donors underwent filgrastim-stimulated PBPC collection coordinated by the NMDP. No donors had splenic bleeding or rupture or other abdominal catastrophe during mobilization (D. Confer, personal communication, 2007). Worldwide, only 6 PBPC donors were identified as experiencing splenic rupture after G-CSF mobilization in a comprehensive literature review (Table 4). Therefore, splenic rupture is uncommon in PBPC donors mobilized by G-CSF. The question of why the spleen enlarges may be best answered from the cases of splenic rupture identified (D.C. Dale, personal communication, 2008). Four of the 6 donors with splenic rupture required a splenectomy. The histology of each of the removed spleens indicated extramedullary myelopoiesis possibly leading to expansion of the hematopoietic tissue in the spleen with the new blood cell formation (D.C. Dale, personal communication, 2008). This hypothesis has also been considered by others [23].

Approximately 10% of donors had ≥2-fold increases in spleen volume. These increases did not correspond with spleen palpability on physical examination. The most frequently reported adverse event among donors with ≥2-fold increase in spleen volume was back pain, which occurred in 38% of donors in this subgroup compared with 25% of the overall study population.

The utility of palpating for the spleen on physical examination was not evident. Spleen palpability did
not correlate with increased spleen volume. Splenic examinations where the spleen was considered palpable were often not the assessment at which the spleen was at its largest volume. Similarly, guarding, pain, or tenderness on examination did not correspond with maximum increases in spleen volume. In some cases, these events may have resulted from the process of the examination itself (ie, pressure applied during examination) and not by splenic changes. Consistent with previous reports, physical examination may be ineffective in diagnosing splenomegaly, in part because of differences in donor body size and habitus [27].

The frequency of adverse events observed in this study was similar to those observed in other studies of filgrastim, and were consistent with previously reported data [28]. Reported adverse events were largely events previously associated with either filgrastim use or the leukapheresis procedures.

In summary, increase in spleen size during PBPC mobilization with filgrastim was common, but transient. Spleen palpability was rare, and did not correlate with increase in splenic volume. Spleen volume fold change was not correlated with filgrastim dosage or schedule, ANC values, CD34+ yield, or donor weight.

**FINANCIAL DISCLOSURE STATEMENT**

L.D., M.G., and W.L. are employees and stockholders of Amgen Inc. A.N. has acted as a consultant to Genzyme, W.B. has acted as a consultant to Amgen, and M.H.A. has acted as a consultant to Millennium Pharmaceuticals. P.J.S., W.B., M.H.A., and B.B. have received research funding from Amgen, Celgene, and Genzyme, and A.N. has received research funding from Biogen Idec. T.B.S. has received honoraria from Millennium Pharmaceuticals. M.H.A. is a member of the board of directors for the National Bone Marrow Transplant Link.

**STATEMENT OF PRIOR PRESENTATION**


**ACKNOWLEDGMENTS**

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**AUTHORSHIP**

Contribution: P.J.S., W.B., M.H.A., R.G., A.S.A., A.N., K.S.H., C.S., C.C., B.B., T.B.S., H.M.L, and A.M.Y. performed the research, interpreted the data, and wrote the paper. M.G. collected, analyzed,
Table 4. PBPC Donors with Splenic Rupture after G-CSF Administration Identified in the Literature

<table>
<thead>
<tr>
<th>Body weight / height</th>
<th>Treatment for spleen rupture</th>
<th>Age (years) / Sex</th>
<th>Splenic rupture</th>
<th>History of the spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kro¨ ger et al 2002 [20]</td>
<td>Splenic rupture</td>
<td>Not stated / M</td>
<td>Leukapheresis</td>
<td>Extramedullary myelopoiesis and rare megakaryocytes</td>
</tr>
<tr>
<td>Nuamah et al 2006 [9]</td>
<td>Splenic rupture</td>
<td>Not stated / F</td>
<td>Splenectomy</td>
<td>Extramedullary myelopoiesis and rare megakaryocytes</td>
</tr>
</tbody>
</table>

CT indicates computed tomography; d, day; F, female; M, male; NA, not applicable; Q12 h, every 12 hours; US, ultrasound; G-CSF, granulocyte-colony stimulating factor.

REFERENCES


