For the past 30 years, marrow transplantation has been used successfully to treat an ever-increasing number of diseases. Over the past 10 years, peripheral blood progenitor cell (PBPC) concentrates have replaced marrow as the source for autologous progenitor cell transplants. Since 1995, some centers have been using PBPC concentrates in place of marrow for transplants involving HLA-compatible siblings,1-4 and trials using PBPC concentrates for transplants involving HLA-compatible unrelated donors for second transplants were begun by the National Marrow Donor Program (NMDP) in early 1997.

Experience with transplants involving HLA-compatible sibling donors has shown that PBPC concentrates can be collected safely and that these transplants are effective. From the donor’s point of view, the collection of PBPC concentrates has several advantages over that of marrow: avoiding the potential risk of anesthesia, the painful aspiration procedure, and the prolonged postcollection recovery period. PBPC concentrates may also be advantageous for transplant recipients, in that they contain more hematopoietic precursors than marrow does.5 Preliminary studies on transplants involving HLA-compatible sibling donors have found that, when PBPC concentrates are used in place of marrow, both neutrophil and platelet engraftment times and acute transplant-associated morbidity are reduced, while the incidence of acute GVHD is similar.5 Recipients of PBPC concentrates also have a more rapid reconstitution of T- and B-cell proliferation responses to phytohemagglutinin, pokeweed mitogen, tetanus toxoid, and Candida sp.6 Although results are promising, some studies have suggested that the incidence of chronic GVHD in recipients of PBPC concentrate transplants is high,7 and some centers are reducing the number of T cells in PBPC concentrates by antibody-mediated selection of CD34+ cells.8 Preliminary results of allogeneic transplants using PBPC concentrates enriched with CD34+ cells have been encouraging, in that engraftment has remained rapid, while the incidence of acute and chronic GVHD has been low.8

Transplants using marrow from HLA-compatible unrelated donors have been used to successfully treat acute myelogenous leukemia, acute lymphocytic leukemia, chronic myelogenous leukemia, aplastic anemia, congenital immunodeficiencies, metabolic storage diseases, and many other diseases. The success rates of unrelated-donor marrow transplants are as high as 74 percent for patients with chronic myelogenous leukemia.9 When compared with transplants involving HLA-compatible siblings, however, transplants from unrelated donors are complicated by higher rates of GVHD and transplant-related mortality. It is possible that the use of PBPC concentrates rather than marrow for unrelated-donor transplants could result in higher rates of engraftment and less transplant-associated morbidity and hence better disease-free survival. Trials of PBPC concentrates for unrelated-donor transplants are therefore now warranted. In this review, the procedures used to collect marrow from unrelated donors and to mobilize and collect PBPC concentrates from sibling donors are compared and contrasted. PBPC mobilization and collection issues unique to unrelated donors and precautions that should be taken to ensure the well-being of the unrelated donor in this setting are discussed.

**ABBR eviations:** ECOG = Eastern Cooperative Oncology Group; LT-CiC(s) = long-term colony-initiating cell(s); NMDP = National Marrow Donor Program; PBPC(s) = peripheral blood progenitor cell(s).

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**Marrow Donation**

Marrow was first used successfully for hematopoietic transplants involving HLA-identical siblings in the late 1960s and early 1970s.10 But the first successful transplants using marrow from HLA-matched unrelated donors were not per-
formed until the late 1970s, and they involved small local or regional registries of potential marrow donors. Initially, efforts to develop larger centralized registries were greeted with skepticism. Concerns centered on issues of donor safety, the lack of clear evidence that recipients benefited, and the general belief that a volunteer’s willingness to help an anonymous stranger stopped short of submitting to an operation under anesthesia. In the past 10 years, these concerns have been effectively dispelled by the NMDP in the United States and similar national registries abroad.

With support from a series of federal contracts administered by the US Navy, the National Heart, Lung, and Blood Institute, and the Health Resources and Services Administration, the NMDP has developed into the world’s largest registry of unrelated donors, listing more than 3.2 million of the estimated 5 million volunteers worldwide. Although transplants from unrelated donors are now widely accepted, marrow donation continues to entail significant risks and discomforts for the donor.

Marrow collected from NMDP donors is aspirated under sterile conditions from the donor’s posterior iliac crests. Donors are given general, spinal, or epidural anesthesia. The mean volume of marrow aspirated from the first 493 NMDP donors was 1050 mL, and the range was 180 to 2983 mL. Because of the large volume of marrow that must be aspirated, the median duration of an NMDP marrow collection is about 60 minutes, although up to 3 hours may be required. Because the aspirated marrow contains a significant number of RBCs, most donors receive transfusion(s), but only 3 of the first 493 NMDP donors were given allogeneic RBCs. Autologous RBCs were given to 90 percent of the donors: 27 percent were given 1 unit, 51 percent were given 2 units, and 12 percent were given 3 units or more.

In many cases, donors are admitted to and discharged from the hospital on the day of the procedure, but donors frequently must stay in the hospital for 1 night and occasionally for 2 nights. After the collection, almost all donors experienced one or more symptoms. During the first 72 hours, 63 to 79 percent of the first 493 NMDP donors experienced pain at the collection site, pain on walking, or back pain. Most of these symptoms disappeared within 21 days: 64 percent of the donors felt that they had recovered fully after 14 days and 81.7 percent after 21 days, but 10 percent had problems related to the collection that took more than a month to resolve.

Safeguards for marrow donors

Because unrelated donors derive no direct benefit from the donation, it was critical to make the procedure as safe as possible. The NMDP and other donor registries have therefore established standard policies and procedures to minimize the small risk that a serious complication could arise from anesthesia or from the procedure itself. NMDP standards require that potential donors undergo medical screening, physical examination, and laboratory tests before they are approved to donate. To minimize the risk associated with the anesthesia, donors with health problems that would place them at more than minimum risk are excluded. As a result, only people who are in nearly perfect health can donate. Overweight persons or those over the age of 61 years are also excluded, as they too may be at increased risk from anesthesia.

To minimize the risk to donors from the marrow collection itself, collection teams and centers are required to meet standards for experience and to be approved by the NMDP Membership and Process Improvement Committee. The volume of marrow aspirated is limited to 1500 mL, aspiration from the sternum or anterior iliac crests is discouraged, and autologous RBC units must be collected in proportion to the amount of marrow that will be aspirated. The NMDP collects and reviews data on the volume of marrow collected, the concentration of nucleated cells therein, the duration of the collection, the type and duration of the anesthesia, and the incidence of acute and chronic donor complications. Donors are also monitored weekly after discharge from the hospital to document any symptoms they experience, to determine if a late complication occurs, and to note the length of time they require to recover completely. Donors who experience severe or unexpected complications are followed closely until their problems resolve. The performance of every collection center is reviewed periodically. Centers that perform poorly are identified, and the NMDP works with them to improve.

This cautious approach to handling unrelated marrow donors has proven to be successful. The risks of the donation process have not been entirely eliminated, however, and complications still occur.

Risks of marrow donation

Despite these precautions, marrow donors have a 0.1- to 0.3-percent chance of experiencing a major, life-threatening complication and a 6- to 12-percent chance of a less severe complication. Although deaths have occurred in 4 persons who donated marrow for a relative, from 1987 through mid-1998, over 7000 people donated marrow through the NMDP and no collection-related donor deaths occurred. However, the NMDP has found that serious medical complications have occurred in 0.2 percent of their marrow donors. Complications that have occurred in both unrelated and sibling donors include infections, mechanical injuries, and reactions to anesthesia. Two cases of osteomyelitis have been reported, as has a case of bacterial sepsis. Mechanical injuries include sciatic leg pain, injury to the sacroiliac joint, and fractures of the iliac crest, while reactions to anesthesia include laryngospasm, profound bradycardia, hypotension, hypoxia, and cardiac arrest.

The incidence of minor complications in NMDP donors, including fatigue, back pain, and numbness in the leg...
and foot, was 12 percent. In most cases, these problems resolve without significant treatment, but persistent symptoms related to the collection have kept at least one donor from returning to work for more than a year.

Despite the return of autologous blood, many donors are anemic after the collection. Other laboratory abnormalities that can occur are transient elevations in serum alkaline phosphatase and osteocalcin.

In addition to these risks and discomforts, the donation process requires 20 to 40 hours of the donor’s time for the drawing of up to 3 units of autologous RBCs, for physical examinations and laboratory tests, and for the hospital stay before and after the donation. Marrow donors often miss 1 or 2 days of work, and back pain frequently is aggravated by the lifting of heavy objects or by twisting. As a result, donors’ activities at work and at home are often limited for up to 2 weeks.

DONATION OF PBPC CONCENTRATES

While the number of donors in the NMDP has grown more than 100-fold over the past 10 years, the risks associated with marrow aspiration and anesthesia likely prevent some people from participating. If a safe and convenient alternative to the aspiration of large quantities of marrow under general anesthesia were available, more people would likely join the NMDP and other registries. One alternative is to collect very small quantities of marrow under local anesthesia from donors given hematopoietic growth factors, but experiences with one growth factor, rHu-G–CSF, suggest that the expansion of marrow hematopoietic progenitors during a course of G–CSF is limited. Another alternative is to collect PBPC concentrates from donors given hematopoietic growth factors. These concentrates have been used for many years for autologous marrow transplants and those involving HLA-compatible siblings. Using PBPC concentrates for unrelated-donor transplants is potentially safer and less time-consuming for donors.

PBPC donation requires increasing the concentration of HPCs in the blood and removing them by apheresis. An allogeneic transplant of PBPC concentrates involving HLA-matched siblings was first performed in 1987, but no agents were given to increase the donor’s concentration of circulating HPCs. To obtain enough cells, therefore, it was necessary to perform 10 PBPC concentrate collections and to remove the T-lymphocytes from each one. Now, all people who donate allogeneic PBPC concentrates are first treated with hematopoietic growth factors to increase the concentration of progenitor cells in their blood.

Both the donation and collection of PBPC concentrates present donors with real as well as potential risks. The process for donating PBPC concentrates is similar to that for platelepheresis, and the risks associated with collecting platelets are well known and minimal. Collecting PBPC concentrates, however, presents some additional risks to the donor. The procedure lasts longer, and back-to-back procedures are often needed. PBPC concentrate donors must also be given growth factors. All of the risks associated with mobilizing and collecting PBPC concentrates have yet to be fully defined, but the experience gained from collecting concentrates from HLA-matched sibling and research donors is abundant and growing quickly.

Mobilization of PBPCs

The rHu-G–CSF and rHu-G–CSF have both been used to mobilize PBPCs for allogeneic transplantation. When they are used as single agents, G–CSF is the more effective of the two. The combination of G–CSF and GM–CSF may be more effective at mobilizing earlier progenitors, but G–CSF alone is used almost exclusively for mobilizing progenitor cells for transplants involving HLA-matched allogeneic donors. Chemotherapeutic agents are also used to mobilize PBPCs in autologous donors, but their use is contraindicated in allogeneic donors.

When G–CSF is used to mobilize PBPCs for transplants involving HLA-matched siblings, 10 to 16 µg of G–CSF per kg of donor weight is typically given. Although G–CSF can be given intravenously or subcutaneously, it is almost always given as a subcutaneous injection once or twice a day. G–CSF increases the concentration of circulating progenitor cells in a dose-dependent manner to at least 7.5 µg per kg. Higher doses may further increase the number of circulating progenitor cells, but good comparisons of the relative effectiveness of higher dosing schedules are not available. However, to be sure that an adequate dose of G–CSF is given, most centers administer more than 7.5 µg per kg a day.

G–CSF begins to increase the donor’s WBC count within 4 hours after the first dose is given, but the increase in blood HPCs is delayed. The concentration of CD34+ cells in the blood begins to increase after 3 days of G–CSF and reaches a peak after 5 or 6 days. Although the WBC count remains elevated as long as G–CSF is given, the increase in HPCs is transient. Even if G–CSF is administered daily, the concentration of CD34+ cells in the blood falls slightly after 7 days and decreases to one-third of peak levels after 10 days. If a daily dose of G–CSF is not given, the CD34+ cell counts also fall quickly.

The G–CSF-induced increase in the concentration of CD34+ cells can be very dramatic. When 7.5 to 16 µg per kg of G–CSF is given daily for 5 days to healthy donors, CD34+ cell counts increase 8- to 13-fold. A slightly greater, 9- to 37-fold increase is seen in the concentration of CFU–GM in the circulation. Long-term colony-initiating cell (LT–CIC) assays are thought to be a better measure than CD34+ cells or CFU–GM of primitive progenitor cells. When LT–CIC assays held in culture for 5 weeks were used to assess G–CSF-mediated mobilization, the concentration of circulating LT–CICs increased 60-fold. However, when the increase in circulating progenitors following G–CSF admin-
istration was measured by using LT–CIC assays held in culture for 8 weeks; the increase in LT–CIC was 90 to 95 percent less, but it was similar to the increase in the concentration of CD34+ cells and CFU–GM. These results suggest that G–CSF mobilizes primitive progenitor cells, but it is most potent at mobilizing HPCs of intermediate maturity.

While the G–CSF-induced increase in CD34+ cell concentration in the typical healthy donor is dramatic, the increase in CD34+ cell counts can range widely. When 10 µg per kg of G–CSF is given daily, the CD34+ cell counts increase on average to 77 × 10^6 cells per L after 5 days. However, in 5 to 10 percent of healthy donors, the mobilization of CD34+ cells is poor, and the peak CD34+ cell count may be as low as 8 × 10^6 cells per L. By contrast, in some healthy donors, the CD34+ cell counts can increase to as much as 1100 × 10^6 cells per L.

G–CSF causes almost all donors to experience at least one symptom. The most common symptoms that donors describe are bone pain, headaches, myalgia, and fatigue (Table 1). About 90 percent of PBPC concentrate donors experience at least one of these symptoms, and people who experience three or more of them often feel as if they have contracted an influenza virus or have “the flu.” These symptoms can begin within hours of the first injection and often persist throughout the mobilization. Symptoms may be tempered but not entirely relieved by acetaminophen or ibuprofen. When standardized drug toxicity scales are used to score the severity of the symptoms, most donors feel that they are mild, but some describe them as moderate and a few describe them as severe (Table 2). When the effects of the G–CSF on the donor's activity level were measured by means of an Eastern Cooperative Oncology Group (ECOG) performance status scale, up to 38 percent of the donors given a G–CSF dose of 10 µg per kg a day felt that their ability to perform physically strenuous activities was limited. In approximately 1 to 5 percent of the cases, symptoms can be severe enough to make research donors drop out of a study, but symptoms rarely if ever prevent sibling donors from continuing to receive G–CSF.

Symptoms subside quickly after the last dose of G–CSF is given and usually disappear within 48 hours. However, when an ECOG performance status scale was used to assess recovery, an occasional donor felt that he or she had not fully recovered 9 to 16 days after the last dose was given (Table 3).

G–CSF also induces several changes in blood chemistries. Sodium, LDH, alkaline phosphatase, ALT, and uric acid increase, while potassium, bilirubin, and magnesium decrease. These changes are mild and resolve within a week, and they have had little or no clinical effect on healthy donors, though some donors have been given potassium supplements.

### Collecting PBPC concentrates

Most blood cell separators that are used to collect platelets can also be used to collect PBPC concentrates, and their performance is similar. The procedures used to collect PBPC concentrates are very similar to those used to collect MNCs. The blood cell separators are typically operated at a flow rate of 40 to 60 mL per minute, and citrate is used as an anticoagulant. Usually, 12 to 15 L of whole blood is processed over 3 to 5 hours. Most separators require the use of two venous access sites.

A standard PBPC concentrate has not yet been defined. It is known that PBPC concentrates are rich in MNCs and platelets but have few RBCs or granulocytes. When healthy donors are given 5 to 10 µg per kg of G–CSF and when 8 to 9 L of whole blood is processed, the PBPC concentrates contain 35.0 × 10^6 WBCs, 33.3 × 10^6 MNCs, 1.71 × 10^6 granulocytes, and 7.2 mL of RBCs. The number of platelets in the PBPC concentrates was 480 × 10^9, similar to the number found in a platelepheresis unit. The most abundant

### TABLE 1. Incidence (%) of symptoms in healthy people donating G–CSF-mobilized PBPC concentrates

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Stroncek et al. (n = 21)</th>
<th>Leitman et al. (n = 24)</th>
<th>Grigg et al. (n = 15)</th>
<th>Anderlini et al. (n = 350)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone pain</td>
<td>76</td>
<td>100</td>
<td>87</td>
<td>84</td>
</tr>
<tr>
<td>Headache</td>
<td>67</td>
<td>38</td>
<td>33</td>
<td>54</td>
</tr>
<tr>
<td>Fatigue</td>
<td>42</td>
<td>31</td>
<td>47</td>
<td>31</td>
</tr>
<tr>
<td>Myalgia</td>
<td>66</td>
<td>29</td>
<td>27</td>
<td>NA</td>
</tr>
<tr>
<td>Nausea</td>
<td>24</td>
<td>8</td>
<td>0</td>
<td>13</td>
</tr>
</tbody>
</table>

* 10 µg per kg per day.
† 12 µg per kg per day.

### TABLE 2. Severity of symptoms in PBPC concentrate donors given G–CSF for 6 days

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Number of donors with each grade of toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 µg/kg/day (n = 19)</td>
</tr>
<tr>
<td></td>
<td>0 1 2 3</td>
</tr>
<tr>
<td>Skeletal pain</td>
<td>9 5 5 0</td>
</tr>
<tr>
<td>Headache</td>
<td>5 9 4 1</td>
</tr>
<tr>
<td>Myalgia</td>
<td>13 5 1 0</td>
</tr>
<tr>
<td>Fatigue</td>
<td>12 5 2 0</td>
</tr>
<tr>
<td>Insomnia</td>
<td>16 2 1 0</td>
</tr>
<tr>
<td>Paresthesia</td>
<td>16 3 0 0</td>
</tr>
<tr>
<td>Back pain</td>
<td>16 2 1 0</td>
</tr>
<tr>
<td>Nausea</td>
<td>16 3 0 0</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>17 2 0 0</td>
</tr>
<tr>
<td>Rigors</td>
<td>16 3 0 0</td>
</tr>
<tr>
<td>Rash</td>
<td>17 1 1 0</td>
</tr>
<tr>
<td>Upper respiratory distress</td>
<td>16 3 0 0</td>
</tr>
</tbody>
</table>

* Severity ratings are 0 = none, 1 = mild, 2 = moderate, 3 = severe.

50 × 10⁶ CD34+ cells are collected per L of whole blood processed. Only one-half of all CD34+ cells that pass through it, and 30 to 40 percent of CD34+ cells collected is, however, most closely related to the number of CD34+ cells in the peripheral circulation. In general, the blood cell separator removes about 25 to 30 percent of CD34+ cells that the concentrate will contain. As a result, to increase cell yield, both the volume of blood processed during each procedure and the number of procedures performed must be increased. The volume of blood processed is limited by the rate of flow through the separator and the donor's tolerance of the procedure. The flow rate is limited by separator design and venous access. Separators have a maximum rate above which they no longer operate efficiently, but the size of the donor's veins or intolerance of the citrate anticoagulant often prevents the operation of the cell separator at maximum flow rates. To prevent citrate from limiting the flow rate, heparin alone or in combination with citrate can be used as an anticoagulant. The length of time that the donor can tolerate being seated and attached to the cell separator also limits the duration of the collection.

Unfortunately, the relationship between the concentration of CD34+ cells in the blood and the quantity of CD34+ cells in the PBPC concentrate is not strong enough to use a preapheresis count to reliably predict the quantity of CD34+ cells that the concentrate will contain. As a result, the number of CD34+ cells in each PBPC concentrate must be counted directly before it can be determined that enough cells have been collected.

It is, of course, desirable to obtain enough progenitor cells for a successful transplant from a single collection. Typically, the target dose is 5 × 10⁶ CD34+ cells per kg of recipient weight, but the exact minimum dose necessary for a successful transplant is not certain, and 2 or 3 × 10⁶ cells per kg or less may be sufficient. The average number of progenitor cells that can be collected by processing 9 to 10 L of blood over 2.5 to 3 hours from healthy donors who are given 5 to 10 µg of G-CSF is 412 × 10⁶, enough to provide a transplant for a person weighing 80 kg. But the total number of CD34+ cells in each PBPC concentrate is highly variable. In 14 percent of the cases, a single PBPC concentrate collected from healthy donors under these conditions contains fewer than 150 × 10⁶ CD34+ cells.32 By contrast, a single PBPC concentrate collected under the same conditions can contain as many as 1600 × 10⁶ CD34+ cells.32

When a donor is given a course of hematopoietic growth factor and the CD34+ cell count fails to rise sufficiently, more than one PBPC concentrate must be collected. Other situations in which more than one PBPC concentrate often has to be collected are those involving transplants to heavy recipients and those in which the concentrate is processed to remove T cells. Because initial results of PBPC concentrate transplants involving HLA-matched siblings have shown that recipients experience a high rate of chronic GVHD,7 some centers routinely transplant concentrates that have been treated to remove T cells. When T cells are removed, about one-half of the PBPC concentrate's CD34+ cells are lost. As a result, about 10 × 10⁶ CD34+ cells must be collected to provide a transfusion of 5 × 10⁶ cell per kg.

The PBPC concentrate CD34+ cell yield can be improved by increasing the number of cells mobilized or the volume of blood processed during the collection. Unfortunately, when CD34+ cells are poorly mobilized with G–CSF, there is no good way to increase the level of mobilization. As a result, to increase cell yield, both the volume of blood processed during each procedure and the number of procedures performed must be increased. The volume of blood processed is limited by the rate of flow through the separator and the donor's tolerance of the procedure. The flow rate is limited by separator design and venous access. Separators have a maximum rate above which they no longer operate efficiently, but the size of the donor's veins or intolerance of the citrate anticoagulant often prevents the operation of the cell separator at maximum flow rates. To prevent citrate from limiting the flow rate, heparin alone or in combination with citrate can be used as an anticoagulant. The length of time that the donor can tolerate being seated and attached to the cell separator also limits the duration of the collection.

A typical granulocyte concentrate apheresis procedure processes 7 L of blood and a platelet concentrate apheresis procedure processes 4 to 5 L. To ensure that adequate numbers of cells are collected for all recipients, the typical PBPC apheresis procedure processes 12 to 15 L of whole blood over 3 to 5 hours. Although performing more than one collection can increase the total cell yield even further, the total number of collections that can be performed during a mobilization course is limited. The fact that the peak concen-

<table>
<thead>
<tr>
<th>TABLE 3. Percentage of people who were given 5 days of G–CSF, donated two PBPC concentrates, and reported a restriction in physically strenuous activities (ECOG)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day†</td>
</tr>
<tr>
<td>----</td>
</tr>
<tr>
<td>Before G–CSF</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>14</td>
</tr>
<tr>
<td>21</td>
</tr>
<tr>
<td>28</td>
</tr>
</tbody>
</table>

* An ECOG performance status of 1 is defined as being restricted in carrying out physically strenuous activities but ambulatory and able to carry out work of light or sedentary nature, e.g. light housework or office work.
† G–CSF was given on Days 1 through 5 and PBPC concentrates were collected on Days 5 and 6.

WBCs in PBPC concentrates are lymphocytes (65%), and less than 4 percent are neutrophils.

Typically, only 1 to 2 percent of the MNCs in the PBPC concentrate express the CD34 antigen, but the total number of CD34+ cells collected depends on the volume of whole blood processed and the concentration of CD34+ cells in the blood, is high.32 The concentration of CD34+ cells, WBCs, and neutrophils in the blood at the time of collection is related to the number of CD34+ cells collected, which increases as the cell counts increase.32,38,39 The quantity of CD34+ cells collected is, however, most closely related to the concentration of CD34+ cells in the peripheral circulation. In general, the blood cell separator removes about one-half of all CD34+ cells that pass through it, and 30 to 50 × 10⁶ CD34+ cells are collected per L of whole blood processed.38 Several factors in addition to the concentration of CD34+ cells affect the CD34+ cell yield. More CD34+ cells are collected from younger, heavier, and male donors and donors with higher premobilization WBC counts.32,38,40

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transplantation of CD34+ cells lasts for three days (Days 3-6) limits the number of effective collections to three. Experience with transplants involving HLA-compatible sibling donors and these larger-volume apheresis procedures has found that, in 62 to 68 percent of transplants, only one collection is needed, but, in 14 percent, more than two PBPC concentrates were needed \(^{(37,43)}\) (Table 4).

If two or more PBPC concentrates are collected from a donor and they do not contain enough CD34+ cells, marrow is often collected as well. Most centers collect PBPC concentrates while the recipient is undergoing pretransplant myeloablative therapy, and marrow must be collected immediately after it has been determined that the collection of PBPC concentrates has failed. Sometimes PBPC concentrates are cryopreserved before the recipient is given myeloablative therapy, and in these cases either an additional course of G-CSF can be given and more PBPC concentrates collected, or marrow can be aspirated. Unfortunately, the results of the collection of PBPC concentrates after a second course of G-CSF are very similar to those of collection after the first one. When healthy donors have been given a course of G-CSF on two occasions separated by a few months to more than a year, the increases in CD34+ cell counts and in the quantity of CD34+ cells collected were remarkably similar \(^{(44,45)}\). Therefore, if a PBPC concentrate yield is low because of a small increase in the concentration of CD34+ cells, a second mobilization with G-CSF is not likely to be helpful. If, however, the yield was low because of a problem with the apheresis procedure, a second mobilization and collection would likely be effective. It is not certain how much time should elapse between mobilizations.

Complications in PBPC concentrate donors treated with G-CSF

The administration of a hematopoietic growth factor and the collection of progenitor cells expose donors to several risks. Not only do PBPC concentrate donors experience symptoms and changes in blood chemistries, but marked changes in blood counts occur as well. When G-CSF is given, the WB count in healthy donors can increase to as many as \(81 \times 10^9\) per L. \(^{(32)}\) The greatest increase occurs in the neutrophil fraction, but the numbers of lym-

<table>
<thead>
<tr>
<th>TABLE 4. Collecting G-CSF-mobilized PBPC concentrates for transplants involving HLA-identical siblings: number of collections required and the need to use a central venous catheter</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>G-CSF dose (µg/kg/day)</strong></td>
</tr>
<tr>
<td>Blood volume processed during each collection</td>
</tr>
<tr>
<td>Target CD34+ cell dose ((x \times 10^9/\text{kg}))</td>
</tr>
<tr>
<td>Transplants requiring 1 donation (%)</td>
</tr>
<tr>
<td>Transplants requiring 2 donations (%)</td>
</tr>
<tr>
<td>Transplants requiring 3 or more donations (%)</td>
</tr>
<tr>
<td>Donors requiring a central venous catheter (%)</td>
</tr>
</tbody>
</table>

* Percentage requiring two or more collections.

phocytes and monocytes increase as well. People given G-CSF have developed cutaneous vasculitis \(^{(46)}\), iritis \(^{(47)}\), and acute gouty arthritis \(^{(48)}\), although rarely, and it has been hypothesized that the extremely high neutrophil counts induced by G-CSF could harm the donor by enhancing inflammatory lesions. As a result, some centers reduce the G-CSF dose if counts rise above 50 or \(75 \times 10^9\) cells per L \(^{(49)}\), but it is not certain whether dose reductions are necessary. It is clear, though, that a dose of G-CSF should not be skipped entirely: if it is not given, the number of CD34+ cells in the blood will then drop quickly.

Transient drops in blood counts occur after mobilized PBPC concentrates are collected. The extent of this reduction depends on the volume of blood processed during each collection and the total number of concentrates collected. The donor’s platelet counts fall 20 to 30 percent with each collection and remain reduced for 4 to 6 days after the last collection \(^{(36,50,51)}\). If one concentrate is collected, counts rarely fall below \(100 \times 10^9\) cells per L, but, if two concentrates are collected, platelet counts fall to between \(100 \times 10^9\) per L and \(77 \times 10^9\) cells per L in 22 percent of donors (Table 5). \(^{(36)}\) While spontaneous bleeding has not been a problem when counts have fallen to these levels, donors may be at increased risk for bleeding if they are injured. It is possible to reduce the drop in platelet counts by recovering platelets from the PBPC concentrates and giving them to the donor. However, the quantity of platelets that can be recovered decreases as the platelet count falls with each collection. The drop in platelet counts is predominantly due to the loss of platelets in the PBPC concentrate, but G-CSF administration alone reduces platelet counts. One study found that the platelet count fell approximately 25 percent in healthy donors who were given G-CSF but did not donate PBPC concentrates. \(^{(36)}\)

Neutrophil counts in PBPC donors fall below baseline levels for 1 to 2 weeks after the collection \(^{(50,51)}\). The absolute neutrophil count falls below \(1.0 \times 10^9\) per L in 10 percent of donors, but it is low for only a few days (Table 6). Infections have not been a problem, so PBPC concentrate donors should not be at risk of infection.

Otherwise, PBPC concentrate donors are exposed to the same risks all apheresis donors face: blood loss if the cell separator fails, hematoma or nerve injury at the site of the intravenous line placement, air emboli, hypersensitivity to ethylene oxide, and citrate toxicity. Citrate toxicity is the most common, but it can be controlled by giving the donor calcium, by reducing the dose of citrate the donor receives by decreasing the blood flow rate, or by using heparin. One survey found that symptoms of citrate toxicity—that is, numbness or tingling—were experienced by donors in 45 per-
a myocardial infarction after donating PBPC concentrates, given 6 daily doses of 10 µg per kg of G–CSF and had doing a G–CSF mobilized PBPC concentrate.52 He had been PBPC concentrates for transplants involving siblings. A 22-
curred in persons who have donated G–CSF-mobilized
venous access, a central venous line is placed to collect PBPC
access. When HLA-compatible sibling donors have poor
component must be collected even if the donor has poor venous
granulocyte apheresis concentrate collections in that a compo-
cent of the procedures.43
PBPC concentrate collections differ from platelet and
granulocyte apheresis concentrate collections in that a compo-
ment must be collected even if the donor has poor venous access. When HLA-compatible sibling donors have poor
venous access, a central venous line is placed to collect PBPC
concentrates. This is the greatest potential risk that PBPC
concentrate donors face: an estimated 1 percent of central line
placements are associated with hemorrhage, pneumotho-
rax, and femoral vein catheters are well tolerated for a short time. The number of donors needing a cen-
ral venous catheter has varied from 5 to 22 percent.34,37,43

Other rare but more serious complications have occurred in persons who have donated G–CSF-mobilized
PBPC concentrates for transplants involving siblings. A 22-
year-old man suffered a ruptured spleen 3 days after donat-
ing a G–CSF mobilized PBPC concentrate.52 He had been
given 6 daily doses of 10 µg per kg of G–CSF and had do-
ated two PBPC concentrates. A 64-year-old man suffered
a myocardial infarction after donating PBPC concentrates,
and a 54-year-old woman died of a cere-
brovascular event 2 days after her dona-
tion.49 There have also been reports of
arterial thrombosis53,54 and an anaphyl-
lactoid reaction55 during G–CSF admin-
istration.

PBPC concentrate donors also face
another potential risk. It is theoretically pos-
sible that G–CSF could induce the growth of
an abnormal or malignant clone of cells that could result in the expansion of those cells into a leukemia. There is little
evidence, however, to support this. Some patients with congenital neutropenias have been given G–CSF for many years
and have developed leukemia, but this may be part of the natural progression of their disease,56 as G–CSF has been given
to patients with chronic idiopathic neu-
tropenia for many years without induc-
ing leukemia.46,57 There has also been a
report that G–CSF induces remissions of
acute myeloblastic leukemia.58

No long-term hematologic abnor-
malities have been reported in the thou-
sands of healthy people who have been
given G–CSF. When the blood counts and
the marrow of PBPC concentrate donors
have been evaluated 1 or more years af-
fter G–CSF was given, no abnormalities have been de-
tected.45,49 However, thousands of donors would have to be
followed for many years for even a 10-fold increase in the
risk of leukemia or malignancy to be detected.50 Therefore,
it cannot be stated with complete certainty that G–CSF does
not place the donor at some risk of a future complication.
The long-term risks, if any, associated with G–CSF must be
compared with the risks of the alternatives: marrow dona-
tion and anesthesia. In addition, the long-term effects of
marrow donation have not been carefully studied, and it too
could be associated with some long-term risks.

### Table 6. Neutropenic* donors among healthy people who were given 5 µg
per kg per day (n = 19) or 10 µg per kg per day (n = 21) of G–CSF for 5
days and who donated 2 PBPC concentrates

<table>
<thead>
<tr>
<th>Donor number</th>
<th>G–CSF dose (µg/kg/day)</th>
<th>Absolute neutrophil count (× 10⁹/L)</th>
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<tbody>
<tr>
<td></td>
<td>Before filgrastim</td>
<td>4 days after 2nd collection</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8 days after 2nd collection</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15 days after 2nd collection</td>
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<tr>
<td></td>
<td></td>
<td>21 days after 2nd collection</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>1.5</td>
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<tr>
<td></td>
<td></td>
<td>0.86</td>
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<tr>
<td></td>
<td></td>
<td>0.64</td>
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<tr>
<td></td>
<td></td>
<td>2.16</td>
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<tr>
<td></td>
<td></td>
<td>2.18</td>
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<tr>
<td>17</td>
<td>5</td>
<td>1.8</td>
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<td></td>
<td></td>
<td>1.64</td>
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<tr>
<td></td>
<td></td>
<td>1.01</td>
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<tr>
<td></td>
<td></td>
<td>0.97</td>
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<tr>
<td></td>
<td></td>
<td>1.44</td>
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<tr>
<td>26</td>
<td>10</td>
<td>2.4</td>
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<tr>
<td></td>
<td></td>
<td>3.20</td>
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<tr>
<td></td>
<td></td>
<td>2.00</td>
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<tr>
<td></td>
<td></td>
<td>0.97</td>
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<td></td>
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<td>30</td>
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<td></td>
<td>0.95</td>
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<td>2.48</td>
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<td></td>
<td></td>
<td>1.58</td>
</tr>
</tbody>
</table>

* A neutrophil count under 1.0 × 10⁹/L was defined as neutropenia.

In many ways, the magnitude of risks connected with the
two collection procedures is similar. Both present donors
with discomfort, inconvenience, and potentially serious
risks, and donors will experience fatigue and pain regard-
less of the collection procedure used, because of either G–
CSF administration (in PBPC concentrate donors) or aspi-
rations (in marrow donors). However, the symptoms do not
last as long in PBPC concentrate donors (6-8 days vs. 2-4
weeks in marrow donors).
Both procedures require many hours of the donor's time and are inconvenient. Marrow donors must undergo a physical examination, donate units of autologous blood, and spend 1 or 2 days in the hospital, and they may miss 1 or 2 days of work after the collection. PBPC donors must undergo a physical examination, be given daily injections of growth factor, and spend a day or two in the apheresis center. If only one apheresis collection is needed, then donating PBPC concentrates will likely require less time and be more convenient. However, it may be possible to streamline and improve the PBPC donation procedure. In the future, it may be possible to give G-CSF injections at home or at work, to simplify the donor work-up, and to establish procedures to ensure collection of the necessary HPCs in a single collection. Unfortunately, there are few opportunities to improve marrow collection processes and procedures.

Both procedures can rarely result in chronic donor health problems. Marrow collection can lead to chronic back pain and nerve injury, while placing apheresis needles in the arms very rarely can result in median nerve damage. Otherwise, apheresis results in few injuries.

Both types of donors face a small risk of serious complications. Marrow donors face a well-documented 1-in-10,000 chance of a serious complication secondary to anesthesia, while PBPC concentrate donors who have poor venous access and require a central line face a 1-in-100 chance of a complication from the placement of the central line.

Thus far, the data suggest that, if an adequate quantity of HPCs can be collected in a single concentrate and no central venous catheter is required, PBPC concentrate donation will likely be safer and better tolerated. But if the increase in the donor's PBPC count is poor and two or more PBPC concentrates are collected, or if the donor must give marrow in addition to PBPC concentrates, then the donor would likely have spent less time and been exposed to fewer risks had he or she just donated bone marrow. Potential donors who have good venous access, whose progenitor cells mobilize well, and who do not experience severe symptoms when G-CSF is given will tolerate the PBPC concentrate donation procedure very well. However, those who have poor veins or in whom PBPCs are not mobilized will find marrow donation a better experience.

SAFEGUARDS FOR UNRELATED PBPC CONCENTRATE DONORS

PBPC concentrates are being collected by the NMDP from unrelated marrow donors for the treatment of graft failure and disease relapse after unrelated-donor marrow transplants. While only 29 unrelated persons have donated PBPC concentrates under this protocol, preliminary results suggest that unrelated donors will donate PBPC concentrates and that donor centers can administer G-CSF and collect PBPC concentrates.

A trial of PBPC concentrates for initial transplants is now warranted. Because of the unresolved medical, scientific, and practical issues, initial trials should limit the rate at which donors and recipients are enrolled. It may also be best to restrict cohorts in initial trials to recipients undergoing transplantation for diseases associated with high transplant-related morbidity and disease relapse, such as advanced leukemia. These patients may benefit from an increased rate of engraftment and a possible increase in anti-leukemic effect provided by PBPC concentrates. Ideally, the results of PBPC concentrate and marrow transplants should be compared in prospective trials.

Although trials of PBPC concentrates for unrelated-donor transplants are warranted, donor and apheresis centers are obliged to ensure that the process is as safe as possible. Trials should include provisions to minimize the risks to donors and to study important medical issues that have not yet been resolved. To best protect and assess the effects of the process on the donors, standard protocols should be used to qualify donors and recipients, mobilize PBPCs, collect PBPC concentrates, and follow donors.

In addition, PBPC concentrate donors should be required to undergo the same predonation medical history, physical examination, and laboratory testing as marrow donors. The evaluation should include an assessment of the potential donor's veins. Those who are felt to have poor veins and in whom a central venous catheter will need to be placed for collection of the PBPC concentrate may be better off donating marrow. All donors must have normal blood counts and chemistries and no evidence of hypertension, diabetes, or cardiac or pulmonary problems. When appropriate, a pregnancy test should be performed, as pregnant women must not be given G-CSF. People with a history of malignancy, lymphoma, leukemia, or dysmyelopoietic syndrome must not donate PBPC concentrates or marrow. In fact, at this time, only healthy people should be allowed to donate PBPC concentrates. A potential donor who cannot donate marrow probably should not donate PBPC concentrates either. The exception to this may be people with back or iliac problems that could be exacerbated by the aspiration of marrow or potential donors with well-controlled asthma that could be exacerbated by anesthesia.

People more than 61 years old are not permitted to donate marrow. It is appropriate at this time to follow the same practice for PBPC concentrate donors, as PBPC concentrate CD34+ cell yields are lower in donors over 55 or 60 years of age.

PBPC concentrate donation protocols should specify which agents can be used to mobilize progenitor cells in unrelated donors, what the minimum and maximum doses are, what method of administration is permitted, and how long it should be given. The mobilization agents and protocols used in unrelated donors should already be proven to be safe and effective. Guidelines are also needed as to
when to reduce or stop the administration of the mobilization agents because of adverse effects or extreme changes in blood counts or chemistries. At this time, it is probably appropriate to give unrelated donors G–CSF or GM–CSF only.

The minimum quantity of progenitor cells that must be collected and the maximum quantity that can be collected must be specified. The number of collections, the volume of blood processed, and the duration of each procedure must be limited. Those responsible for the administration of hematopoietic growth factors must be appropriately trained, and all apheresis centers handling unrelated donors must have experience with the collection of PBPC concentrates. Physicians placing central venous catheters must be experienced. A physician should be immediately available if PBPC concentrates are collected through a central line. In some situations, it may be necessary to collect more than two PBPC concentrates or to collect marrow in addition to PBPC concentrates, but this should only rarely be necessary, and the situations in which this is permitted must be clearly defined.

After the collection, donors should be followed until they have made a full recovery. Blood counts and chemistry assessments should be made at least once after the donation to be sure they have returned to normal. The psychological and social impact on the donor of the PBPC mobilization and collection process on the donor should be evaluated.

To ensure that donating PBPC concentrates does not result in any long-term problems, donors should be followed periodically for many years. This likely is best done through the establishment of a registry of donors given G–CSF. This registry should include control groups such as unrelated marrow donors and people who, though enrolled in marrow donor registries, have not donated marrow or PBPC concentrates and who match the age, sex, and race of the PBPC concentrate donors. The incidence of hematologic malignancies, hematologic disorders, and solid tumors in each group should be compared.

The value of a method of collecting progenitor cells depends on the effects of the collection procedure on the donor and the outcome of the transplants. The survival of PBPC concentrate transplant recipients should be monitored to ensure that it is comparable to that of marrow transplant recipients. In addition, the rate of engraftment, the incidence of acute and chronic GVDH, the incidence of disease relapse, and long-term disease-free survival should be compared.

CONCLUSIONS

Although trials of PBPC concentrates for unrelated-donor transplants are now warranted, measures should be taken to safeguard the welfare of donors. This is best accomplished by using standardized mobilization and collection protocols. A protocol to collect PBPC concentrates for initial unrelated donor transplants has been developed by the NMDP.

While standardized protocols are critical for unrelated donors, the need for standardization must not prevent unrelated-donor HPC programs from changing. The collection of progenitor cells from unrelated donors for transplantation should be considered a dynamic process in which marrow collection was used first and PBPC concentrates are now being used. It is important to continue to search for methods to enhance the mobilization of progenitor cells, especially in the 10 percent of donors who respond poorly to G–CSF. However, because of the importance of maintaining the safety and trust of donors, new mobilization agents and new uses of available agents should be adopted with caution for unrelated donors.

In the future, other donation procedures will likely be possible. These could include the transplantation of HPCs obtained from small amounts of blood or marrow collected from donors treated with other growth factors and the ex vivo expansion of progenitor cells present in small amounts of blood or marrow collected from unstimulated donors. Each change in the process will, it is hoped, result in increased donor safety and convenience while improving transplant efficacy.

REFERENCES


