Filtration Versus Gravity Leukapheresis in Febrile Granulocytopenic Patients: A Randomized Prospective Trial

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Forty-eight patients with fever >38.3°C for at least 24 hr despite broad spectrum antibiotics and an absolute granulocyte count <1000/μl were randomly allocated to 4 days of granulocyte transfusions obtained by leukapheresis using filtration (n = 27) or gravity (n = 21) techniques, the latter permitting simultaneous nonmechanical collection of granulocytes and platelets utilizing hydroxyethyl starch as a sedimenting agent. Patient characteristics and dose of granulocytes obtained from both techniques were similar. Complete response to granulocyte transfusions was established by a reduction in temperature to <37.2°C sustained for at least 48 hr after the fourth transfusion with sterilization of cultures where previously positive and diminution of measurable infection when present. This occurred in 6/21 (29%) for gravity leukapheresis and 9/27 (33%) for filtration leukapheresis. An additional group had diminution in temperature and clinical improvement during transfusion (6/21 gravity leukapheresis versus 10/27 filtration leukapheresis). Eighty-six percent of patients transfused with gravity leukapheresis cells were alive at day 20 compared with 81% for filtration leukapheresis cells. Transfusion reactions were comparable. Thus, gravity leukapheresis appears to be as efficacious as filtration leukapheresis for treating granulocytopenic febrile patients, with the added advantages of availability to any blood bank without new equipment, of having platelets as by-products, and of not requiring donor heparinization.

The major cause of death in patients with impaired bone marrow function resulting either from neoplastic disease, chemotherapy, or radiation therapy is infection. Granulocyte transfusions have been utilized increasingly in the treatment of these granulocytopenic patients. The methods of procuring granulocytes have consisted of buffy coats from multiple donors, continuous flow centrifugation,1,2 discontinuous centrifugation with the Haemonetics Model 30,3,4 and filtration leukapheresis, the reversible adhesion of granulocytes to nylon-wool filters with their elution by calcium-chelating agents.5,6 Of the four methods, filtration leukapheresis permits the highest yield of granulocytes. However, much controversy exists in the literature concerning the integrity and function of granulocytes obtained by the different methods.7,11 Transfusions of granulocytes along with broad spectrum antibiotics have been shown in numerous studies to be effective in protecting patients with fever and granulocytopenia.12-19 Unfortunately, the techniques available for harvesting granulocytes are unavailable for the majority of granulocytopenic febrile patients because of the need for expensive machinery, variable needs of patients, and the requirement of a large team of skilled personnel. For these reasons, Dr. Isaac Djerassi, designer of filtration leukapheresis, reported on the collection of granulocytes and platelets by gravity leukapheresis, a technique almost as simple as donating a unit of blood.20

We present the results of a randomized prospective trial of 4 days of granulocyte transfusions to granulocytopenic febrile patients, comparing gravity leukapheresis with filtration leukapheresis, one of the standard harvesting techniques for granulocytes.

MATERIALS AND METHODS

A total of 48 patients with temperature greater than 38.3°C (101°F) for at least 24 hr despite broad spectrum antibiotics and an absolute granulocyte count less than 1000/μl were strictly randomized to 4 days of granulocyte transfusions obtained by filtration leukapheresis (27 patients) or gravity leukapheresis (21 patients). Four days of granulocyte transfusions were chosen since the time period produced a significant clinical response in a study reported by our earlier team20 and was a reasonable choice for the number of donors available. No numerical limit on the extent of transfusions is implied when they are used as part of routine care, however. Prior to entering the study, all patients had a complete blood count, automated chemistry profile, chest x-ray, at least two blood cultures (including cultures with isotopically labeled glucose for early determination), other appropriate cultures, and a serum for viral titers. All patients were placed on combined broad spectrum antibiotics usually utilizing cefazolin and gentamicin and/or carbenicillin at the beginning of the febrile episode, unless clinical or microbiologic evidence indicated more specific antibiotic therapy, and were observed for a minimum of 24 hr. If fever persisted, patients were eligible for this study. Our study parameters included an evaluation of response by fever abatement, negative cultures, improvement of localized infection, and short-term survival in the presence or absence of fever. Concomitant antibiotic changes and marrow regeneration manifested by peripheral blood counts (CBCs) and bone marrow changes were closely monitored. In addition, patients were closely monitored for transfusion reactions, coagulation abnormalities, graft versus host reactions, and immunization.

Donors came from the family of the patients, their friends or from...
normal volunteers. In all cases, the donors were red cell compatible with the recipient and in vitro crossmatching must have failed to demonstrate red cell incompatibility. Donors met the criteria for blood donation of the American Association of Blood Banks and gave full and informed consent. Prior to leukapheresis, all donors had a CBC with platelet count, type and crossmatch with recipient blood, VDRL, Protime, HBsAg, and automated chemistry profile. In addition, a health questionnaire and vital signs were obtained. Eligibility for leukapheresis was limited to those donors whose hematocrit was greater than 35, white blood cell count greater than 5000/μl, and platelet count greater than 125,000/μl. After leukapheresis, a CBC with platelet count was performed in order to evaluate the effect of the procedure on the donor and to establish eligibility for further donations.

Filtration Leukapheresis

The filtration leukapheresis system is one in which blood from one arm of the donor is passed through two filters in parallel. The blood is brought into the system by a peristaltic pump so that a continuous flow is maintained. The estimated extracorporeal volume is approximately 200 cc. Donors were premedicated with 10 mg dexamethasone and 2500 U of heparin intravenously just prior to starting the procedure. Heparin was continuously administered into the input line at a dose of approximately 60 U/min. An average run consumed less than 20,000 U of heparin. Ten liters of blood were usually processed in the 2.5-hr period. The granulocytes were separated from filters by elution with a solution made up of 1 U plasma together with 250 cc of normal saline. When 45% of the platelets and the plasma was returned to the donor. The red blood cells were in the lower layer, usually taking about 15 min. The red blood cells were removed and spun for 6 mm at 2000 rpm for 10 mm to obtain the eluents were centrifuged at 1500 rpm for 20 min and the granulocytes separated into 2 administration bags.

Gravity Leukapheresis

The donor was premedicated with 10 mg of dexamethasone and 0 U of whole blood was removed and spun for 6 min at 2000 rpm to remove the platelet-rich plasma from the red and white blood cells. Platelet rich plasma was spun at 3000 rpm for 10 min to obtain platelets and the plasma was returned to the donor. The red blood cells and white blood cells were placed in a 2-liter bag with 250 cc of 6% hydroxyethyl starch and 250 cc of normal saline. When 45% of the red blood cells were in the lower layer, usually taking about 15 min, the red blood cells were removed and returned to the donor. The white blood cells were separated from hydroxyethyl starch by centrifuging at 600 rpm for 18 min. The efficiency of the technique separated approximately 80% of the granulocytes removed from the donor. To process 6 U of blood (~3 liters), the amount ordinarily harvested, required 5.5–6 hr.

After completion of the study we used precollection of a unit of blood to allow double unit processing during gravity leukapheresis in several donors to shorten the time of the procedure. A unit of whole blood was removed 1–2 days prior to granulocyte donation and stored. Just prior to initiating gravity leukapheresis, the stored unit was transfused and 2 U of blood were withdrawn, processed, and returned. This procedure was repeated two additional times, thus processing a total of 6 U of blood in 3 hr.

Granulocyte Administration

Approximately 1 × 10^10 granulocytes were infused over 60 min, followed by a 30-min rest period. This was repeated until all granulocytes were infused. The infusion was interrupted if chills and fever became evident and the patient was treated with meperidine, antihistamines, and/or steroids as was clinically indicated. Leukocyte infusion was then restarted. Infusion thus required from 2.5 to 3 hr, including a 30-min rest period for completion. Temperature and blood pressure in the recipient was monitored hourly and for at least 6 hr after transfusion.

Response

A response was established by a reduction in temperature to less than 37.2°C (99°F), sustained for at least 48 hr after the fourth leukocyte transfusion, with sterilization of blood cultures if previously positive and diminution of measurable infection when present. Improvement included all elements of a complete response except that the temperature although decreasing did not stay consistently below 37.2°C (99°F) for the 48-hr period.

Statistics

Patients were randomized from a table of random numbers to treatment with 4 days of granulocyte transfusions obtained by filtration leukapheresis or gravity leukapheresis. With 25 patients in each group, a difference between the 2 response rates from 30% to 70% would be detectable at the 10% significance level with a power of 75%. (The observed difference of 4% would require more than 1000 patients in each group to be significant at the 10% level with a power of 75%.) Thus, it is unlikely that an important difference in response has been undetected in this series.

The characteristics of patients randomized to the two treatment groups were comparable using methods of descriptive statistics. Chi-square tests and t tests to compare the 2 treatment groups (F tests with corrections for unequal variances if required using Welch procedure) were used in an exploratory fashion to determine areas for further study and to determine whether any of the characteristics under study might be associated with differences in response rate. Response rates in the two groups were compared using chi-square tests.

RESULTS

Patients randomized to the two treatment groups were comparable with respect to characteristics at entry. Table 1 shows the age and sex of the granulocyte recipients. Sixty-seven percent of both groups were male. The mean age was 47 yr for filtration leukapheresis with a range of 21–70 yr and 43 yr for gravity leukapheresis with a range of 7–68 yr. Table 2 reveals that the underlying diseases in both groups were also comparable. Nineteen of 21 (91%) patients with gravity leukapheresis had acute leukemia of all types, and all of the patients who received filtration leukapheresis cells did also.

Eleven of 21 gravity leukapheresis patients (52%) had an unknown site of infection without an organism cultured. Similarly, for the filtration leukapheresis patients, 18 (59%) had febrile episodes without finding

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<th>Table 1. Age and Sex of Granulocyte Recipients</th>
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<td>Gravity Leukapheresis</td>
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an organism. Bacteremia was present in four patients in each group. Other types of infections included abscesses, urinary tract infection, pneumonia, and biopsy-proven invasive fungus (Table 3). Three of 21 (14%) patients who received gravity leukapheresis cells had positive blood cultures compared to 5 of 27 (19%) for filtration leukapheresis patients. Measurable infection was present in 4 of 21 (19%) of gravity leukapheresis recipients compared with 5 of 27 (19%) for filtration leukapheresis recipients. The antibiotics used were considered entirely appropriate based on sensitivities of cultured organisms in all of the known 24% of the known episodes in which gravity leukapheresis cells were used and in all of the known 30% for filtration leukapheresis cells (Table 4).

The duration of fever over 38.3°C (101°F) prior to instituting granulocyte transfusion was a 3 days median for gravity leukapheresis recipients (range 2–13 days) compared with 5 days for filtration leukapheresis recipients (range 1–14 days). The median duration of granulocytopenia with total leukocyte counts less than 1000/µl prior to transfusion was 10 days for gravity leukapheresis cell recipients (2–48 days) compared with 8.5 days for filtration cell recipients (range 1–45 days).

The average donation (granulocytes x 10⁹/sq m of recipient) was 87.2 (SE, 4.34) for filtration leukapheresis and 77.6 (SE, 6.11) for gravity leukapheresis for 4 days of transfusion. This small difference was not statistically significant (t₄₃ = 1.32, p = 0.1). Granulocytopenia despite transfusion persisted a median of 11 days for gravity leukapheresis cell recipients and 14.5 days for filtration leukapheresis cell recipients.

Transfusion reactions defined as spiking fever and/or chills were comparable in both groups. Approximately 60% of recipients had at least one transfusion reaction. These are summarized in Table 5.

Responses and improvements, as summarized in Table 6, were seen in 58% of gravity leukapheresis recipients and 70% of filtration leukapheresis cell recipients (χ² = 0.90, 0.5 > p > 0.25). Thirty-three percent of gravity leukapheresis patients did not respond compared to 26% for filtration leukapheresis recipients. The unknown group included three patients who died without receiving the full 4-day trial of granulocyte transfusions.

Survival for 20 or more days after transfusion also showed comparability: 86% of those who received gravity leukapheresis cells survived compared to 81% for filtration leukapheresis cells, as indicated in Table 7. Thus, gravity leukapheresis has been shown to be as efficacious as filtration leukapheresis for the treatment of febrile granulocytopenic patients. After 4 days of granulocyte transfusions, patients were observed for 48 hr. If fever still continued, further transfusions obtained by filtration leukapheresis could be used at
the discretion of the attending physician. Analysis of those 11 patients who were given additional transfusions revealed responses in 1 of 5 gravity and 2 of 6 filtration leukapheresis (Table 8).

On further evaluation of the response for both treatment arms, males had a slightly lower response rate than females (25% versus 44%). Discriminate analyses were used to determine whether some combination of characteristics might enable prediction of those patients who would benefit from granulocyte transfusions. The mean number of granulocytes donated to responders in both arms of the study was 9.6 x 10⁹/sq m (SE, 0.08), substantially higher than for nonresponders 7.3 x 10⁹/sq m (SE, 0.03). (Welch procedure for unequal variances $F_{0.05} = 4.6, p = 0.05$.) No predictions could be made for age, sex, diagnosis, type of infection, duration of fever, or days of granulocytopenia, however.

Thirty-seven patients with acute myelocytic leukemia were undergoing remission induction when administered granulocytes. Twenty patients received filtration leukapheresis derived cells and 17 patients received gravity leukapheresis derived cells. The complete remission rate for filtration leukapheresis patients was 55% (11/20), and for gravity leukapheresis patients it was 53% (9/17). The method of obtaining granulocytes did not alter the remission rates of these patients with acute myelocytic leukemia.

**DISCUSSION**

Data are available to document the clinical efficacy of granulocyte transfusions for febrile granulocytopenic patients. In the first randomized prospective trial of granulocyte transfusion, our earlier team showed that 4 days of granulocyte transfusion derived from filtration leukapheresis caused significant defervescence, improved clinical outcome, and produced greater survival of granulocytopenic febrile patients. Other studies by Alavi et al.,13 Herzig et al.,15 Lowenthal et al.,17 and McCredie et al.,19 also found a therapeutic role for granulocyte transfusion, which was dramatically evident in those with proven bacterial infection. These studies have utilized continuous flow centrifugation, discontinuous flow centrifugation, or filtration leukapheresis, all techniques that utilize expensive equipment and highly trained personnel. These requirements have precluded utilization of leukocyte transfusions by many hospitals.

Filtration leukapheresis produces the highest granulocyte yields per unit of donor time and minimizes unwanted lymphocytes, platelets, and red blood cells. Large quantities of heparin are required, however, producing a minor risk of bleeding in the donor and even in the recipient. Several studies have revealed defects in chemotaxis, enzymatic reactions, bactericidal activity, as well as abnormal circulation of granulocytes collected by filtration leukapheresis.8,21,22 These abnormalities may be ameliorated by steroids.23–25 In addition, adhesion of leukocytes to nylon-wool activates complement that may alter circulation kinetics and function of granulocytes.24,25 A recent report indicated a serious complication in a male donor when priapism occurred.26

Differential sedimentation of granulocytes does not cause significant alteration of granulocyte function.8 It does produce a smaller granulocyte yield per unit time and requires steroids to increase circulating granulocytes27,28 and hydroxyethyl starch29 to increase rouleaux formation of red blood cells for better separation from the white blood cells. Hydroxyethyl starch has no effect on platelet or granulocyte function.30

Gravity leukapheresis derived granulocytes have been studied by Aisner et al.31 Granulocytes were normal in appearance when stained with Wright-Giemsa and were greater than 95% viable when studied with Trypan blue. The phagocytic index and bactericidal capacity was found to be normal. These workers found gravity leukapheresis to be a highly efficient technique for harvesting granulocytes with an effi-
ciency equal to discontinuous flow leukapheresis after either steroid or nonsteroidal stimulation of the donor.

We have found in a randomized prospective trial that gravity leukapheresis is clinically as efficacious as filtration leukapheresis for febrile granulocytopenic patients. Comparing both techniques in terms of simplicity of operation, initial investment, complicated machinery, need for heparin, and universal applicability, gravity leukapheresis has marked advantages. The functional integrity of the granulocyte collected by gravity is preserved. In addition, platelets can be obtained as a byproduct. Filtration leukapheresis has the advantages of requiring less extracorporeal volume (200 versus 450 ml) of being a completely closed system thereby reducing the risks of bacterial contamination and of requiring less donor and personnel time (2.5 versus 6 hr). In our hands, the time requirement has been the major drawback to utilizing gravity leukapheresis more frequently. However, when other harvesting techniques are not available, this takes on minor significance. Rarely, donors can tolerate double unit processing, (900 ml extracorporeal volume). It is safer, however, to remove a unit of red blood cells the day before and to transfuse them back on the day of granulocyte donation to allow double unit processing without acute hypovolemia. This procedure was not followed in the present study but has subsequently been found feasible and useful.

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REFERENCES

Filtration versus gravity leukapheresis in febrile granulocytopenic patients: a randomized prospective trial

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