Turk J Hematol 2007; 24:117-126

RESEARCH ARTICLE

The effect of granulocyte colony-stimulating factor on engraftment in patients with lymphoproliferative malignancies and solid tumors undergoing autologous peripheral stem cell transplantation

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Received: Sep 22, 2006 • ***Accepted:*** Apr 19, 2007

ABSTRACT

The effects of granulocyte colony-stimulating factor (G-CSF) and engraftment quality on the rate of hematopoietic system recovery were assessed in patients with solid tumors and lymphoproliferative diseases after autologous hematopoietic stem cell transplantation.

Treatment with autologous hematopoietic stem cell transplantation was performed in 40 patients for non-Hodgkin's lymphoma (n= 20), Hodgkin's lymphoma (n= 8), multiple myeloma (n= 8), and breast cancer (n= 4). In 20 patients with solid tumors and lymphoproliferative diseases, treatment with autologous hematopoietic stem cell transplantation was followed by G-CSF therapy (experimental group). Another 20 patients with solid tumors and lymphoproliferative diseases were treated with autologous hematopoietic stem cell transplantation without G-CSF therapy (control group). The two patient groups were matched for age, sex, diagnosis, number of chemotherapy courses, and engraftment quality (nucleated cells or CD34+ cells). The experimental group received G-CSF in a dose of 5 µg/kg body weight subcutoneous from day 1 of autologous hematopoietic stem cell transplantation until leukocyte count increase to > 1.0 x 10^9/L over three consecutive days. Nonparametric tests (x^2-test, median test, extended median test) were used in statistical analysis.

The hematopoietic system showed rapid recovery. Leukocyte count > 1.0 x 10^9/L was recorded on median day 11 (range 8-21) in the experimental group vs median day 12 (range 9-16) in the control group; gra-

nulocyte count > 0.5 x 10^9/L on median day 12 (range 8-23) vs day 14 (range 10-16) in the control group; and platelet count > 20 x 10^9/L on median day 13 (range 8-80) vs day 11 (range 7-15) in the control group.

Differences between the two patient groups were not statistically significant. G-CSF therapy administered after autologous hematopoietic stem cell transplantation did not result in faster leukocyte, granulocyte or platelet recovery. The patients receiving a relatively lower number of cells showed the same rate of recovery as those who received a higher cell number. The number of CD34+ cells per kg body weight did not correlate with the rate of leukocyte, granulocyte or platelet recovery.

Key Words: CD-34 antigen, leukocyte, mononuclear, hematopoietic function recovery, stem cell, transplantation, G-CSF

INTRODUCTION

Hematopoietic stem cells are found in bone marrow and circulate in peripheral blood [1-4]. Their peripheral blood count is low [5]. The presence of hematopoietic stem cells in the circulation and the possibility of their peripheral count to considerably increase by the procedures of mobilization have stimulated the introduction of patient treatment with autologous hematopoietic stem cell transplantation [2,5,6-8]. Autologous hematopoietic stem cell transplantation has recently been routinely used in the management of many malignant tumors, lymphomas, and solid tumors in particular [6-9]. The main precondition for treatment with autologous hematopoietic stem cell transplantation is an adequate hematopoietic stem cell count and tumor sensitivity to the scheduled cytotoxic agents. Assessment of the stem cell number and quality is currently done by testing nucleated cell and CD34+ cell count, and their ability to give rise to colonies, primarily granulocyte-macrophage colonies [colony-forming unit (CFU), granulocyte-macrophage (GM)]. CD34 antigen is a stage-specific glycoprotein, which is found in cells in the earliest stage of hematopoietic differentiation. Therefore, the CD34+ cell population contains precursor cells directed towards the myeloid, lymphoid and erythroid compartments, as well as primordial progenitor cells capable of long-term hematopoiesis restitution [10]. However, this marker is found both on immature pluripotent stem cells (which are also Thy 1+, HLA-DR, and lacking specific second lineage markers) and on more mature directed cells (which are also Thy 1-, HLA-DR+, CD38+, and carry particular specific lineage markers) [11-13]. The CD34+ cell population also contains hematopoietic stem cells, since it is capable of establishing the hematopoietic system function. Most authors believe that determination of CD34+ cell count in a transplant can well indicate their quality [8,14,15].

In most studies, 2.0 x 10^6/CD34+ cells/kg body weight and 5.0 x 10^4 CFU-GM cells/kg body weight have been considered adequate counts to establish hematopoiesis in humans after transplantation [14,15]. Reinfusion of a greater number of immature precursor cells does not lead to faster hematopoietic system recovery because the bone marrow homing and proliferation sites appear to be limited [14]. Experiments in rats have shown that less than 10 pluripotent stem cells were capable of establishing hematopoiesis within several weeks of complete bone marrow, lymph node and blood destruction by radiotherapy and chemotherapy. It remains obscure whether the same model is possible in humans; however, it appears that only a few hematopoietic stem cells are inadequate to establish hematopoiesis in humans.

The aim of the study was to assess the effect of granulocyte colony-stimulating factor (G-CSF) on the rate of leukocyte, granulocyte and platelet recovery in patients with solid tumors and lymphoproliferative diseases after autologous hematopoietic stem cell transplantation.

MATERIALS and METHODS

Materials

This clinical trial included 40 patients with lymphoproliferative diseases or solid tumors, treated with autologous hematopoietic stem cell transplantation at the Department of Hematology, Faculty of Medicine, University Hospital Center, Zagreb, Croatia. Twenty patients received G-CSF, whereas the other 20 patients did not receive G-CSF and served as a control group. Informed consent was obtained from all patients and protocol was approved by the Zagreb University Hospital Center Institutional Review Board. We evaluated data retrospectively from two groups of patients: one that received G-CSF after transplantation and the other that did not.

Patient characteristics are presented in Table 1. There were 21 (52.5%) male and 19 (47.5%) female patients, median age 32.5 (range

Table 1. Characteristics of patients with malignant tumors of the hematopoietic system and solid tumors treated with autologous hematopoietic stem cell transplantation followed by granulocyte colony-stimulating factor administration

Patient characteristic	Experimental group	Control group	p
Number of patients	20	20	1.0
Age median (range) (yrs)	32.5 (16-58)	32.5 (17-55)	1.0
Sex, male/female (n)	11/9	10/10	0.75
Diagnosis:			
Non-Hodgkin's lymphoma	12	12	
Hodgkin's lymphoma	4	4	
Multiple myeloma	1	3	0.57
Breast cancer	3	1	
Chemotherapy courses			
1 course (n= 15)	8	7	
2 courses (n= 13)	7	6	0.79
≥ 3 courses (n= 12)	5	7	
Engraftment quality parameters			
Nucleated cell count ($x10^8$/kg b.w.) median (range)	4.655 (2.32-11.47)	6.19 (2.57-15.59)	0.11
Mononuclear cell count ($x10^8$/kg b.w.) median (range)	3.445 (1.46-9.33)	4.17 (1.60-12.39)	0.75
CD34+ cell count ($x10^6$/kg b.w.) median (range)	3.66 (0.81-45.19)	5.675 (0-29.7)	0.61
CFU-GM ($x10^6$/kg b.w.) median (range)	14.655 (1.38-31.6)	20.7 (2.09-60.21)	0.08
Parameter of blood cell recovery			
Leukocytes > 1.0 x 10^9/L (days*) median (range)	11 (8-21)	12 (9-16)	0.75
Granulocytes > 0.5 x 10^9/L (days*) median (range)	12 (8-23)	14 (10-16)	0.75
Platelets > 20 x 10^9/L (days*) median (range)	13 (8-80)	11 (7-15)	0.34
Reticulocytes (days**) median (range)	13 (9-28)	12 (9-16)	0.34
Number of days* with fever > 38°C median (range)	4 (0-18)	3.5 (0-11)	0.51
Length of hospital stay (days*) median (range)	19 (12-43)	19.5 (15-31)	0.75
Blood culture			
Positive	6	9	0.51
Negative	14	11	
Number of antimicrobials administered			
Median (range)	4 (0-7)	4 (0-7)	1.0

b.w.: Body weight. CFU-GM: Colony-forming unit-granulocyte-macrophage.

*days after transplantation;

**reticulocyte occurrence after transplantation.

16-58) years. Twenty-four (60%) patients were treated for non-Hodgkin's lymphoma, eight (20%) for Hodgkin's lymphoma, four (10%) for multiple myeloma, and four (10%) for breast cancer. The experimental and control groups did not differ according to the number of previously received therapies, considering radiotherapy as a course of chemotherapy (Table 2). Patients from the two groups also did not differ according to the stage of disease at the time of autologous hematopoietic stem cell transplantation. Sixteen (40%) patients underwent treatment in remission I, 12 (30%) patients in disease relapse, i.e. remission II, and 12 (30%) patients in the advanced, refractory stage of disease.

Methods

Mobilization was performed by administration with:

a. Cyclophosphamide 4 g/m^2 in one infusion of 5% glucose during 90 minutes-1st day, and G-CSF (Neupogen) 5 μg/kg/d subcutaneous (sc) from 6th day after administration of cyclophosphamide till the last day of leukapheresis;

b. Cyclophosphamide 4 g/m^2 in one infusion of 5% glucose during 90 minutes-1st day, etoposide (VP 16, Vepeside) 1 g/m^2-2nd day and G-CSF 5 μg/kg/d sc from 6th day after administration of cyclophosphamide till the last day of leukapheresis;

c. VIP (VP 16, ifosfamide, cisplatin) + G-CSF [6];

d. Mini-BEAM: BCNU 150 mg/m^2 iv 1st day, etoposide 100 mg/m^2 from 2nd till 5th day, cytosine-arabinoside 100 mg/m^2 iv 2 times a day from 2nd till 5th day, melphalan 70 mg/m^2 iv 6th day, G-CSF 5 μg/kg/d sc from 6th day after administration of cyclophosphamide till the last day of leukapheresis.

Cyclophosphamide is administered along with hyperhydration, but prior to administering, a urinary catheter is placed in urinary bladder, and it is washed out with NaCl solution in order to prevent hemorrhagic cystitis.

Prevention and, if necessary, treatment of infection is done in every granulocytopenic patient. In most of the patients, we expected increase in number of cells between the 10th and 15th days, four days after G-CSF and first leukapheresis. Leukapheresis is usually done 2-5 times, 3 on average. G-CSF is used until the day prior to last leukapheresis.

During the mobilization of hematopoietic stem cells in the blood, the number of leukocytes and thrombocytes and proportion of CD 34+ cells in the blood (immunologic laboratory, FACS) are measured daily from the 6th day after cyclophosphamide is administered.

Table 2. Effect of engraftment CD34+ cell count on the rate of leukocyte, granulocyte and platelet recovery in patients with malignant tumors of the hematopoietic system and solid tumors treated with autologous hematopoietic stem cell transplantation

	Finding in patients administered			
Parameter of blood cell recovery	**≤ 2.0 x 10^6 CD34+ cells/kg b.w. (n= 13)**	**2.0-5.0 x 10^6 CD34+ cells/kg b.w. (n= 13)**	**≥ 5.0 x 10^6 CD34+ cells/kg b.w. (n= 13)**	**p**
Leukocytes > 1.0 x 10^9/L (days*) median (range)	12 (10-21)	12 (8-18)	11 (8-16)	0.47
Granulocytes > 0.5 x 10^9/L (days*) median (range)	13 (12-23)	14 (9-18)	12 (8-16)	0.81
Platelets > 20 x 10^9/L (days*) median (range)	13 (11-17**)	12.5 (8-30)	10 (3-14)	0.90

b.w.: Body weight.

*days after transplantation;

**one patient failed to achieve platelet count > 20 x 10^9/L.

Stem cells were collected on a COBE Spectra Blood Cell Separator using original closed system for leukapheresis, ADC as anticoagulant, and instrument parameters set to the mode for stem cell collection (collection of mononuclear cells, MNC mode). A leukocyte count increase to > 1.0 x 10^9/L, the occurrence, i.e. overt increase in the proportion of CD34+ cells in the blood (> 1%) relative to previous values, and platelet count > 50 x 10^9/L were the necessary criteria before starting the hematopoietic stem cell collection from the blood. Two blood volumes (7-10 L) were processed per apheresis procedure. Determination of nucleated cell count, hematocrit, mononuclear cell count (cytologic smear differentiation), CFU-GM cell count, CD34+ cell count (possibly with additional markers, e.g., CD38), and microbiologic control testing were performed in each apheresis product. Upon collection from the blood, stem cells were frozen at 196°C and stored in liquid nitrogen by use of a Planer Biomed, Kryo 10 Series II device. Decision on the use of myeloablative therapy and autologous hematopoietic stem cell transplant reinfusion required the latter to contain ≥ 2 x 10^6 CD34+ cells/kg body mass and/or ≥ 5 x 10^4 CFU-GM/kg body mass.

Treatment with intensive cytostatic chemotherapy-conditioning

Laboratory tests were performed before the introduction of intensive cytostatic therapy as well as before mobilization for the occurrence of hematopoietic stem cells in the blood. Patients with Hodgkin's and non-Hodgkin's lymphoma received cytostatic therapy according to BEAM protocol: BCNU 300 mg/m^2, etoposide 200 mg/m^2 x 4, cytosine arabinoside 400 mg/m^2 x 4, and melphalan 140 mg/m^2.

Patients with breast cancer were treated according to VICE protocol as follows:

- Day 5, etoposide 500 mg/m^2 in 3-hour infusion, carboplatin 250 mg/m^2 in 4-hour infusion, and epirubicin 50 mg/m^2 iv;

- Day 4, etoposide 500 mg/m^2 in 3-hour infusion, carboplatin 250 mg/m^2 in 4-hour infusion, epirubicin 50 mg/m^2 iv, and ifosfamide 4000 mg/m^2 in 2-hour infusion; and

- Day 3, etoposide 500 mg/m^2 in 3-hour infusion, carboplatin 250 mg/m^2 in 4-hour infusion, epirubicin 50 mg/m^2 iv, and ifosfamide 4000 mg/m^2 in 2-hour infusion.

Patients with multiple myeloma were treated with melphalan [17], 140 mg/m^2 on days -3 and -2. Upon conditioning, the graft was allowed to thaw and reinfusion of autologous hematopoietic stem cells was performed on day 0. Engraftment quality was assessed on the basis of nucleated cell count, CFU-GM cell count, and CD34+ cell count.

G-CSF therapy after autologous hematopoietic stem cell transplantation

G-CSF was administered from day 1 of autologous hematopoietic stem cell transplantation, in a dose of 5 μg/kg body weight in infusion, until leukocyte count exceeded 1.0 x 10^9/L on three consecutive days.

Criteria for engraftment acceptance and function

The effect on the rate of engraftment acceptance and function was assessed according to the number of days to leukocyte increase to > 1.0 x 10^9/L, number of days to neutrophil increase to > 0.5 x 10^9/L, number of days to platelet increase to > 20.0 x 10^9/L, number of days with fever > 38°C, number of days on antibiotic therapy, and length of hospital stay.

Statistical analysis

Data were entered in a PC and analyzed by statistical nonparametric tests (χ^2-test, median test, and extended median test).

RESULTS

Engraftment quality in experimental and control group patients is illustrated in Table 1. Experimental group patients received less nucleated cells, mononuclear cells and CD34+ cells than control group patients; however, the difference was not statistically significant. The number of cells in CFU-GM culture was also greater, the difference approaching statistical

significance. The rate of leukocyte, granulocyte, platelet and reticulocyte recovery in experimental and control group patients is presented in Table 1. Results showed that G-CSF had no impact on the rate of hematopoietic system recovery. Difference between the groups was not statistically significant. The rate of leukocyte, granulocyte and platelet recovery in experimental and control groups is shown in Table 2. Results yielded no difference in the rate of bone marrow functional recovery irrespective of CD34+ cell count. Difference between the groups was not statistically significant. The patients administered a relatively lower number of cells showed the same rate of recovery as those that received a higher amount of cells. The effect of nucleated cell count on the rate of leukocyte, granulocyte and platelet recovery is illustrated in Table 3. There were also no between-group differences according to the number of days with fever and length of hospital stay (Table 1). The incidence of positive blood cultures is shown in Table 1, as well as the number of antimicrobial agents administered to patients. Difference between the groups was not statistically significant. Only gram-positive agents were isolated from blood cultures of both patient groups, whereas the causative agent remained undefined in two patients. *Staphylococcus epidermidis* was isolated in five patients and *Staphylococcus aureus* in one patient from the experimental group.

DISCUSSION

Many studies have demonstrated engraftment of autologous hematopoietic stem cells collected from patient blood upon mobilization for their appearance in the blood by chemotherapy, chemotherapy and growth factors, or growth factors alone as being preferable to bone marrow transplantation according to the rate of hematopoietic system recovery, and as efficient in establishing long-term hematopoiesis [8,16]. In the majority of patients treated with autologous bone marrow transplantation, pancytopenia persisted for three to four weeks, and the mean length of hospital stay was 40-50 days [17]. However, there are few studies investigating factors that may influence the rate of hematopoietic system recovery after myeloablative therapy and autologous hematopoietic stem cell engraftment. Some studies found a correlation between the number of CFU-GM cells infused and parameters of graft acceptance, whereas others did not [12,15]. Data on the role of the number of CD34+ cells in the graft in hematopoietic system recovery are also contradictory. Furthermore, the effect of growth factors on hematopoietic system recovery after homologous hematopoietic stem cell engraftment has not yet been examined in a large patient series.

The aim of the present study was to assess the efficacy of granulocyte growth factor on the

Table 3. Effect of engraftment nucleated cell count on the rate of leukocyte, granulocyte and platelet recovery in patients with malignant tumors of the hematopoietic system and solid tumors treated with autologous hematopoietic stem cell transplantation

	Finding in patients administered		
Parameter of blood cell recovery	**$\leq$ 5.0 x 10^8 nucleated cells/kg b.w. (n= 16)**	**$\geq$ 5.0 x 10^8 nucleated cells/kg b.w. (n= 24)**	**p**
Leukocytes > 1.0 x 10^9/L (days*) median (range)	11 (8-21)	12 (8-16)	0.37
Granulocytes > 0.5 x 10^6/L (days*) median (range)	12 (8-23)	13.5 (10-16)	0.72
Platelets > 20 x 10^9/L (days*) median (range)	11.5 (11-30**)	13 (9-15)	0.09

b.w.: Body weight.
*days after transplantation.
**one patient failed to achieve platelet count > 20 x 10^9/L.

hematopoietic system recovery rate after homologous hematopoietic stem cell transplantation. The efficacy of G-CSF and other growth factors on the hematopoietic system prior to bone marrow transplantation or homologous hematopoietic stem cell engraftment (in the stage of stem cell mobilization) has been investigated in numerous studies; however, there are few studies assessing the efficacy of G-CSF after homologous hematopoietic stem cell engraftment.

In the present study, the group of patients administered G-CSF showed a tendency to faster leukocyte and neutrophil recovery than the control group of patients who did not receive G-CSF (leukocyte count > 1.0 x 10^9/L on median day 11 vs day 12; neutrophil count > 0.5 x 10^9/L on median day 12 vs day 14); however, the difference did not reach statistical significance. In a group of 54 patients (breast cancer 22, non-Hodgkin's lymphoma 18, multiple myeloma 7, and other tumors 7 patients), Bensinger et al. [18] recorded fast recovery of both neutrophils and platelets (> 1.0 x 10^9/L and > 20.0 x 10^9/L on median day 12 and day 10, respectively); however, also without a statistically significant difference between the patients who did or did not receive G-CSF after homologous hematopoietic stem cell engraftment (median day 11 vs day 14). Furthermore, they reported the graft CD34+ cell count to correlate with the rate of granulocyte and platelet recovery as well as a positive correlation between previous radiotherapy and granulocyte recovery, and between the diagnosis of breast cancer and rate of platelet recovery.

In their study of 28 patients with Hodgkin's lymphoma treated with autologous hematopoietic stem cell engraftment in "sensitive relapse", Haas et al. [14] demonstrated rapid recovery of leukocytes (> 1.0 x 10^9/L on median day 13) and granulocytes (> 0.5 x 10^9/L on median day 16), which is consistent with the results obtained in our study. In their study including 24 multiple myeloma patients treated with autologous hematopoietic stem cell engraftment after myeloablative therapy, Kröger et al. [19] also observed fast recovery of granulocytes (> 1.0 x 10^9/L on median day 10) and platelets (> 20.0 x 10^9/L on median day 14). The patients received a median dose of 3.9 x 10^6 CD34+ cells/kg body weight.

Advani et al. [13] reported a randomized study including patients with Hodgkin's lymphoma and non-Hodgkin's lymphoma, in which they demonstrated the favorable effect of granulocyte-macrophage colony-stimulating factor (GM-CSF) on neutrophil recovery after autologous hematopoietic stem cell transplantation. However, autologous hematopoietic stem cell transplantation was performed in only 19% of patients, whereas others underwent transplantation of bone marrow, or of bone marrow and autologous hematopoietic stem cells. In patients administered GM-CSF, neutrophil count of > 1.0 x 10^9/L was recorded on day 15 vs day 24 (median) in those administered placebo; however, analysis of the GM-CSF effect was complicated by a great number of clinical variables (the patients administered GM-CSF did not receive autologous stem cells alone but also received bone marrow). On multivariate analysis, the use of non-purified bone marrow, autologous hematopoietic stem cells and GM-CSF were the factors most significantly associated with the rate of granulocyte recovery. Shpall et al. [8] investigated the rate of hematopoietic system recovery and effect of growth factors on this recovery in breast cancer patients. Group 1 patients received bone marrow alone; group 2 bone marrow + G-CSF; group 3 bone marrow + GM-CSF; group 4 bone marrow + autologous hematopoietic stem cells + G-CSF; and group 5 autologous hematopoietic stem cells + G-CSF. Granulocyte count exceeding 1 x 10^9/L was achieved at a significantly slower rate in group 1 than in group 2 patients (median, day 23 vs day 10). However, there was no correlation between the use of GM-CSF or G-CSF and granulocyte recovery rate in groups 2 to 5. The median time to achieve platelet count of > 20 x 10^9/L was significantly shorter in groups 4 and 5 as compared with groups 1 to 3. In a group of 52 patients transplanted with autologous hematopoietic stem cells and bone marrow (n= 22) or autologous hematopoietic stem

cells alone (n= 30), Mangan et al. [20] observed faster neutrophil recovery in those who received G-CSF or GM-CSF than in those who did not (leukocytes > 0.5 x 10^9/L on median day 11.5 vs day 13.5). Watts et al. [21] demonstrated a statistically significantly faster neutrophil recovery in patients administered G-CSF than in those who did not receive it, however, only 27 of 81 study patients received G-CSF (leukocytes > 1.0 x 10^9/L on median day 10 as compared with day 13 in the remaining study patients). Klumpp et al. [22] reported on a randomized study including 41 patients (27 with breast cancer, 1 with Hodgkin's lymphoma, 5 with multiple myeloma, and 8 with non-Hodgkin's lymphoma), of which 22 patients received G-CSF and 19 patients did not. Faster neutrophil recovery was recorded in patients that received G-CSF than in those who did not (> 0.5 x 10^9/L on median day 10.5 vs day 16). In a study that included 692 patients, Weaver et al. [23] found the use of growth factors following autologous hematopoietic stem cell transplantation to correlate with the rate of neutrophil recovery but not with the rate of platelet recovery.

In the present study, the use of G-CSF had no effect on the rate of platelet recovery. The median time for platelet recovery to > 20.0 x 10^9/L was 13 days in the experimental group and 11 days in the control group, i.e. faster by two days in the latter, although the difference was not statistically significant. Similar results have been reported from a number of other studies. In the study by Bensinger et al. [18], growth factors were found to exert unfavorable effects on platelet recovery, yet without statistical significance. These authors investigated the impact of G-CSF on granulopoiesis in healthy donors and observed the platelet count to be statistically significantly lower in the group of subjects that received G-CSF than in those who did not. Advani et al. [13] also reported no statistically significant difference in platelet recovery between the patients who did or did not receive GM-CSF. Klumpp et al. [22] showed that the use of G-CSF was not associated with a statistically significantly faster platelet recovery. In the study by Watts et al. [21], the median time to platelet recovery to > 20.0 x 10^9/L was 11 days and there was no statistically significant difference between the patients administered or not administered G-CSF.

In our study, there was no statistically significant difference between the experimental and control groups with respect to the number of days with fever > 38°C (median, 4 days vs 3.5 days), length of hospital stay (median, 19 days vs 19.5 days) or number of antimicrobials used. Blood culture was positive in six experimental group patients and nine control group patients (p= NS). Advani et al. [13] demonstrated the incidence of bacterial infection to be significantly lower in the group of patients administered GM-CSF than in the group of patients that did not receive GM-CSF; however, there was no significant between-group difference in the length of hospital stay. Klumpp et al. [22] found the length of hospitalization and median days on non-prophylactic antibiotic therapy to be significantly shorter in the group of patients administered G-CSF, whereas the number of days with fever, the symptom-free period, and overall survival did not differ significantly between the patients who received G-CSF and those who did not.

In the present study, there was no correlation between the rate of neutrophil or platelet recovery with the engraftment CFU-GM cell count or CD34+ cell count. This may have been due to the small number of study patients, or because all our study patients, i.e. those from both experimental and control groups, received a greater number of CFU-GM and CD34+ cells per kg body weight than generally considered adequate (median > 2.6 x 10^6 CD34+/kg body weight, i.e. > 5.0 x 10^4/kg body weight). Comparable results have been reported by Stadtmauer et al. [24], who found no correlation between the rate of neutrophil or platelet recovery and number of CFU-GM or CD34+ cells infused in patients with metastatic breast cancer. Klumpp et al. [22] also found no correlation between granulocyte recovery and number of CD34+ cells infused in patients, but reported a correlation between granulocyte recovery and number of CFU-GM cells in the graft. Shpall et

al. [8] also found no correlation between neutrophil or platelet recovery rate and number of CD34+ cells infused. However, Weaver et al. [23] observed the dose of CD34+ cells infused to clearly correlate with the rate of neutrophil and platelet recovery, yet all their patients received a CD34+ cell number (> 5.0 x 10^6/L) that by far exceeded the dose generally considered adequate for hematopoietic system recovery.

Furthermore, Bensinger et al. [18] showed the number of CD34+ cells infused to significantly correlate with the rate of neutrophil and platelet recovery (although they were unable to determine the minimal CD34+ cell count necessary for hematopoietic system recovery, since good recovery occurred in at least two patients who had received less than 1.0 x 10^6 CD34+ cells/kg body weight), but found no correlation between the neutrophil or platelet count and number of CFU-GM cells infused in patients. In our study, four patients also received less than 1.0 x 10^6/kg body weight, and had a comparably good recovery of their hematopoietic system.

The lack of positive correlation between the number of CD34+ cells infused and rate of neutrophil recovery may in some studies be due to having set relatively narrow limits, thus making any conclusion more difficult.

The relatively small difference in the rate of granulocyte recovery (1-3 days in the present study, like in most other studies) between the patients administered or not administered G-CSF after autologous hematopoietic stem cell transplantation might be explained by the low dose of G-CSF used in our experimental group, or by the high number of mobilized precursor cells to granulocytes in the graft, which then could additionally respond to G-CSF only to a limited extent. Ho et al. [25] investigated endogenous concentration of G-CSF, interleukin (IL)-3, IL-6 and GM-CSF in 12 patients with breast cancer after myeloablative therapy and autologous hematopoietic stem cell transplantation. Blood concentrations of G-CSF and IL-6 were statistically significantly higher than pre-therapeutic levels, reaching peak on day 7 of engraftment. This finding may in part be the reason for the failure of additional exogenous administration of G-CSF after autologous hematopoietic stem cell transplantation to lead to faster recovery of the hematopoietic system.

Whether the slightly faster neutrophil recovery (in most studies by 1-3 days) justifies the use of the very expensive therapeutic option with growth factors, and whether their potentially undesired, unfavorable effect on platelet recovery is real, remain to be elucidated in additional randomized studies that will also include the cost-benefit analysis.

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