Quantity and Origin of Transplanted Autologous Blood Cells Are Independent Factors Associated with Speed of Posttransplant Hematological Reconstitution

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Aim. Multivariate analysis of the prognostic significance of clinical and laboratory parameters on hematological recovery after autologous hematopoietic stem cell transplantation.

Methods. Sixty-two patients suffering from hematological and non-hematological malignancies entered the study. After conditioning therapy, 28 patients received bone marrow stem cells, 21 received peripheral blood stem cells, and 13 received both. The dynamic of hematological engraftment was calculated as recovery probability of leukocytes and neutrophils. Statistics was done using Kaplan-Meier method and multivariate Cox’s proportional regression.

Results. Numerous clinical and laboratory parameters correlated with hematological recovery, but only two variables were found to be independently associated. Faster reconstitution correlated with greater number of progenitors and patients who received bone marrow cells recovered significantly later than others. Faster recovery could be expected in patients receiving >13x10⁴ CFU-GM/kg body weight, and significantly slower in those receiving <8.5x10⁴ CFU-GM/kg.

Conclusion. The quantity of progenitor cells and transplant type are variables significantly associated with the speed of postransplant engraftment, but these two parameters are mutually independent. The number of stem cells estimated by CFU-GM assay is a good and reliable routine test for predicting hematopoietic recovery.

Key words: bone marrow transplantation; colony-forming units, hematopoietic; hematopoietic stem cell transplantation; hematopoietic neoplasms; stem cells, hematopoietic; transplantation, bone marrow; transplantation, conditioning

Transplantation of autologous hematopoietic stem cells after intensive chemotherapy is an effective method in the therapy of malignant diseases (1,2). Before the transplantation it is important to estimate the dynamic of hematological recovery. Recovery has been proved to be faster after transplantation of peripheral blood stem cells (PBSC) compared to transplantation of bone marrow (2-7). This is usually explained with greater number of progenitors in a PBSC transplant compared to a bone marrow stem cells (BMSC) transplant (1,3-7).

The aim of this study was to evaluate, using a multivariate statistical approach, the prognostic significance of clinical and laboratory parameters on hematological recovery.

Patients and Methods

Sixty-two patients were treated with high-dose chemotherapy followed by transplantation of hematopoietic stem cells, suffering from hematological (n=46; acute myeloid and lymphocyte leukemia, non-Hodgkin lymphoma, Hodgkin's disease, and plasmocytoma) and non-hematological malignancies (n=16), mainly breast and testicular cancer. Basic patients' characteristics are shown in Table 1. All patients were previously treated with conventional chemotherapy commonly used for their diseases. PBSC were collected after a mobilization treatment with high-dose chemotherapy with or without growth factors. Thirty-four patients underwent mobilization therapy: 20 patients received cyclophosphamide with granulocyte colony-stimulating factor (G-CSF), 5 patients citarabine with mitoxantrone, 3 patients mini-BEAM (lower doses of bichloretilnitrosourea, carmustine, etoposide, cytarabine and cyclophosphamide) with G-CSF, and 6 patients received therapy according to other protocols (cyclophosphamide and mini- BEAM alone, CTX, VP-16).

In conditioning regimens, patients were treated with intensive chemotherapy or radiochemotherapy. Ten patients were conditioned with cyclophosphamide and total body irradiation (TBI), 13 received busulfan with cyclophosphamide, 25 BEAM (bichloretilnitrosourea, carmustine, etoposide, cytarabine and cyclophosphamide), and 14 other protocols, mostly melphalan with or without total body irradiation and CARBOPEC (carboplatinum, etoposide, cyclophosphamide). After conditioning therapy, 28 patients (45.2%) received BMSC, 21 (33.9%) received PBSC, and 13 (20.9%) received
both BMSC and PBSC. After reinfusion, 30 patients (18 PBSC recipients, 9 BMSC recipients and 3 BMSC/PBSC recipients) received granulocyte-macrophage colony stimulating factor (GM-CSF) in a dose of 5 mg/kg of body weight as daily subcutaneous injections until the neutrophil count was greater than 0.5x10^9/L in three successive days.

Number of granulocyte/macrophage colony forming unit (CFU-GM) progenitors in transplants was assessed with cultivation in vitro, as described previously (8). The dynamic of hematological engraftment was calculated as recovery probability and set as the day posttransplant on which patients reached leukocyte and granulocyte concentrations in peripheral blood to ≥0.5x10^9/L, ≥10^9/L and ≥2x10^9/L. Day of transplantation was set as day 0. Patients lost to follow-up were censored. Statistics was done using Kaplan-Meier method and multivariate Cox’s proportional regression (forward stepwise model) with original numerical data and qualitative parameters set as dummy variables. Parameters are listed in Table 2. All reported p-values refer to two-sided test and were considered significant if less than 5%.

Results

Probability of hematological recovery of patients in this study is presented in Figures 1 (total leukocytes) and 2 (granulocytes).

**Figure 1.** Cumulative probability of attaining leukocyte counts of 0.5x10^9/L (thick line), 10^9/L (dashed line) and 2x10^9/L (thin line) after autologous hematopoietic stem cell transplantation in 62 patients with hematological and non-hematological malignancies.

**Figure 2.** Cumulative probability of attaining granulocyte (neutrophils) counts of 0.5x10^9/L (thick line), 10^9/L (dashed line) and 2x10^9/L (thin line) after autologous hematopoietic stem cell transplantation in 62 patients with hematological and non-hematological malignancies.

**Table 1.** Patients' characteristics

**Table 2.** Effects of parameters affecting the speed of recovery to three granulocyte concentrations (greater or equal to 0.5, 1, and 2 x10^9/L) in the peripheral blood after hematopoietic stem cells transplantationa

Numerous clinical/laboratory parameters correlated with the recovery of granulocytes (Table 2, for marginal effect p<0.05): number of cycles of previous chemotherapy, transplant volume, cyclophosphamide therapy, etc., but only two variables in the multivariate approach were found to be independently associated with granulocyte recovery (Table 2, partial effect): No. of CFU-GM per body weight in thawed control specimen and type of transplant (when adjusted to progenitors quantity). Faster reconstitution correlated with greater number of CFU-GM/kg (p<0.001 for all granulocyte concentrations), and patients who received BMSC recovered significantly later than others (p<0.02; Table 2). In fact, all 6 hematopoietic reconstitution curves proved faster recovery in patients receiving PBSC only compared to both BMSC (p<0.05) and BMSC/PBSC recipients (p<0.05), but the difference in speed of engraftment between BMSC/PBSC and BMSC only recipients was not always significant (Figure 3, data presented for reaching the granulocyte concentration to ≥2x10^9/L in peripheral blood, with no difference between BMSC/PBSC and BMSC only groups; p>0.05).

The same final result presented for granulocytes in Table 2 was obtained for the recovery of total leukocytes (data not shown).

Concerning CFU-GM/kg variable (median 9.1x10^4/kg, range 1.4-87.3x10^4/kg for all patients), two cut-off values were calculated from the data. Faster recovery could be expected in patients receiving >13x10^4 CFU-GM/kg body weight, and significantly slower in those receiving <8.5x10^4 CFU-GM/kg (Fig. 4).

**Figure 3.** Cumulative probability of attaining granulocyte counts of 2x10^9/L after autologous transplantation in 62 patients with hematological and non-hematological malignancies, related to the transplant type: peripheral blood stem cells (thick line), bone marrow stem cells (thin line), and both peripheral blood and bone marrow stem cells (dashed line).

**Figure 4.** Cumulative probability of attaining granulocyte counts of 2x10^9/L after autologous transplantation in 62 patients with hematological and non-hematological malignancies, related to the quantity of progenitor cells transplanted: more than 13x10^4/kg (thick line), less than 8.5x10^4/kg (thin line), and between 8.5 and 13 x10^4/kg (dashed line).
Discussion

High-dose chemotherapy followed by hematopoietic stem cell transplantation is established therapeutic procedure for many hematological and non-hematological malignant diseases (3,4). Our study showed that number of hematopoietic stem cells transplanted, estimated with No. of CFU-GM/kg of body weight, was highly and significantly associated with faster postransplant recovery (Table 2, p<0.001). The finding is consistent with the literature (1-8), proving that the speed of postransplant engraftment is dependent on quantity of hematopoietic cells with repopulating ability. The reconstitution of BMSC recipients was the slowest compared to all others (Table 2, p<0.02 for all concentrations evaluated). This variable had significant partial effect after adjusting for CFU-GM/kg quantity, meaning that the effects of these two parameters are independent. As stated before, hematopoietic reconstitution curves for all six evaluated cell concentrations showed statistically faster recovery in those patients receiving PBSC only compared to both recipients and BMSC/PBSC recipients (Fig. 3, for granulocytes ≥2x10^9/L in peripheral blood), whereas the difference was not found consistently significant between BMSC/PBSC and BMSC groups. Patients who received both BMNSC/PBSC were at first randomized for PBSC transplantation but the number of stem cells in leukocyte concentrate, estimated with CFU-GM/kg, was assumed not sufficient for safe engraftment, so that they received BMSC with PBSC.

Results shown in Table 2 clearly prove that progenitors quantity (i.e., No. of CFU-GM/kg) and transplant type are variables significantly associated with the speed of postransplant engraftment, but even more important is the finding that these two parameters are mutually independent. It might suggest differences between mobilized BMSC and PBSC progenitors. Several authors investigated characteristics of hematopoietic stem cells harvested from different samples. Lemoli et al (9) reported that mobilized progenitors from peripheral blood and progenitors from bone marrow indeed have some different kinetic and functional profiles, while the study of Ponchio and coworkers (10) failed to prove any difference in proliferative activity between long-term culture-initiating cells and colony-forming cells in BMSC/PBSC.

Lemoli’s results support the findings of our study. Multivariate data analysis approach, based on engraftment kinetics, proved independence of progenitors’ quantity and origin, which might rely on the ability of PBSC to differentiate and repopulate faster (9). However, only engraftment kinetic of leukocytes and granulocytes was evaluated in this investigation as an independent factor and exact differences between BMSC and PBSC characteristics are still not explained and have to be investigated in future.

This study also confirmed that the number of hematopoietic stem cells estimated by CFU-GM assay is a good and reliable routine test for prediction of hematopoietic recovery, as suggested before (8). Efficient in vitro procedure for estimating the functional ability of the pluripotent stem cell is still not known as a routine laboratory test (5). No. of CD34+ cells is widely accepted as a phenotyping procedure for estimating the quality of hematopoietic stem cells autografts. It positively correlates with CFU-GM quantity and some authors use it to replace the CFU-GM assay (1), although it can not evaluate exact proliferative capacity of hematopoietic stem cells. In future extension of this study, both CFU-GM and CD34+ contents will be evaluated.

Concerning the quantity of CFU-GM progenitors as a laboratory parameter, our results showed two disparate values significantly related to the engraftment kinetics (8.5 and 13x10^4/kg; Fig. 4). Data in the figure clearly show that half of the patients receiving more than 13x10^4 CFU-GM/kg reach granulocyte concentration to 2x10^9/L in approximately 15 days, while half of those receiving less than 8.5x10^4 CFU-GM/kg reach the same concentration in approximately 30 days (recovery time is doubled). We feel that the real benefit of this finding will be in planning the hospitalization of patients after transplantation.

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References


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