

AUTOLOGOUS BONE MARROW TRANSPLANTATION IN NON-HODGKIN'S LYMPHOMAS: PREDICTION OF MONONUCLEAR CELL YIELD IN BONE MARROW HARVESTS

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ABSTRACT

We retrospectively analyzed the factors influencing the mononuclear cell (MNC) yield of bone marrow (BM) harvests in a cohort of 15 non-Hodgkin's lymphoma patients undergoing autologous bone marrow transplantation. All the patients were treated with the F-MACHOP regimen and four of them also received radiotherapy on bulky disease. Before harvesting, the patients underwent complete peripheral blood (PB) count, BM biopsy and aspirate. WBC and MNC/ μ L were determined on the pre-harvest PB and BM aspirate samples using an automated counter. The following variables were examined in univariate and multivariate regression analysis as having possible influence on the MNC yield in the harvests: age, sex, number of cycles of CT, previous radiotherapy, state of the disease at the time of harvest, interval between the end of therapy and BM harvest, cellularity of the BM biopsy, absolute WBC and MNC counts in the PB before harvesting, absolute WBC and MNC counts in the BM aspirate performed before harvesting. The amount of marrow harvested was constant for all patients: 21.2 ± 0.24 mL/kg B.W. Among the factors analyzed, two correlated strongly with the MNC yield/kg B.W. in the harvests: the MNC count in pre-harvest PB ($r = 0.823$; $p = 0.0001$) and the MNC count in pre-harvest BM aspirate ($r = 0.802$; $p = 0.0003$). A regression equation was generated that allows calculation of the MNC/kg yield prior to harvesting.

Key words: mononuclear cell yield, bone marrow harvest, NHL, ABMT

The practice of autologous bone marrow transplantation (ABMT) as consolidation treatment has become widespread in non-Hodgkin's lymphomas (NHL) in an attempt to improve remission rate and long-term disease-free survival.¹

Hemopoietic reconstitution after marrow ablative therapy is correlated to the number of granulocyte-macrophage colony-forming units (CFU-GM) harvested and then reinfused into patients.² But the CFU-GM assay, which is used to quantify the number of CFU-GM in a given sample, can be evaluated only retrospectively.

Since hematopoietic stem cells are mononuclear cells (MNC), a simple and reliable method

for calculating the number of cells that need to be collected to ensure adequate engraftment is to count the MNC in the harvest.

Expressed per kilogram of patient body weight (B.W.), 0.9×10^8 MNC is considered a safe amount while 0.4×10^8 MNC is the minimum transplantable dose.³

We retrospectively analyzed the factors influencing the MNC yield of BM harvests in a cohort of NHL patients undergoing ABMT, and found that the number of MNC/ μ L of peripheral blood (PB) and bone marrow (BM), as calculated prior to harvesting, correlated significantly with the yield of MNC/kg B.W. in the harvests.

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Materials and Methods

Fifteen consecutive patients affected by non-Hodgkin's lymphomas (diffuse centroblastic-centrocytic, centroblastic, immunoblastic or anaplastic large-cell) entered the study (11 males and 4 females); median age was 39 years (range 22-56). They were treated with the 3rd generation regimen F-MACHOP for a total of 6 cycles (only 3 cycles in 1 patient and only 4 cycles in 2 others). Four patients were also given radiotherapy (RT) on mediastinal bulky disease.

BM was harvested in complete remission (CR) (N = 8), partial remission (PR) (N = 6) or relapse (R) (N = 1), at a median of 113 days (range 43-253) from the end of therapy (either CT or CT + RT).

None of the patients presented disease involvement of the bone marrow at the time of harvesting. Before harvesting (usually 10 days before), all patients underwent complete PB count and BM aspirate and biopsy.

WBC and MNC/ μL were determined on the PB and BM aspirate samples using an automated cell counter (Cell Dyn 3000, Abbott, Santa Clara, CA, USA) with an incorporated high resolution flow cytometer. Besides traditional forward and perpendicular light scattering, the Cell Dyn 3000 technology, referred to as M.A.P.S.S. (*multi-angle polarized scatter separation*) introduces two additional dimensions to differentially classify WBC without the need for cytochemical staining or monoclonal tagging. The PB and BM aspirate differentials were performed simultaneously with the counter and on a light microscope: only lymphocytes and monocytes were included in the MNC. The percentage of MNC in the BM aspirate was slightly higher with the automated counter due to the presence of 1-3% erythroblasts.

The cellularity of the bone marrow biopsy was determined at low power ($10\times$) and recorded as normocellular (when $> \frac{2}{3}$ of the total space was represented by hemopoietic tissue) or hypocellular ($< \frac{2}{3}$).

Patients then underwent bone marrow harvesting under general anesthesia. Marrow was aspirated from the posterior iliac crest in a sterile operating room, and the same automated

cell counter as above was used to perform cell counts on the collected marrow.

Statistical analysis

Univariate and multivariate regression analyses were performed (using the stepwise regression method for the multivariate analysis) to test the correlation between the yield of MNC/kg B.W. in the harvests and the following variables: age, sex, number of CT cycles, previous radiotherapy, state of the disease at the time of harvest, interval (days) between the end of therapy and BM harvest, cellularity of the BM biopsy, absolute WBC and MNC counts in the PB before harvesting (pre-harvest PB), absolute WBC and MNC counts in the BM aspirate performed before harvesting (pre-harvest BM).

Results

Overall, the yield of MNC/kg B.W. in the harvests was $0.42 \pm 0.13(\text{SD}) \times 10^8/\text{kg}$ B.W. (median 0.42; range 0.17-0.68). Table 1 shows the results of univariate analysis. Of all the factors analyzed, two correlated strongly with the MNC yield/kg B.W. in the harvests: the MNC count in the pre-harvest PB ($r = 0.823$; $p = 0.0001$) and the MNC count in the pre-harvest BM aspirate

Table 1. Correlation between MNC $\times 10^8/\text{kg}$ B.W. harvested and other variables analyzed in univariate analysis.

	r	p
PreH PB MNC $\times 10^3/\mu\text{L}^*$	0.823	0.0001
PreH BM MNC $\times 10^3/\mu\text{L}^*$	0.802	0.0003
State of disease at harvest ^o	0.856	0.080
Previous radiotherapy [#]	0.351	0.198
BM cellularity [@]	0.338	0.510
PreH PB WBC $\times 10^3/\mu\text{L}^{\wedge}$	0.220	0.420
PreH BM WBC $\times 10^3/\mu\text{L}^{\wedge}$	0.189	0.498
Days from end of Tx [§] to harvest	0.153	0.580
Number of CT cycles	0.140	0.590
Sex	0.140	0.610
Age	0.130	0.640

*MNC/ μL as calculated by an automated counter in the PB and BM just prior to harvest (PreH); ^oComplete remission vs. partial remission and relapse; [#]No RT vs. RT; [@]Normocellular vs. hypocellular marrow; [^]WBC/mL as calculated by an automated counter in the PB and BM just prior to harvest (PreH); [§]Tx=therapy.

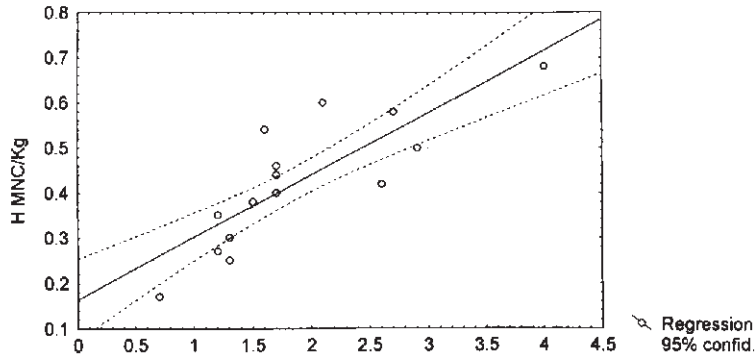


Figure 1. Comparison of harvested MNC×10⁸/kg b.w. (H MNC/kg) and pre-harvest peripheral blood MNC×10³/μL (PreH PB MNC/μL). Correlation: r=0.823; p = 0.0001.

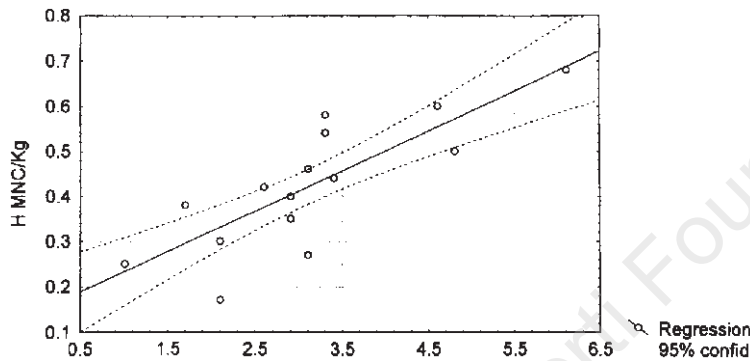


Figure 2. Comparison of harvested MNC×10⁸/kg b.w. (H MNC/kg) and pre-harvest bone marrow MNC×10³/μL (PreH BM MNC/μL). Correlation: r = 0.802; p = 0.0003.

(r = 0.802; p = 0.0003). A regression equation was generated that allows calculation of the MNC yield when either of these two values is known (see also Figure 1 and 2):

$$\begin{aligned} \text{harvest MNC} \times 10^8 / \text{kg} &= 0.16337 + \\ & (0.13792 \times \text{pre-harvest PB MNC} \times 10^3 / \mu\text{L}) \\ \text{or} \\ \text{harvest MNC} \times 10^8 / \text{kg} &= 0.14363 + \\ & (0.08905 \times \text{pre-harvest BM MNC} \times 10^3 / \mu\text{L}). \end{aligned}$$

Since the amount of marrow harvested was constant for all patients (21.2±0.24 [SE] mL/kg B.W.; median 21.1 mL/kg B.W., with a 95% confidence interval of 20.1-23.2 mL), these equations are valid for a harvest of 21.2±0.24 mL/kg B.W. If, according to the equations, the calculated MNC yield is outside the desired range (i.e. the number of MNC in the pre-harvest PB or BM is too high or too low), one could easily calculate the number of MNC

needed in the harvest to ensure adequate engraftment by modifying the amount of mL/kg B.W. to be harvested:

$$\begin{aligned} \text{MNC/kg}_{21.2 \text{ mL}} : \text{MNC/kg}_{\text{XmL}} &= 21.2 \text{ mL} : \text{X mL} \\ \text{MNC/kg}_{\text{XmL}} &= \frac{\text{MNC/kg}_{21.2 \text{ mL}}}{21.2 \text{ mL}} \times \text{X} \end{aligned}$$

where X is the estimated mL of marrow harvested/kg B.W., MNC/kg_{21.2 mL} is the amount of MNC harvested/kg when 21.2 mL/kg are collected and MNC/kg_{XmL} will be the amount of MNC harvested/kg when the desired volume of BM (X) is collected.

Multivariate analysis showed that each of these two factors independently of the other was so strongly correlated to the MNC yield that the addition of any other variable (stepwise regression method) was not able to significantly increase the correlation.

Discussion

The quality of a harvest can be evaluated only retrospectively by analyzing the number of CFU-GM, CD34⁺ cells and even LTC-IC/mL present in the processed BM.⁴ Nevertheless, during BM collection the number of MNC/mL in the harvest is the most simple and reliable method for predicting the amount of marrow needed. Knowing in advance the amount of bone marrow to be harvested is very important in that it would allow collection of a sufficient number of hemopoietic stem cells to ensure adequate engraftment, eliminate the need for several cell counts during harvesting, and avoid harvesting excessive (and not needed) amounts of marrow. The factors that influence the MNC yield in BM harvests have not been established, so protocols used in bone marrow transplant centers vary widely in their assessment of an adequate cell yield at harvest. Approximately half the centers determine harvest volume on the basis of intraoperative cell counts, while other centers harvest a predetermined volume of bone marrow (ranging from 10 to 30 mL/kg B.W.) and intraoperative cell counts are not performed.⁵ Bone marrow biopsies are frequently performed before harvesting to ensure that the marrow is free of malignancy and has adequate cellularity. The relationship between biopsy cellularity and a cell yield sufficient to produce engraftment has been reported. According to Vukelja *et al.*⁶ BM cellularity correlates with nucleated BM cell recovery from marrow harvests; however, these investigators provided neither actual marrow cellularity nor marrow cell yield. More recently, Rosenthal *et al.*⁷ reported the same relationship but the correlation they found between biopsy cellularity and marrow cell counts was very low: $r = 0.24$ for unprocessed marrow and $r = 0.36$ for the final cell count after processing (not unlike the correlation we found between marrow cellularity and MNC yield: $r = 0.338$). Moreover, in their study the variability in marrow cellularity and cell yield was very great (from 2 to 80% and from 0.6 to 8.2×10^8 nucleated cells/kg B.W. respectively), and a lower limit of biopsy cellularity below which an adequate cell count was unlikely to be obtained could not be estab-

lished. They concluded by indicating that more than 65% cellularity as the one consistently yielded at least 2×10^8 nucleated cells/kg B.W., but emphasized that even 20% marrow cellularity usually yields the same amount of cells.

These data illustrate the need for better predictive factors of cell yield. Nevertheless, a clear correlation has still not been established between cell yield in BM harvests and the peripheral blood or bone marrow MNC counts found before harvesting. Our analysis was carried out on a homogeneous group of patients all affected by the same disease and all treated with the same chemotherapy regimen. We tried to correlate the MNC yield in the harvest to all variables known to possibly affect it, and found a strong and statistically significant correlation with the number of MNC/ μ L in the PB and BM aspirate performed a few days before harvesting. These two factors were so closely correlated (independently of each another) that the addition of any other factor in the multivariate analysis was not able to add any further significance.

A regression equation was generated that allows advance calculation of the number of MNC harvested/kg B.W. on the basis of the absolute number of MNC/ μ L in the PB or BM aspirate. This equation is valid for harvests of 21.2 ± 0.14 mL/kg B.W. If the predicted MNC yield is outside the desired range, one could easily calculate the mL of marrow to be harvested with a simple equation in which the mL of marrow/kg to be harvested (X) are progressively increased or decreased with respect to the 21.2 mL/kg used in this study.

Though this study was retrospective and carried out on a small sample of patients, the correlation we found was very strong and significant. The predictive power of this equation must be evaluated on a larger sample, and a prospective study is currently in progress.

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