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# Multiple small versus few large amount aspirations for bone marrow harvesting in autologous and allogeneic bone marrow transplantation

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ABSTRACT

For successful bone marrow transplantation it is necessary to obtain enough progenitor cells during the bone marrow (BM) harvesting procedure. Most centers are using multiple aspirations of maximum 2 ml BM (A), while other centers are using few larger amount aspirations for BM harvesting (B). There is still a discussion about possible differences in graft composition between A and B. To evaluate the feasibility in children we evaluated twenty BM harvestings that were performed in 18 donors, 7 autologous (median age 6.93y; 2.48–16.6) and 13 allogeneic donors (median age 19.75y; 6.45–50.7). A and B were performed crosswise by 2 operators starting with A (2 ml) or B (100 ml) changing to B or A, collecting identically amounts with both methods. We found no statistically significant difference between A and B for MNC, T-cells, and CFU (MNC/ml 824572 versus 725000, $p = 0.728$; MNC/kg 3.1 $10^7$ versus 2.9 $10^7$, $p = 0.296$; CD3/ml 162500 versus 300000, $p = 0.310$; CFU/$10^5$ MNC 1678 versus 1315, $p = 0.094$), but for CD34+ cells (CD34/kg 2.62 versus 2.09, $p = 0.045$). BM harvest by the large amount few punctures method (B) is as sufficient as the commonly used small amount frequent punctures method (A), and could be therefore used equally.



## 1. Introduction

Bone marrow harvested by multiple aspirations from the iliac crest was the main source of human progenitor cells in the early times of stem cell transplantation. With the introduction of GCSF as mobilizing agent and the use of leukapheresis for stem cell harvest bone marrow harvest became less important, and over the time leukapheresis developed to the predominantly used method for stem cell harvesting. Recently the higher incidence of chronic graft versus host disease (GVHD) after peripheral blood progenitor cell transplantation in contrast to bone marrow derived cells led to a rethinking about the use of bone marrow as a source for stem cell transplantation [1]. Therefore, the old discussion about how to harvest stem cells from the bone marrow was brought again to our attention. Especially the question whether multiple small or a few large amount aspirations lead to a higher contamination of the bone marrow harvest with “peripheral blood” and therefore to a higher CD3 positive T-lymphocytes content, associated with higher incidence of chronic GVHD. In order to elucidate this, we retrospectively analyzed our data from a cross over study where we compared multiple small versus few large amount aspirations. Data were collected in 1996 but not published at that time. However, due to the regained significance of this issue, the lack of data in children and the unsolved questions we decided to restart an evaluation of these data. At the time of donation the donors and/or their guardians were asked to provide their consent to the study design. Ethical committee was not necessary at that time.

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## 2. Patients

Twenty procedures of bone marrow harvesting for eighteen patients (median age 8.78, range 2.48–16.6, F 5, M 13) were enrolled from 1991 to 1993. All patients were transplanted in the bone marrow transplantation unit of the St. Anna Children's Hospital, Vienna. There were seven bone marrow harvestings for autologous BMT and thirteen for allogeneic BMT. The median age of the autologous donors was 6.93 years (range 2.48–16.6, 1 female, 5 male), and of the allogeneic donors, the median age was 19.75 years (range 6.5–50.7 years, 8 female, 5 male). The median amount of harvested bone marrow was 900 ml (range 440–2380 ml) corresponding to 37.66 ml/kg body weight of the recipient (range 20–55.3 ml/kg).

## 3. Methods

Bone marrow harvesting was performed in our local surgery theatre under sterile conditions and general anesthesia. The multiple small amount aspiration technique (method A) was defined as an aspiration of 2 ml bone marrow at most before the position of the needle in the bone was changed. The large amount aspiration technique was defined as an aspiration of at least 100 ml from one puncture before the location of the needle was changed (method B). The bone marrow was collected in two separate bags. After gentile squeezing samples were drawn from each of the bags for laboratory analysis. The following cell populations were investigated: mononuclear cells (MNC), CD 34 positive progenitor cells, CD34 positive and CD 45 RA negative early myeloid progenitor cells, CD 34 positive and CD 45 RA positive late myeloid progenitor cells and CD 3 positive mononuclear cells, and colony forming units (CFU/GM). Two operators carried out the bone marrow harvesting. One began with the multiple small amount aspiration (Method A) and changed in the second part to the few large amount aspirations technique (Method B). The second operator carried out the procedure in the opposite way. The bone marrow was collected in two different bags, one for method A and one for method B. After a sample was collected from both bags each, the marrow was transferred into one bag before transplantation. Both operators collected the same amounts for each harvesting method until the amounts of bone marrow cells harvested with both methods were identical. The total volume of harvested bone marrow depends on the cellularity of the bone marrow. Routinely a collection of $0.3 \times 108$ nucleated cells/kg recipient body weight up to a volume of 20 ml/kg recipient body weight is planned.

### 3.1. Mononuclear cells

Bone marrow samples were diluted in Iscove's modified Dulbecco's medium (IMDM) (1:2–1:5) before density centrifugation. Between 4 ml and 6 ml of the bone marrow suspension were layered on 4 ml of 1,077 g/cm$^3$ density solution (Nycoprep, Nycomed, Oslo, Norway) and centrifuged at 1000 g and at 20 °C for 20–30 minutes. Low density cells are collected from the interface between density solution and medium, washed twice (400g, 4 °C) and re-suspended in IMDM containing 2% fetal calf serum (FCS). The number of MNC was determined in a counting chamber after appropriate dilution in Türk's solution (Merck, Darmstadt, Germany). MNC number was recorded as cells per 1 ml of the original bone marrow sample and as cells harvested per kg body weight. In two cases the information about the MNC numbers was missing.

### 3.2. CD34+, CD34/45RA-, CD34/45RA+ and CD3+ cell typing

MNCs were suspended in IMDM/2% FCS. The $1 \times 10^5$ cells were used for each analysis. Staining was performed in the dark at 4 °C for 30 minutes. After washing, cells were resuspended, filtered through 30 nm nylon mesh (Swiss Silk Bolting Mfg Co. Zurich, Switzerland) and analyzed within 30 minutes. Results were reported as cells per 1 ml of bone marrow and as cells harvested per kg body weight. Staining for CD 34 positive cells was performed in all cases. Staining for CD 45 RA cell typing was performed since April 1992 in 9 cases and the cell typing of CD 3 in 7 cases.

### 3.3. Clonogenic assay

Cells from the bone marrow suspension were cultured with methylcellulose-based semisolid culture medium as described elsewhere. Depending on the flowcytometric CD 34 analysis performed before plating, the number of unsorted mononuclear cells per milliliter of culture medium ranged between $1 \times 10^3$ and $1 \times 10^5$. Cultures were incubated at 37 °C in humidified atmosphere and in presence of 5% $CO_2$ and 3% $O_2$ in N2. The proportions of CFU-GM, CFU-Mix and burst forming unit erythrocyte (BFU-E) were evaluated on day 14. Colonies were recorded as numbers per $1 \times 10^5$ MNC [2,3].

### 3.4. Statistics

The Wilcoxon range test and the Mann–Whitney U test were used to determine the significance (p value) of the difference between two variables or cohorts. A p value of $<0.05$ was accepted as statistically significant. Also used was the Spearman rank correlation coefficient to determine the correlation between two variables. Statistical analysis were performed with WinSTAT® for Microsoft Excel (version 2012.1; Robert K. Fitch©; Germany).

## 4. Results

Detailed results are shown in Table 1. Results are expressed as median (range). For MNC numbers there was no significant difference between method A and method B. The amount per ml bone marrow harvested and the proportion of the CD 34 positive MNC were higher in method A. These results were statistically significant ($p = 0.028$ and $p = 0.019$). However, in 9 of the 18 harvests there was no significant difference for early myeloid progenitor cells (CD 34 positive and CD 45 RA negative MNC) between methods A and B. The same results were shown for MNC, CD 34 positive MNC, CD 34 positive and CD 45RA negative MNC calculated per kg body weight of the recipient. Only the proportion ($p = 0.028$) but not the absolute number