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ORIGINAL ARTICLE

Optimizing BM harvesting from normal adult donors

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The experience at a single institution of BM harvesting (BMH) in general anesthetic for allogeneic transplantation from 49 healthy adult donors since March 2002 is presented in detail, together with an analysis of all the donor complications. In this study, we analyzed the advantages through the change from an aspiration needle with one hole (group A, $n = 18$) to a system with additional five side holes (group B, $n = 31$) in April 2005 for faster aspiration of large volumes of BM. In group B, the operation time was reduced by 50%, which is 12 min to date (1006 ml BM). Furthermore, the collection rate (volume BM/time) was significantly increased, namely to 81.9 ml/min in group B. The yields of total nucleated cells and CD34+ cells are nearly identical and adequate in both systems. The proportion of donors treated as day cases—that is, able to be discharged on the same day as the procedure—was 56% in group A and 81% in group B. There was no significant operative site morbidity. BMH accomplished by trained personal is a safe procedure for healthy adult donors on an outpatient basis as standard in our collection center.

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Introduction

BM aspirated from the iliac crest of the donor was the main source of hematopoietic stem cells for transplantation for several decades. However, during the past decade, G-CSF mobilized PBSCs are increasingly being used. But compared to BM PBSC contain a 10-fold higher content of T cells and therefore carry an increased risk of acute GVHD.[1,2]

The European Group for Blood and Marrow Transplantation activity survey 2006 on hematopoietic SCT (HSCT) provides numbers of HSCT by indication, donor type and stem cell source over the past year in Europe. The stem cell source has changed from BM to peripheral blood (PB) and novel conditioning regimens have been introduced. In 2006, of the 9661 allogeneic first transplants that were performed 24% (2352) were from BM (71% from PB and 5% from cord blood). Within allogeneic HSCT, the only disease indications with more BM than PB donors as stem cell source were BM failure syndromes (57% BM) and congenital disorders (58% BM).[3] There may be two reasons for a renaissance of BM donations. On the one hand, BM remains a significant source of stem cells for transplantation in selected indications. On the other hand, the number of donors feeling insecure will be on the rise by reading publications on findings for healthy volunteers/donors who developed hematological malignancies following G-CSF administration.[4] This may decrease the number of donors' willing to undergo G-CSF-mobilized stem cell harvesting procedures.

An important precondition for successful performance of BMT is the qualitatively and quantitatively efficient BM harvesting (BMH) of the donor's BM with minimal impairment.[2,5] An adequate marrow cell dose is one of the most important factors for successful engraftment, thus improving the survival rate of the patients treated.[6,7] The time length of the BMH treatment should be kept as short as possible to minimize side effects and complications of anesthesia and to use operating theater capacity more economically.[8]

The aim of this retrospective study was to evaluate the factors, for example, yield of nucleated cells, CD34+ cells and time of collection in BMH by different aspiration needles with or without side holes.

Materials and methods

BM donors

Results of all marrow collections originated from unrelated donors ($n = 46$) and siblings ($n = 3$) in the study period from March 2002 to July 2007 (group A, October 2002–August 2005; group B May 2005–July 2007). The donors were evaluated by the local donor registry in Heidelberg (DEHSR) and Mannheim (DEMAN). Donor characteristics are shown in Table 1.

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Table 1 Donor characteristics

	Group A	*Group B*	*Total*
No. of subjects	18	31	49
Male	9 (50%)	19 (61%)	28 (57%)
Female	9 (50%)	12 (39%)	21 (43%)
Median age (range), years	38 (19–46)	38 (20–58)	38 (19–58)

BM collection

The typical collection team consists of five people: two collectors, an anesthesiologist and two nurses (one theater nurse and one circulating nurse). After informed consent, donors underwent BM aspiration simultaneously by two operators from both posterior iliac crests using sterile technique under general anesthesia and additional local anesthesia with 10 ml of scandicaine 2%. Administration of anesthesia and BMH were performed under identical conditions with respect to staff, material, surrounding temperature and operating theater.

In group A, the needle was introduced in the cavity. In the correct position it felt stable. The syringe was attached, and a small volume about 10 ml was aspirated. The needle was rotated 90°, followed by another 10 ml aspiration until a full cycle. The time for a rotation of 360° was only a few seconds to avoid clotting in the syringe. The stylet was reinserted in the needle and introduced a few millimeters deeper into the bone cavity. This collection process was repeated until reaching the opposite cortex. After approximately 100 ml BM a new insertion side was necessary. When informed of an expected high volume (>1000 ml) of BMH, the theater nurse prepared 30 (20 ml) syringes (wetting with heparin).

In group B, the collection procedure was performed in a similar way, but the collection volume per needle insertion was approximately 200 ml BM. The needle was rotated only 45° for each of the 10 ml BM aspirations until a full cycle was archived.

BM was harvested and prepared by the marrow collection center Cytonet Heidelberg, convenient to the donor and when necessary transported by courier to the marrow transplant hospital throughout the world. Cytonet Heidelberg has a manufacturing license for stem cell products issued by the regional council. The harvested volumes varied between 280 (small volumes for pediatric recipients) and 1771 ml. The anesthesiologist was informed of the maximum volume of the individual BMH and compensates decreasing blood pressure by 0.9% sodium chloride infusions. Duration of collection time was defined as the interval between the time at the start of BM collection (first insert of the needle) to the time at the end of the BM collection (needle pulled out). The amount of BM collected was determined by the difference between the final volume of BM and media (Heparin 20 IE per ml BM; Braun, order no. 20472217; ACD-A 1/10 ml BM; Fresenius, order no. 01024945), two measured items. Donors normally sign consent and donate an additional 40 ml BM for research (after the approval by the Heidelberg University Ethical Board; approval nos.: 251/2002 and S-076/2007).

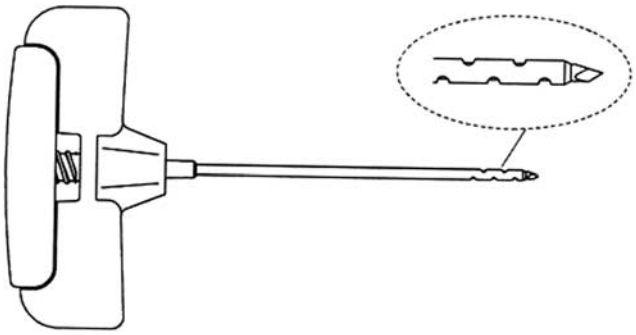

Figure 1 Design drawing of the Somatex Transplant-Aspiration Needle system. Enlarged in detail at the top followed by five additional side holes spaced in height and opposed to each other.

Puncture systems

LMV Medizintechnik reusable aspiration needle (Wiesloch, Germany) with one hole (a diameter of 3 mm and a length of 80 mm; group A) vs Somatex Transplant-Aspiration Needle system (Teltow, Germany; order no. 180635) with a diameter of 3 mm with 11-gauge thickness, a length of 90 mm and additional five lateral side holes (Figure 1; group B).

Data collection

Marrow collection center personnel completed a standard form describing the BMH procedure. Cytonet Heidelberg has among others the manufacturing license for BMTs. This pharmaceutical enterprise keeps complete documentation for all steps of manufacturing a BM transplant including all collecting steps, cell counts, volume analyses and sterile testing. The data collection was carried out in a retrospective and nonrandomized trial.

Nucleated cell counting

The total number of nucleated cells, lymphocytes and hemogram was counted directly in a hemocytometer COULTER AcT diff (Beckman Coulter, Fullerton, CA, USA).

CD34+ cell testing

A total of 50 µl of whole BM was incubated with 10 µl of PE-conjugated monoclonal anti-CD34 antibodies (Clone 2D1, mouse IgG_1; BD Biosciences, Heidelberg, Germany; order no. 345802) for 15 min at room temperature and then depleted of RBCs by lysis with ammonium chloride lysis reagent (15 min at 2–8 °C, dark). The cells were analyzed with a BD FACSCalibur (Becton Dickinson, Franklin Lakes, NJ, USA).[9]

Statistics

The Mann–Whitney *U*-test was used to determine the significance (two-sided *P*-value <0.025) between the different puncture system cohorts. Boxplots represent the smallest observation, the lower quartile ($x_{0.25}$), the median ($x_{0.50}$), the higher quartile ($x_{0.75}$) and the largest observation.

Results

A total of 49 BMHs were performed on healthy donors during the study period. Distribution of BMHs performed

in the year 2002 (1), 2003 (5), 2004 (5), 2005 (7 (group A) + 11 (group B)), 2006 (12) and 2007 (8 until August). The volume of harvest was adapted to the recipients' body weight (BW) with the minimum required total nucleated cells (TNC) yield $\geqslant 2 \times 10^8$ per kg and the donors' BW (maximum 20 ml/kg BW).[2] By change to a needle system with five side holes (group B) in healthy donors the time for collection of 1000 ml BM (median is 1006 ml in group A and 1024 ml in group B) was reduced significantly from 27.5 min in group A (needle system with one hole) to 12 min in group B (Table 2; Figure 2). The harvested BM volume per min (collection rate) was rose significantly from 39.44 ml (group A) to 81.9 ml (group B; Table 2; Figure 3). Regarding the quality of collected BM as determined by total cell count, comprising mononuclear cells (TNC) and CD34+ cells, there was no significant difference between the groups. Lymphocyte concentration in the transplant as a value for contamination with PB was lower in group B (Table 2).

The proportion of donors treated as day cases—that is, able to be discharged on the same day as the procedure—was 56% in group A and 81% in group B (Table 2).

Criteria for an overnight stay were: donor could not be accompanied/driven home and/or could not be discharged with a carer adult for the following 24 h.

There were no life-threatening complications, no severe anesthesia complications and no significant operative site morbidity. No transient neuropathies, bleeding, pain at the collection side, infection, vomiting, dizziness or transient postoperative fever of unknown origin were observed, nor did any of these cause an overnight stay. No autotransfusions were performed.

Discussion

High yield and excellent quality of harvested BM are essential preconditions for successful outcome of allogeneic BMT. In the relevant literature, data on the exogenous conditions during BMH are rare. Increasing room temperature and body temperature, respectively, should lead to a significantly better yield of BM per min without loss of sample quality.[8] In this study, the collection time was in the best group (body temperature was increased by about 1 °C) 38.25 min for 1200 ml BM (36 ml BM per min).

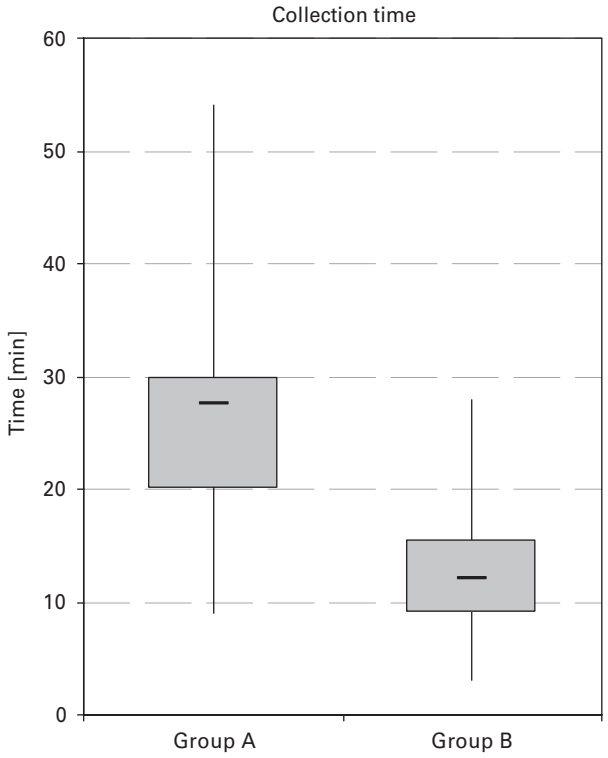

Figure 2 Collection time using LMV Medizintechnik reusable aspiration needle with one hole (group A) vs the Somatex Transplant-Aspiration Needle system with additional five lateral side holes (group B). Time of harvest was more then halved by using the Somatex needle (group B; $P = 0.0001$).

Table 2 Results of bone marrow harvesting

	Group A	*Group B*	P-*value*
Graft, median (range)			
n	18	31	
Volume of harvest (ml BM)	1006 (454–1566)[a]	1024 (280–1771)[a]	0.9283
Collection time (min)	27.50 (9–54)	12.00 (3–28)	0.0001
Collection rate (ml BM per min)	39.44 (19.26–70.00)	81.90 (44.93–150.90)	<0.0001
TNC (10^8 per ml BM)	0.22 (0.11–0.34)	0.18 (0.11–0.29)	0.427
TNC (10^8 per kg BW recipient)	3.36 (1.00–14.80)	3.20 (1.75–13.00)	0.3192
CD34+ (10^6 per ml BM)	0.15 (0.05–0.46)	0.13 (0.04–0.35)	0.166
CD34+ (10^6 per kg BW recipient)	3.00 (1.61–33.00)	2.30 (0.70–13.40)	0.2266
Lymphocyte (10^3 per µl)	5.55 (3.05–8.05)	3.95 (2.45–6.61)	0.0006
Overnight admissions	8 (44%)[b]	6 (19%)[b]	
Donors hemogram, 4 h post-donation; median (range)			
n	18	31	
Hemoglobin (g per 100 ml)	11.15 (9.2–14.4)	11.70 (9.3–13.7)	0.0854
Hematocrit (%)	32 (27–42)	35 (26–42)	0.022
Platelets (10^3 per µl)	269 (161–463)	237 (125–428)	0.3542

Abbreviations: BW = body weight; TNC = total nucleated cell.
[a]Small volumes are grafts for recipients (pediatric patients) of low BW.
[b]Total number of donors.

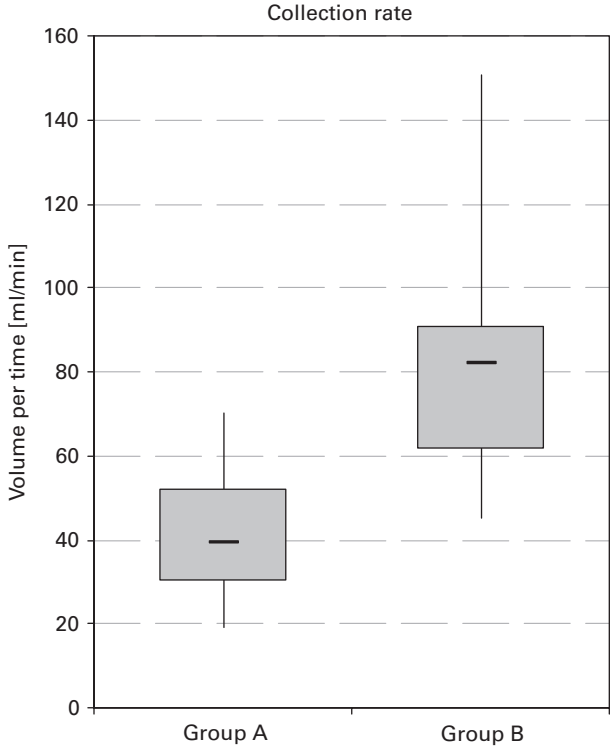

Figure 3 Group A in comparison to group B according to the collection rate. Boxplots represent the median, the smallest and largest observation and the lower and higher quartiles. Rate was increased from 39 to 82 ml/min (median) in group B; $P<0.0001$.

Furthermore, early studies showed that aspiration of multiple small quantities (1–2 ml) of marrow minimized the dilution with PB and resulted in greater number of cells and hematopoetic progenitors.[10,11] This procedure of multiple punctures and lesions of the periosteum may be responsible for side effects, for example, pain at the collection side.[12] In April 2005, we started to optimize our BMH and started to use a new one-way aspiration needle system with additional five side holes thitherto we were using a reusable aspiration needle with one hole. Our retrospective study started in March 2002.

The new needle system (group B) has reduced the time of BM collection by 50%, which is 12 min to date, compared to 27.5 min (group A; Table 2; Figure 3), impacting consequently on the duration and thereby the risk of anesthesia. Furthermore, the collection rate (BM volume/time) was significantly increased from 39.44 up to 81.9 ml/min (Table 2; Figure 3). We attempted to limit aspirate volume to 10 ml or less, but we did not routinely attempt to limit it to 2–5 ml per aspirate, as has been suggested by other BMH centers.[13] In group B, only 2–3 punctures on each side were needed for 1000 ml BMH and the additional local anesthesia of the periosteum may result in low side effects of our collection.

The same well-trained operation team was used throughout, so that variations in techniques between physicians and the fact that some physicians collect BM more rapidly or collecting larger volumes from each aspiration side should not influence the results.[14] The influence of a 'learning effect' on better results in group B could be clearly observed, because since March 2006 the collection rate was always higher than 80 ml/min. This value was never obtained in group A. The quality control was performed by Cytonet Heidelberg. The yields of TNC and CD34+ cells are slightly lower but adequate in group B (Table 2). The increased aspiration volume (up to 10 ml) and higher collection rate in group B did not lead to a higher dilution of the aspirated product with PB (Table 2).

The faster collection and the earlier point of time for reevaluation (physical status and hemogram) of donors 4 h after BMH allows earlier discharge. Groups A (56%) and B (81%) donors were therefore discharged within 8 h after BMH and none have developed long-term complications from the procedure as also reported by Bolwell *et al*.[13] The own increasing experience plus the reports of other centers, for example, Aleem *et al*.,[15] allow BMH in outpatient setting.

There were no life-threatening complications or severe adverse events observed that necessitated an overnight stay. Day case BMH appears safe, cost effective and reduces the pressure on inpatient beds.[15]

In summary, BMH from adult healthy donors, under optimal conditions reduce operation time, risk of complications and risk of anesthesia. In our collection center there is a trend to perform BMH from healthy adult donors on an outpatient basis as standard.

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