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14.1 Introduction

Historically, the bone marrow (BM) has been the first source of stem cells considered since the early 1960s for HSCT (Santos 1990; Thomas et al. 1979; Mathe 1964; Gorin et al. 1990). Parallel attempts at using fetal liver cells at that time have remained unsuccessful. In 1986 the first success of an unrelated cord blood (UCB) transplantation in a child promoted UCB (Gluckman et al. 1997) as an alternative source in certain settings.

Since 1994 and the initial demonstration that PBSC mobilized by cytokines (G-CSF first and more recently when needed plerixafor) could be used as well as BM, the proportion of PB transplants has considerably increased to reach about 70–95% of all stem cell transplants (Passweg et al. 2012, 2016), so that nowadays BM transplantation accounts for a minority of transplants.

There remain however several situations where and when a marrow harvest can still be of interest or even is highly recommended.

This chapter indicates the principal indications of BM transplantation, compares schemati-

cally the advantages of BM versus PB, and details the technique of BM harvesting.

14.2 Indications for Considering and Possibly Selecting BM as a Preferred Source of HSC

It is not the purpose of this chapter to review the benefit/risk ratio of BM versus peripheral mobilized blood as sources of HSC. Several studies, including prospective randomized studies, have shown in general with BM when compared to PB slower engraftment but lower incidence and lower severity of acute and chronic GVHD with in the end similar disease free and overall survivals (Schmitz et al. 2005). However, some retrospective studies for both auto- and allo-HSCT have shown better survival with rich BM collections (Gorin et al. 2003) or BM versus PB (Gorin et al. 2009, 2010). Also, the quality of life has not been carefully analyzed (Sun et al. 2010), and further studies may be in favor of BM (Ruggeri et al. 2018; Lee et al. 2016).

Time and cost constraints have however in general favored leukaphereses and PB transplants which represent about 95% of all auto-HSCT and 70% of allo-HSCT (Passweg et al. 2016). Table 14.1 lists the situations when nowadays marrow may appear as a better choice.

For allo-HSCT, BM is preferred/mandatory whenever the wish to reduce toxicity, NRM, and most of all GVHD (particularly extensive chronic)

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Table 14.1 Preferences for BM as source of stem cells in ALLO-HSCT

Allo-HSCT	Rationale	Justification 1	Justification 2	Reference
Children donors and/or recipients	Administration of G-CSF to and leukapheresis of the donor more difficult to set	More cGVHD and NRM after PB compared with BM	In some countries, the use of G-CSF (and plerixafor) is not allowed in children	Simonin et al. (2017)
Aplastic anemia	BM mandatory, associated with better results	Higher risk of GVHD with PB	Included in EBMT and CIBMTR guidelines	Bhella et al. (2018), Schrezenmeier et al. (2007), Bacigalupo et al. (2012), Eapen et al. (2011), and Barone et al. (2015)
MAC with a MUD and no ATG	BM associated with better results and less cGVH	Randomized trial with no ATG showing less cGVH and better QOL with BM	If a suitable BM donor is available. Otherwise PB with ATG	Anasetti et al. (2012), Lee et al. (2016), Eapen et al. (2007), and Walker et al. (2016)
Haploidentical transplantation	BM or combination of PB and BM favored by some teams	Team choice or clinical trial	High-dose CY for GVH prevention	Kasamon et al. (2017) and Luznik et al. (2010)

is considered as priority, such as for children with aplastic anemia and for some teams for haploidentical transplantation (see Table 14.1). The outcome considered to favor this choice is the GRFS (GVHD and relapse-free survival) as defined by EBMT (Ruggeri et al. 2016).

Conversely, PB can be a first choice in patients with hematological malignancies at high or very high risk of progression/relapse, such as AML FLT3ITD positive, lymphomas in progression after relapse from auto-HSCT, etc. for whom the risk of relapse is considered as first priority despite the risk of increasing NRM. Usually, this choice is made in parallel to the decision whether the conditioning regimen should be MAC or RIC (Gilleece et al. 2018).

For autologous HSCT (Table 14.2) the two major reasons for using BM are autologous transplantation for AML in remission and attempts at increasing the stem cell dose infused following poor marrow collections.

14.3 Mobilized or Primed Marrow

Following the discovery of cytokines, G-CSF in particular, the use of BM collected after 2–4 days of G-CSF administration has been investigated in the year 2000–2005. G-CSF-primed marrow harvesting results in a graft with more mononuclear

cells collected and higher CD34(+) stem and progenitor cell doses (Grupp et al. 2006). The clinical significance of different HSC sources (primed marrow, mobilized blood, and steady-state marrow) in auto- and allo-HSCT was reviewed in 2004 (Elfenbein and Sackstein 2004). Mobilized marrow speeds up engraftment for both auto- and allo-HSCT, with a possible (unproven) reduction of GVHD rate and severity. Its use nowadays is rare, but it is for some teams the preferred stem cell source or part of a combination of primed marrow + PB as stem cell source for haploidentical donor transplantation (Huang et al. 2009; Ly et al. 2015).

14.4 Technique of BM Collection and Impact of the Dose of Nucleated Cells Infused

Marrow is collected from the posterior superior iliac crests, usually under general anesthesia, although few teams have used sedation or locoregional anesthesia (Fig. 14.1).

Marrow is aspirated with bone needles with multiple holes all around, which makes collection easier and the procedure more rapid. However, to avoid large dilution with blood, it is recommended to limit each aspiration to a volume of less than 5 mL, before transferring the



Fig. 14.1 Bone marrow harvest

aspirate through a three-way tap to the collection bag. The collection bag contains ACD anticoagulant solution and the syringes are rinsed with heparin (5,000 U/mL).

The goal is nowadays to obtain typically at least 3×10^8 nucleated cells/kg, although 2×10^8 nucleated cells/kg has long been in the past the usual target and remains acceptable. However, it should be kept in mind that old studies in the early development of BMT have indicated better results both in terms of engraftment but also decrease in NRM and RI and better outcome, with higher marrow doses (Gorin et al. 1999, 2003; Sierra et al. 1997):

- For Allo-HSCT with identical siblings, an early EBMT retrospective study evaluated the impact of the marrow cell dose infused: The median BM cell dose was 2.7×10^8 /kg ($0.17\text{--}29 \times 10^8$ /kg). In multivariate analyses, high-dose BM compared to PB was associated with lower NRM, better LFS, and better OS (RR = 0.64; 95% CI, 0.44–0.92; $P = 0.016$). Results in patients with AML receiving allografts in CR1 indicated a better outcome with BM as compared to PB, when the dose of BM infused was above the median value.
- For Allo-HSCT fully with matched unrelated donors (Sierra et al. 1997), transplantation of a marrow cell dose above the median value of 3.65×10^8 /kg was associated with faster neutrophil and platelet engraftment and decreased

incidence of severe acute GVHD. Transplant in remission of acute leukemia with a high dose of marrow cells was associated with the best outcome in both children and adults.

If the targeted goal cannot be achieved, additional collection can be made from the anterior iliac crests, although it is time consuming and potentially more harmful for the patient or the donor, who must be turned over with all sterile fields to be reinstalled.

All things considered the maximum accepted volume collected should not go over 20 mL/kg donor body weight. Depending on the volume collected, three attitudes regarding transfusion during marrow collection may be followed: no transfusion and liquid replacement is the first option for many teams. Autotransfusion (to prepare beforehand in the 3 weeks preceding marrow collection, which adds some constraint) is the other recommended transfusional option. In rare circumstances allo-transfusion remains possible; usually two packs of concentrated red cells are enough.

Another option to consider to increase the stem cell dose to infuse when marrow collection has been insufficiently productive is the addition of PBSC. This however can be a complex decision which should take into account the disease and disease status, whether it concerns an autograft or an allograft or in this last situation whether a possible increase in the incidence and severity of GVHD associated with GVL/tumor is potentially beneficial or harmful. Two examples of this dilemma are summarized below:

1. In the context of auto-HSCT for leukemias or lymphomas, when analyzing patients receiving combinations of BM and PBSC (either because PBSC were collected to supplement poor BM or the reverse), outcomes are poor. One likely explanation is the existence of a bias since in most of these patients, poor collections (either BM or PBSC or both) are surrogate markers of multiple previous lines of chemotherapy for resistant/progressing diseases.
2. In contrast, for some teams, the combination of BM and PBSC has become the standard stem cell source for HSCT (see Table 14.1).

Table 14.2 Preferences for BM as source of stem cells in AUTO-HSCT

Auto-HSCT	Rationale	Justification	Comments
Poor PB collection	Increase the dose of HSC in the autograft	Ensure safer engraftment	However, poor mobilizers are likely to also produce poor marrow collection*
AML	Outcome better when compared to PB		Several EBMT retrospective studies

*Although there are biases, data from the EBMT registry indicate that poor mobilizers (often previously heavily treated with chemotherapy) have a poor outcome Shouval et al. (2018)

14.5 Complications of Bone Marrow Collections

One cannot ignore that on theoretical grounds two major hazards of marrow collection although very rare are death secondary to general anesthesia (<1/200,000) and major organ damage by mechanical mismanipulation of the bone needles that may sideslip if sufficient expertise and caution are not present.

The NMDP analyzed in 1993, 493 volunteers who donated unrelated marrow from October 1991 in 42 centers (Stroncek et al. 1993). The median volume of marrow collected was 1050 mL (range 180–2983 mL). Autologous red blood cells were transfused to 90% of donors, but only three donors received allogeneic blood. Apnea during anesthesia occurred in one donor. Other acute complications related to the collection procedure occurred in 6% of donors. Following marrow collection 75% experienced tiredness, 68% experienced pain at the marrow collection site, and 52% of the donors experienced low back pain. Mean recovery time was 16 days, but 42 donors felt that it took them at least 30 days to recover fully. The duration of the marrow collection procedure and duration of anesthesia both positively correlated with donor pain and/or fatigue following the collection. The

recommendation of this study was the duration of the collection procedure and probably the duration of anesthesia, and therefore the volume of marrow collected should be kept to a minimum, but this conclusion is to be weighed against the wish to collect stem cell doses as high as possible to ensure fast engraftment and improve outcome.

14.6 Bone Marrow Cryopreservation

In the context of auto-HSCT, BM and PBSC are almost always cryopreserved and stored either in liquid nitrogen (−196 °C) or the gas phase of liquid nitrogen (−140 °C). The technique of freezing at −1 °C/min with dimethyl sulfoxide (DMSO) and the technique of rapid thawing are well established (Gorin 1986). Harmless long duration of storage has been reported up to 11 years (Aird et al. 1992). Recently some attempts at avoiding cryopreservation to replace it by storage at 4 °C in the refrigerator have produced interesting results, but using refrigerator storage is not presently validated and cannot be recommended by EBMT (Sarmiento et al. 2018).

Cryopreservation and storage of a marrow in view of an allo-HSCT is possible. However, it should be kept in mind that any cryopreservation procedure, would it seem perfect, results in some measurable (CFU-GM, BFU-E) and many less measurable (immune functions, etc.) damages. Therefore, it should be reserved to special situations when, for instance, the donor cannot be available at the very time of the transplantation procedure. As a rule, fresh marrow is preferable to frozen marrow.

14.7 Quality Control for BM Harvesting and Cryopreservation

The major indicator for successful BM collection is the dose collected, as discussed above, i.e., the number of nucleated cells expressed per kg of

body weight for the recipient. It is very usual to have a blood count done at the mid time of the collection to ensure proper richness. With a goal of a minimum of 3×10^8 nucleated cells/kg, any richness above this value can be seen as a bonus. Harvest below this level, around 2×10^8 or even lower, however has been associated with correct engraftment.

CD34+ evaluation is not routinely performed for BM, while it is the rule for PB.

For cryopreserved marrow, some teams routinely cryopreserve small samples in minibags or ampoules, enabling viability testing before thawing the graft (usually an autograft). However, and importantly, a technical bias has been observed with ampoules since differences in cooling rates prevent ampoules from being a reliable index of HSC cryopreservation in large volumes (Douay et al. 1986a).

More pertinent testing consists in the evaluation of CFU-GM which represents in this setting the most reliable functional viability indicator (Douay et al. 1986b). Although there is no guideline, experience has shown that the results in CFUGM/kg are about 1–1.5 log below the expected or calculated results in CD34+ cells /kg (therefore expressed in 10^5 /kg). CFU-GM evaluation is not a consensual prerequisite since it is an additional time-consuming effort, but it may bring important information in some cases, for instance, when dealing with marrow collections below 2×10^8 /kg.

14.8 Conclusions

PB collections and transplantation nowadays represent 70–90% of all stem cell sources for transplants.

However, BM transplantation has not disappeared and is likely to persist in some limited situations and indications.

Further studies may revisit and increase the choice of marrow as stem cell source.

The Five Key Points of Marrow Collection

- Harvest with small (2–5 mL) aspirate volumes to avoid dilution with blood.
- The goal should be at least 3×10^8 nucleated cells per kg, but the more the better. The maximum volume collected should not go over 20 mL/kg donor body weight. Decision for no transfusion with liquid replacement (recommended) or autotransfusion (second best option) or in rare cases Allo-transfusion during collection relies on the judgment of the local medical team.
- Cryopreservation is the rule for auto-BMT, while it should be avoided and used only in rare specific conditions for allogeneic transplantation.
- BM is mandatory in children and patients with aplastic anemias. It is presently favored by some teams in the context of haploidentical transplantation.
- BM when compared with PBSC results in less NRM, less GVHD (in particular less chronic extensive GVHD), but less GVL/lymphoma/tumor effect.

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