INTRODUCTION

The objective of hematopoietic stem cell transplantation (HSCT) is to achieve a complete restoration of normal lymphohematopoiesis in a patient with bone marrow (BM) damage, primary immunodeficiency or metabolic disease after conditioning with chemotherapy and/or radiotherapy. This conditioning regimen is given as a part of the treatment of the disease, like in autologous transplantation, but also as a preparative schedule to accept the new hematopoietic stem cells (HSC), like in the allogeneic setting. With the exception of some non-myeloablative conditionings, the BM is not able to recover by itself after the conditioning treatment, and HSC must be infused to restore the normal BM function. This recovery depends in part on the number and quality of hematopoietic progenitors infused. Usually, when normal hematopoiesis is restored by the infusion of HSC, it can be said that successful engraftment has been achieved.

HSC can be obtained from different sources, including the BM, mobilized peripheral blood and umbilical cord blood (UCB). The preferential source of these stem cells remains controversial, at least for specific diseases and donor types (unrelated vs related and minor vs adult donor). The perfect human leukocyte antigen (HLA)-identical sibling donor is usually available in only 30% of all patients who need a transplant. For the remaining patients, it becomes necessary to look for a well-matched unrelated volunteer donor. Since such volunteer donors are not always available, a longer search time is required, which might compromise the final transplant outcome. For these cases, an HLA-mismatched, T-cell-depleted graft from a related or unrelated donor, or an unrelated, already cryopreserved UCB offers an alternative stem cell source.

Over the years, SCT, mainly in the allogeneic setting, has become a complex procedure, and restoring a normal BM function is not sufficient to ensure a successful transplant. For example, maintaining a graft-versus-tumor effect while at the same time preventing a severe graft-versus-host disease (GVHD) remains a key target in allogeneic transplantation for leukemias. Therefore, a T-cell depletion might be meaningful in some cases to reduce the risk of GVHD, while in others, the infusion of T-cells to enhance a graft-versus-tumor effect might be the optimal approach.
In this chapter, a basic review of the commonest sources of HSC is presented, together with a discussion of how to collect and process these cells.

**HEMATOPOIETIC STEM CELLS**

Initially, from a functional perspective, HSC were defined as those cells having a self-renewal capacity, and therefore, the capability to restore a long-lasting normal BM function in lethally irradiated mice. This functional approach could also be transferred to humans by demonstrating that stem cells are able to restore a defective lymphohematopoiesis, for example, in patients with primary immunodeficiency or aplastic anemia.

It has been of special interest over the years to identify and characterize these cells either in ex-vivo culture systems or by defining specific cell surface epitopes, which can be recognized by monoclonal antibodies and are not only characteristic markers for specific cell lines but also the maturation stage of these cells. Although the final phenotype of the most immature human HSC is not completely defined yet, it is well known that the infusion of highly purified CD34+cells is capable of a rapid and sustained engraftment and restoration of the lymphohematopoietic system. For practical transplant reasons, therefore, progenitor cells expressing the CD34+ surface antigen are considered to contain the relevant stem cell populations. There are probably other cell subpopulations capable of acting as stem cells, for example CD133+ cells. However, they are not commonly used as a transplant source or to characterize the stem cell quantity of a transplant.

**BONE MARROW AS STEM CELL SOURCE**

Bone marrow (BM) has been the typical source for HSC collection for more than 40 years. Since 1990, a dramatically increased use of mobilized peripheral blood stem cells (PBSC) in the autologous as well as allogeneic setting has been seen worldwide. Today, almost all autologous transplants are performed from PBSC in pediatric as well as adult patients. For allogeneic transplants some controversies remain. While the
CD34+ cell number is higher in PBSC compared to BM, resulting in a faster engraftment, the T-cell number is also higher, resulting in a higher rate of especially chronic GVHD, as demonstrated in several studies. Although many adult donors would prefer a PBSC harvest over BM, which requires general anesthesia, it has to be stressed that in minor family donors, BM harvests remain the method of choice, and mobilized PBSC collections are not allowed within that age group in some countries because of the required cytokine application.

**Bone marrow collection**

Bone marrow (BM) is typically collected from the posterior iliac crest of the donor. The procedure is usually performed under general, or rarely, regional anesthesia. The collection starts at the posterior superior iliac spine. A normal collection will require about 200 to 300 punctures performed directly through the skin or across a small incision. Once the needle has passed the bone cortex, aspirations should be made by vigorous suction of not more than 5-10 ml of BM using a heparinized syringe. The aspirated product is then filtered and transferred into an anticoagulant solution, usually anticoagulant citrate dextrose (ACD), in a concentration of 1 : 10 vol (ACD : BM) and/or 10 IU heparin per ml BM.

The harvested BM is always contaminated with normal blood. The degree is related to the total volume of BM harvested, but clearly also to the collection technique, being lower after vigorous short aspirations. The required cell dose empirically established over four decades relies on the amount of nucleated BM cells, which should be at least 1–2 \( \times \) 10^8/kg for autologous transplants and at least 2 \( \times \) 10^8/kg, or better 4–6 \( \times \) 10^8/kg, for allogeneic transplants.

The anterior iliac crest can be used if necessary, but the quantity that can be collected is clearly lower than that collected using the posterior iliac crest.

**Adverse events related to bone marrow collection**

Bone marrow (BM) collection is a safe procedure mainly related to mild and transient side effects. The vast majority of donors experienced pain in the puncture area or
simply backage. Mild adverse events could also include fever, nausea, vomiting, or light headache.

Serious adverse events are rare after marrow donation, with an expected frequency of about 0.1%-0.3%, and they can be classified into five risk categories as anesthesia, infection, mechanical injury, transfusion, and others. Allogeneic blood transfusion is not used routinely because blood loss usually does not require this practice. The total volume of marrow collected is recommended not to be higher than 15-20 ml/kg of recipient body weight. Some centers use an autologous blood collection prior to the procedure that is re-transfused during or after marrow collection.

PERIPHERAL BLOOD STEM CELLS AS STEM CELL SOURCE

Peripheral blood stem cells (PBSC) are increasingly used today as a source of stem cells for HSCT. However, under normal conditions, the number of CD34+ cells circulating in peripheral blood is too low for a sufficient collection. Thus, a mobilization treatment is required in order to increase the number of circulating CD34+ cells in the blood.

Mobilization of peripheral blood stem cells

The most common means of mobilizing circulating stem cells into the peripheral blood is the use of growth factors, mainly granulocyte colony-stimulating factor (G-CSF). A dose of 10-16 mcg/kg/day over four consecutive days is usually sufficient for a good PBSC mobilization followed by an apheresis on day five. In most instances, one collection is enough in the autologous or allogeneic setting. Rarely, a second donation is required on day six, mainly when high stem cell doses are required for T-cell-depleted mismatched transplants. Poor mobilizers, where a BM harvest might have to follow a frustrating PBSC collection, are also seen rarely; thus, a number of alternative growth factors, like stem cell factor, have been proven. In recent years, plerixafor has emerged as an effective chemokine in addition to G-CSF for such poor mobilizers. Stem cells have CXCR4 receptors preventing their release to the circulating blood by interacting with a corresponding chemokine. Plerixafor has a strong and reversible affinity to these receptors and blocks the interaction and BM niche, thereby releasing stem cells to the circulating blood via this mechanism.
In randomized clinical trials, the combination of plerixafor and G-CSF has indeed demonstrated better results than G-CSF alone.

A different alternative for stem cell mobilization is the use of chemotherapy in the autologous setting. A significant increase in circulating stem cells in the peripheral blood has been observed following BM recovery after standard dose chemotherapy, mainly if additional G-CSF is administered. Therefore, the most common mobilization schedule is a combination of cyclophosphamide plus G-CSF. Sometimes, the chemotherapy used specifically for the treatment of the underlying disease is used for PBSC collection, although in some cases, hematologic recovery is very slow and did not allow a sufficient PBSC collection.

**Collection of peripheral blood stem cells**

After mobilization, circulating PBSC must be collected using an apheresis device. There are different devices that yield similar results in terms of collected CD34+ cells. The most important differences are the numbers of contaminating platelets or red blood cells in the final product.

In order to estimate a sufficient PBSC collection, the number of CD34+ cells should be at least 10 cells/µL at the day of harvest following mobilization. Some centers would not start the collection if this minimal number of circulating CD34+ cells is not achieved. However, one should take into account that the measurement of CD34+ cells in peripheral blood, especially in case of low levels, might reflect a significant error.

**Adverse events related to peripheral blood stem cell collection**

Like in BM collection, PBSC donors usually suffer from mild adverse events, while severe or life-threatening side effects are infrequent.

The most common side effect is bone pain related to the treatment with G-CSF,
present in up to 85% of donors. It is typically more prominent in the pelvis, hips, spine, and ribs. Other mild effects include nausea, vomiting, myalgia, fatigue, or insomnia.

Alterations in chemistry and blood cell counts are seen (e.g. increase in lactate dehydrogenase (LDH) and transaminases, decrease in platelets). Typical side effects also include a hypocalcemia if citrate is used as anticoagulant. An adequate venous access is always necessary. In the majority of healthy donors, PBSC collections are performed using a peripheral venous access, but sometimes a central line has to be implanted, with a subsequent higher rate of adverse events. Like in BM collections, serious adverse events are also rare after PBSC donations, with an expected frequency about 0.1%, and are mostly related to cytokine treatment, the central line or cardiovascular stress. Autoimmune diseases, rupture of the spleen and other side effects have been observed. Of concern was the development of leukemia following G-CSF administration. All careful follow-ups in large series of PBSC donors suggest that this risk is not higher than in the age-matched general population.

STEM CELL MANIPULATION

Cryopreservation of hematopoietic stem cells

When HSC, whether BM or PBSC, are going to be infused up to 72 hours following withdrawal, cryopreservation is not necessary, and the material can be stored at room temperature or for an extended time at 4ºC, according to local standards (standard operating procedures, SOPs).

For extended storage, HSCs should be cryopreserved, according to local SOPs, in a cryopreservation solution (usually 10% dimethyl sulfoxide (DMSO) in autologous plasma, hydroxyethyl starch (HES) or albumin). After controlled freezing, cells can be stored in liquid nitrogen for up to at least 10 years.

T-cell depletion

In general, manipulations of grafts can be divided into removal techniques of lymphocytes mainly for GVHD prevention in allogeneic transplants or positive stem cell
selections for the same reason or for removal of tumor cells in autologous transplants. In the allogeneic setting, ex-vivo T-cell depletion is mandatory in transplants with major mismatched donors in order to prevent a lethal GVHD, or it is performed to reduce the incidence and severity of GVHD in better-matched donors. The success of the procedure depends on the separation technique, the total number of CD34+ cells infused, and the number and composition of residual lymphocytes. The final objective in allografts is to reduce GVHD without risking a significant increase in graft failure. Reduction in T-cells to less than $1 \times 10^4$/kg of recipient body weight is able to prevent a severe GVHD even in HLA-haploypmismatched transplants. On the other hand, while a minimal dose of CD34+ cells of $2 \times 10^6$/kg should be reached in HLA-matched related or unrelated transplants in order to avoid graft failure, in HLA-haploidentical transplants, a minimal dose of $10 \times 10^6$/kg or higher is necessary.

Today, the most commonly used method for ex-vivo lymphocyte depletion or CD34+ positive selection is an immunomagnetic sorting technique using monoclonal antibodies directed to specific lymphocyte or stem cell epitopes and labeled with magnetic beads. This method allows the production of transplants with large numbers of highly purified CD34+ cells or other stem cell compositions, combined with defined numbers of low or very low T-cells, T- and B-cells or lymphocyte subsets.

It must be pointed out, however, that the loss of critical T-cell numbers or -subsets in the transplant carries the risk of delayed immune reconstitution and loss of graft-versus-tumor effect.

CORD BLOOD CELLS

From 1988, cord blood (CB) stem cells emerged as an additional alternative cell source for allogeneic transplantation either from unrelated donors, or in rare instances, from a sibling donor. The clear advantage is the high concentration of immature stem cells, the immediate availability of already characterized and cryopreserved products, and the low rate of acute GVHD in transplant recipients even when more HLA differences are accepted compared to adult volunteer donors. However, there are also disadvantages, like a longer period of hematologic and immune reconstitution. Therefore, transplant results of CB transplants resemble those of T-cell-depleted, HLA-haploidentical transplants with respect to infection and relapse risks, as well as chronic GVHD.
Another major problem is the low total cell dose in a single CB unit, which allows the use of this stem cell source in infants and small children, but requires additional measures in adult patients, like combining more than one CB unit.

Cord blood (CB) can be collected after delivery from the placenta either in utero or ex utero. The last method is preferred and technically easier and safer. Although the attempt should be made to collect as much CB as possible from the placental vein, it is important to remember that the CB collection must never compromise the security of the mother or baby during delivery.

For in-utero collection, a collection kit is prepared before the delivery. After delivery of the infant and before the placental delivery, the cord is clamped and cut as usual. Then, using a specific needle and under aseptic conditions, the blood is transferred by gravity into a bag with the anticoagulant solution. The ex-utero collection is performed after the placental delivery in a separate room using a similar technique. Ideally, a median CB volume of approximately 120 ml but not less than 40 ml should be collected.

After collection, the CB unit is shipped to the processing laboratory, and tested, processed and stored in liquid nitrogen within 48 hours after collection. The majority of laboratories prefer to remove the majority of plasma and red blood cells to store the CB unit in a smaller volume, which allows a lower DMSO content and an application without washing procedures after thawing.

If a single CB unit is selected for a pediatric patient, the HLA-antigen identity, if ever possible, should be at least 4/6 including HLA-A, -B, and DR, the nucleated cell number should be above $3.7 \times 10^7$/kg, and the CD34+ cell number should be above $1.7 \times 10^5$/kg body weight of the recipient.

References


- **Internal revision by:** Prof. Dr. Petr Sedlaček, Charles University, Prague, Czech Republic

- **External revision by:** Prof. Dr. Wolfram Ebell, Charite University, Berlin, Germany

- **Edited by:** Corinne Can