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REVIEW

Hematopoietic stem and progenitor cell harvesting: technical advances and clinical utility

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Abstract: Hematopoietic stem and progenitor cell (HSPC) transplantations require prior harvesting of allogeneic or autologous HSPCs. HSPCs are usually present in bone marrow (BM) during the entire life, in cord blood (CB) at birth, or in peripheral blood (PB) under particular circumstances. HSPCs were first harvested in BM and later in CB and PB, as studies showed interesting features of such grafts. All harvesting methods were in use throughout the years, except BM harvesting for HSPC autologous transplantation, which was replaced by PB harvesting. BM, CB, and PB harvesting methods have been developed, and materials and devices technically improved to increase the number of HSPCs harvested. In parallel, knowing the features of the donors or patients associated with successful numbers of HSPCs allows the adaptation of appropriate harvesting methods. Moreover, it is important to ensure the safety of donors or patients while harvesting. This review describes the methods used for harvesting based on recent studies or developments around these methods, and more particularly, the means developed to increase the numbers of HSPCs harvested in each method. It also explains briefly the influence of technical improvements in HSPC harvesting on potential changes in HSPC graft composition.

Keywords: hematopoietic stem cell, harvesting, cord blood, bone marrow, mobilization, peripheral blood, apheresis

Introduction

Hematopoietic stem and progenitor cell (HSPC) transplantation, which was initially considered as an experimental therapy, has been performed and studied over the last 40 years. It has become a referent treatment of severe hematological diseases.

As HSPCs are localized in the bone marrow (BM), the first HSPC transplantations in the 1950s used that as source of cells.[1] Over the last three decades, allogeneic BM transplantations have become a referent therapy for severe malignant or nonmalignant hematologic diseases.[2]

HSPC transplantations evolved after HSPCs were detected in other sites such as peripheral blood (PB) or cord blood (CB).[3–5] The first allogeneic CB transplantation was successfully performed at the end of the 1980s.[5] In parallel, the development of apheresis devices enabled teams to harvest sufficient PB HSPCs for transplantation. Over the last 20 years, numerous HSPC transplantations have been performed. In all types of HSPC transplantations (BM, CB, PB), it was demonstrated that the outcome for the transplanted patients depended on the number of HSPCs contained in the graft. HSPC harvesting methods have, therefore, been improved to transplant higher numbers of HSPCs. In this review, we focus on the recent technical advances in

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HSPC harvesting, recent studies or developments that have brought new knowledge, and their consequences on the graft composition and their clinical utility.

HSPC harvesting methods

BM harvesting

Although HSPC harvesting was performed for the first time more than 50 years ago, BM harvesting was developed mainly to perform allogeneic HSPC transplantations and later autologous transplantations. Nowadays, BM is harvested to perform only allogeneic HSPC transplantation.

Protocol for BM harvesting

The current protocol recommended for BM harvesting consists in aspirating BM from the posterior iliac crest in a donor under general anesthesia using a needle with multiple side holes, which should be performed by one or two hematologists. The level of aspiration is restricted to 15–20 mL per puncture into sterile syringes previously rinsed with a heparin/saline solution. While harvesting, regular gentle agitation of the harvesting bag containing an anticoagulant solution prevents clotting. A total nucleated cell (TNC) count performed at midway predicts the optimal BM volume to be harvested within the limit of the maximum volume. The BM harvested is sent to the cell therapy unit where it is filtered and processed in case of ABO incompatibility.

The acceptable cell dose harvested in BM and required for allogeneic transplantation is $3–5\times10^8$ TNCs per kilogram of recipient body weight (BW). However, when harvesting and transplanting higher numbers of TNCs, better outcomes, such as improved overall survival, were shown in patients.[6] This occurred particularly in patients allogeneically transplanted for acute myeloid leukemia (AML).[6] Therefore, hematological teams have developed strategies to harvest higher numbers of TNCs.

How to increase numbers of HSPCs harvested in BM

It was suggested that priming donors with granulocyte colony-stimulating factor (G-CSF) enhanced the number of TNCs harvested, but that approach was not developed.[7] Two other ways to harvest higher numbers of BM TNCs and HSPCs, ie, by harvesting larger volumes of BM or by increasing the cell density of the BM harvested, have been developed. The total volume of BM harvested, within the limit of 20 mL/kg to prevent excessive blood loss, depends on the donor's BW. In standard procedures, hematologists usually harvest the highest possible volume, which could be deleterious inducing a hemodilution of the BM harvested. Indeed, it was clearly shown that the volume of BM harvested was inversely correlated to the cell density.[8]

To obtain a higher cell density and higher number of cells, it is necessary to change the needle position at short intervals. It is also recommended to optimize the level of aspiration at each site, repetitive aspirations of small volumes of BM enhancing the numbers of TNCs and HSPCs harvested.[9] Moreover, using needles with multiple side holes combined with harvesting small, repetitive BM volumes induced a high BM cell yield.[10,11]

Parameters other than harvesting techniques, such as the characteristics of donors, could influence the cell density and numbers of TNCs in the BM harvested. Among them, the donor BW and baseline white blood cell (WBC) levels were correlated to the cell density in BM harvests.[8] Other characteristics, including lower age and cytomegalovirus-negative donors, smoking, higher hemoglobin and mononuclear cell blood levels, higher number of whole blood donations in the year preceding the BM harvest, and higher body surface area, were associated with higher numbers of TNCs harvested in BM.[12] A higher hemoglobin level, higher number of whole blood donations, and smoking were probably associated with a more active hematopoiesis.

CB harvesting

CB at delivery was found to contain HSPCs.[4,13] These HSPCs displayed interesting features such as high-potential clonogenicity, but their absolute numbers were, however, not sufficient for allogeneic transplantation in adults and were only appropriate to transplant low-BW recipients, ie, children.[5] Such allogeneic transplantations displayed advantages over allogeneic BM and PB HSPC transplantations, such as low incidence of graft-versus-host-disease. Considering the advantages of CB transplantations, hematologic teams created a European organization for CB banking.[14] The aim was to allow transplantation from unrelated children and possibly from low-BW adults if the numbers of cells contained in CB units (CBU) were sufficient for transplantation. For that purpose, the numbers of HSPCs harvested in CBUs had to be increased by optimizing harvesting methods or obstetrical conditions. In 2005, an American team developed the concept of transplanting two CBUs partially matched together, which induced lower duration of aplasia and fewer infectious complications than after single CBU transplantations in adults.[15] This reinforced the need for standardizations in the stages of the CBU banking process and for studies to increase the number of HSPCs in CBUs.

Protocol for CB harvesting

The initial method for harvesting CB was described at the end of the 1980s.[13] Although a few attempts were made to improve it, the current protocol recommended is very similar to the initial protocol and is described as follows: After the birth of the infant, the umbilical cord is double-clamped from the umbilicus and transacted between the clamps. The umbilical cord vein is punctured under sterile conditions, and the blood flows freely by gravity into an anticoagulated sterile closed harvesting system.[16,17] Birth unit staff should be trained in CB harvesting to reduce the rejection rate due to labeling problems, bacterial contamination, and clotting.[16] The mean volume and numbers of HSPCs harvested in CBU can vary from one study to another, but can be described as follows: 108±28 mL, 12.5±5.3×10^8 TNC, and 3.6±3.3×10^6 CD34+ cells.[18] The harvest must be stored and transported under controlled temperature. The time interval between CBU harvesting and processing is limited to 24–36 hours corresponding to sufficient cell viability.

How to increase numbers of HSPCs harvested in CB

Initially, the numbers of HSPCs contained in CBUs or transplanted were defined by measuring the numbers of TNCs and colony forming unit-granulocyte macrophage (CFU-GM).[5,13] After 2000, the CD34+ immunomarker was used to characterize the population of HSPCs contained in BM, PB, and in CBU, in addition to TNCs.[19] A strong correlation was found between the numbers of HSPCs and the CB volume harvested.[20,21] Obstetrical and technical methods were then developed to harvest the highest possible volume of CB. CB volume was initially considered as – and was later proven to be – one of the best predictive data for acceptable CBU banking.[20,21]

A variety of potential CB harvesting methods have been described to harvest large volumes of CB. Closed, semiclosed, and open systems were developed using blood bags, syringes/flushed or not, or drains/flushed or not, with no significant difference in the harvested volume.[22,23] Since 1998, the US Food and Drug administration (FDA) invited professional groups to submit proposed standards, data, and information to have available CBUs appropriate for allogeneic transplantation. Providers developed systems to obtain more CBU volume and TNCs harvested (Table 1). Some systems were likely to increase the volume or the numbers of cells harvested, but these were not extensively used, and the protocol for CB harvesting remained as described above.

In order to increase the volumes and numbers of cells harvested, obstetrical teams assessed and compared CB harvesting methods. CB can be harvested before or after the placenta delivery, ie, in utero or ex utero, and during cesarean. In utero, harvesting is done by midwives in the delivery room, the placenta being compressed by the uterus at the third stage of labor. Ex utero, harvesting is done by cord bank employees in an adjacent room right after the placental delivery. Therefore, the obstetrical CB harvesting methods vary from one team to another (Table 1). Some studies have shown higher volumes and HSPC numbers when harvested in utero, while other studies have shown comparable volumes and HSPCs numbers in both conditions.[18,29–31] Both methods have advantages and disadvantages, which generated controversies. Therefore, early cord clamping is associated with higher volumes harvested, but this may hamper the normal process of delivery. Harvesting CB after delivery is easier, but only lower volumes can be harvested. Moreover, comparisons between harvests performed during vaginal and cesarean delivery showed either identical numbers or higher numbers of HSPCs harvested during the latter.[32,33]

The factors that influence the yield of CB volume and cells have been studied. Therefore, primigravidae, higher birth weight, Caucasian race, young (34–37 weeks) or old (40 weeks), gestational age, and female sex were associated with higher volumes and numbers of CD34+ cells harvested.[20,33,34] Prenatal sonografic parameters can estimate fetal weight and are correlated with CB hematological parameters.[35] Knowledge of these factors will help in banking and harvesting CB efficiently, but the banking levels depend on the cell threshold and strategy of each CB bank. The main reason for excluding the CBUs harvested is the low volume or low numbers of cells contained in a CBU. The other reasons for excluding the CBU are preparation or logistical complications, abnormal biological result in donor, and CBU microbial infection (Table 2).[18,36,37] The rate of CBU microbial contamination varies from 0% to 48%.[23,24,38]

In order to limit the exclusion of the CBUs, it is necessary to select suitable donors. Successful selection requires the collection of accurate information when gathering and obtaining informed consent from the mother. Parents with previous history of cancers or hematologic, genetic, or autoimmune diseases are excluded from donation. Serological tests (for hepatitis, human immunodeficiency virus [HIV], cytomegalovirus, syphilis, and Epstein–Barr virus) must be performed in the mother's blood at delivery. However a genetic, hematologic, or oncological disease can occur in newborns several years after CB harvesting and storing, which requires information about the CB donor's health before sending the CBU for transplantation.

Table 1 Recent studies performed to improve amounts of hematopoietic stem and progenitor cell (HSPC) harvested in cord blood (CB)

Type of improvement	Reference	Year	Goal	Results
Technical	Elchalal et al[24]	2000	Compare three methods of harvesting process (with or without flushing by a syringe and sodium chloride into an open sterile container or a blood bag). Results on volume, TNC, and bacterial contamination	Flushing increased the volume and total nucleated cell (TNC) numbers Bacterial contamination was lower when harvesting in a blood bag
	Belvedere et al[25]	2000	Evaluate a harvesting system (pressure application system) by inducing additional pressure after delivery	Increase in volume and CD34+ cell numbers (40%). The last fraction harvested by the device contained more HSPCs than the first fraction harvested by gravity
	Bornstein et al[26]	2005	Evaluate a second fraction harvested after placenta perfusion	This fraction contributed to 32% volume and 15% TNC of the whole CB unit
	Takebe et al[27]	2009	Describe a pulsatile machine reperfusion of a placenta to improve harvesting yield	Improved harvest with 1.5-fold increase in CD34+ cells
	Tan et al[28]	2012	Describe an auto-perfusing CB harvesting instrument	Generate vibrations during the perfusion phase and a control platform to integrate all systems
Obstetrical	Surbek et al[29]	1998	A randomized comparison of harvest while placenta is still in the uterus before vs after placenta delivery	More volume and mononuclear cells harvested before placenta delivery
	Larsky et al[30]	2002	Compare the CB harvest while placenta is still in the uterus vs after placental delivery	Both methods produced comparable hematological parameters (volume, TNC, CD34+, CFU-GM)
	Solves et al[18]	2003	Compare the CB harvest in the delivery room (while placenta is still in the uterus) vs in an adjacent room after placental delivery	CB harvesting before placental delivery allows the best TNC and HSPC harvest
	Wong et al[31]	2001	Compare the CB harvest in the delivery room (while placenta is still in the uterus) vs in an adjacent room after placental delivery	CB harvesting before placental delivery allows the best TNC and HSPC yield
	Omori et al[32]	2010	Compare CB collection in cesarean and vaginal delivery	Higher volume when cesarean, but higher CD34+ cells after vaginal delivery
	Cairo et al[33]	2005	Analyze the factors associated with better cell yields	Cesarean section is associated to higher total CFU

Abbreviations: GM, granulocyte macrophage; CFU, colony forming unit.

In 1998, the foundation NetCord was developed to establish an international registry for CB banks and procedures with standards for the safe exchange and transplantation use of CBU. In 2000, the NetCord Foundation for the Accreditation of Cellular Therapy released international standards for accreditation not only for harvesting but also for testing, processing, and storing CBU.[39]

Table 2 Reasons for excluding the CBUs harvested

Exclusion criteria	%
Low volume or low total nucleated cell amounts	52–85
Bacterial cord blood unit (CBU) contaminations	5–17
Storage/transport time >48 h	1–16
Abnormal transport temperature	1–2
Incomplete documentation in hospital	2–3
Parents medical history	2–5
Abnormal maternal infectious disease testing	1–5
Problems during CBU processing	3–5
Clots	2–4

Notes: Data from recent unpublished experience in France and three large cohorts recently published with, respectively, 7,921 CBUs harvested and 2,014 CBUs stored; 31,128 CBUs harvested and 7,056 CBUs stored; and 1170 CBUs harvested and 735 CBUs stored.[17,36,37]

PB HSPC harvesting: mobilization and apheresis techniques

The presence of HSPCs in PB was detected in 1971.[3] In parallel, over the last decades, the development of apheresis devices has allowed the harvesting of PB HSPCs. As no HSPCs are present or detected in PB under normal conditions, it is necessary to mobilize HSPCs from BM to PB. These mobilization treatments are not the same in donors for allogeneic transplantations and in patients for autologous transplantations.

HSPC mobilization in healthy donors

Related or unrelated donors usually receive G-CSF (filgrastim or lenograstim) 10 µg/kg/day from 4–5 days before

apheresis.[40] The factors associated with a better efficiency in mobilizing CD34+ cells are male sex, higher body mass index, higher G-CSF dosage, higher premobilization WBC, and the use of lenograstim rather than filgrastim.[41,42] Higher age, female sex, white ethnicity, and donors lighter than their recipient are factors associated with a poorer mobilization.[43,44] Knowing the risk factors for poor mobilization allows processing larger blood volume or anticipating a possible rescue by BM harvesting. It is difficult to determine the percentage of donors who fail to mobilize an adequate number of HSPCs for harvest because this minimal number is different among centers. In case of insufficient mobilization and, therefore, insufficient HSPCs harvest, the BM harvest rescue can be replaced by using a new mobilization agent named plerixafor, but this treatment is not approved by the FDA and in most countries.[45]

HSPC mobilization in patients

The following three broad strategies are usually followed to mobilize HSPCs from BM to PB in patients:

1. Combined chemotherapies associated with the hematopoietic growth factor, ie, G-CSF currently used to treat the underlying disease, ie, Hodgkin's disease (HD), non-Hodgkin lymphoma (NHL), or solid tumors, and inducing aplasia allow mobilization of CD34+ cells into PB.[46–48] Randomized studies have shown that doubling the dose of filgrastim improved the CD34+ cell harvest and decreased the median number of apheresis procedures.[49]
2. Cyclophosphamide associated with hematopoietic growth factors can be used in the treatment of multiple myeloma (MM) and HSPC mobilization. Mobilization by cyclophosphamide after new chemotherapy regimen is possible, although some of these agents (thalidomide, lenalidomide) were suspected to cause harvest failure.[50,51] Randomized studies have shown that the addition of growth factors (GM-CSF or G-CSF) to cyclophosphamide resulted in a significant increase in the numbers of CD34+ cells harvested.[52] The use of biosimilar G-CSF instead of G-CSF seemed to induce the same levels of CD34+ HSPCs harvested and the same harvesting duration.[53]
3. Mobilization by hematopoietic growth factors alone can be efficiently used to mobilize HSPCs. Randomized studies compared growth factors, ie, filgrastim to molgramostim and to pegfilgrastim, without demonstrating any superiority for a growth factor or a scheme.[54,55]

Over the last 15 years, numerous studied have shown the factors that affect HSPC mobilization, ie, age, sex, underlying disease, interval between diagnosis and harvest, exposure to alkylating agents, prior irradiation, marrow involvement, blood-platelets baseline, and cancer relapse (Table 3).[56–62] Megakaryocyte-platelet lineage is particularly sensitive to damage in the BM microenvironment. Therefore, premobilization blood platelet baseline appears to be an indicator for autologous HSPC mobilization.[59,61] Moreover, agents (thalidomide, bortezomib or lenalidomide, and fludarabine) used in new therapeutic schemes could affect HSPC mobilization. Indeed, the number of HSPCs harvests after short courses of chemotherapy using these agents is lower than when control groups receive

Table 3 Main factors that may negatively affect successful HSPC harvest

Factors	Results	Commentary	Reference
Older age	58 years was the cutoff 70 years has been described as another cutoff	Study performed in myeloma patients Factor also described in other diseases	Lacativa et al[61]
Diagnosis	NHL, myeloma and AML were alternatively described	More difficult in AML patients	Mendrone et al[59] Koenigsmann et al[60]
Prior irradiation	Mainly bone irradiation	Factors described in MM and in most studies	Bensinger et al[56]
Bone marrow involvement	Presence and importance of involvement	Factors described in most studies Factors described in most diseases	Bensinger et al[56] Cesaro et al[48]
Stage of the disease	Advanced stage	Factors described in NHL and cancers	Weaver et al[57]
Number of previous chemotherapy regimens	More than three lines of chemotherapy	Fewer lines are associated to better harvest Factor also described as chemotherapy load	Bensinger et al[56] Ketterer et al[58] Mendrone et al[59]
Alkylating agents	Cisplatin is described in most studies Mitoxantrone is described	Factors associated to the number of chemotherapy cycles	Mendrone et al[59]
Platelet baseline count	Cutoff: 150,000–161,000 platelets/μL	Factors described in most recent studies	Mendrone et al[59] Lacativa et al[61]
Fludarabine exposure	Exposure to several lines	Factor described in NHL Factor confirmed in most studies	Ketterer et al[58] Waterman et al[62]
Cancer relapse	More difficult to harvest when relapse	Factors associated to other factors (lines of chemotherapy)	Cesaro et al[48]

Abbreviations: AML, acute myeloid leukemia; MM, multiple myeloma; NHL, non-Hodgkin lymphoma; HSPC, hematopoietic stem and progenitor cell.

older combination of chemotherapies. Moreover, fludarabine exposure hampers adequate HSPC mobilization probably by causing stem cell damage.[58,62] However, the regimen used to treat MM does not significantly hamper HSPC mobilization during G-CSF or during cyclophosphamide mobilization.[63,64] Knowing all these factors will enable teams to feature poor mobilizers (PMs).

There is no consensus on the definition of PM patients. The patients in whom poor mobilization induces HSPC harvest failure for low blood peak of circulating CD34+ cells associated or not with factors likely to affect HSPC mobilization were named PMs. The patients suspected to become PMs were named predicted PMs. The criteria used for defining the status of PM were different among different studies (Table 4). Whatever the definition or the criteria chosen, the overall incidence of mobilization failure varies from 5% to 40%.[67–69]

Table 4 Definition and criteria of poor mobilizers (PMs)

Reference	Year	Definition	Incidence
Predicted PM			
Li et al[65]	2011	Lower than 15 CD34+ circulating cells/μL while WBC count is $>10\times10^9$/L	Nd
Attolico et al[66]	2012	Failed attempt to collect 2×10^6 CD34+ cells/kg BW under three consecutive apheresis Failure to reach a threshold of at least ten CD34+ circulating cells/μL	Nd
Olivieri et al[67]	2012	Failed a previous harvest attempt Risk factors for failure in HSPC harvest	Nd
Proven PM			
Perseghin et al[68]	2009	Failure to reach a threshold of at least 20 CD34+ circulating cells/μL Among them failure to achieve a CD34+ cell dose of 2×10^6/kg BW	335/2,177 (15%)
Wuchter et al[69]	2010	Borderline PMs: between 11 and 19 CD34+ circulating cells/μL Relative PMs: between six and ten CD34+ circulating cells/μL Absolute PMs: <5 CD34+ circulating cells/μL	129/840 (15.3%)
Olivieri et al[67]	2012	Lower than 20 CD34+ circulating cells/μL after usual mobilizing regimens	15%
Lacativa et al[61]	2012	Failure to collect 2×10^6/kg BW in 3 days of apheresis	22/157 (14%)
Cesaro et al[48]	2013	Failure to collect 2×10^6/kg BW in 3 days of apheresis	24/145 (17%)

Abbreviations: BW, body weight; HSPC, hematopoietic stem and progenitor cell; Nd, not determined; PMs, poor mobilizers; WBC, white blood cell.

How to manage poor mobilizers

After revealing a PM patient, different solutions can be used to perform appropriate mobilization.

1. BM harvesting is no more used to perform autologous HSPC harvests because it was proven to be ineffective in patients with insufficient PB HSPC mobilization and/or harvest.[70]
2. Remobilization can be performed with high-dose chemotherapy associated with growth factors or with growth factors only.[71]
3. High-dose administration of growth factors can be used following the principle of dose-dependent response to mobilization.[72,73]
4. Stem cell factor has been successfully used in combination with G-CSF to mobilize HSPCs.[74] Stem cell factor is the ligand for c-kit, which is a membrane receptor with tyrosine kinase activity expressed on several tissues including the hematopoietic system. This treatment induced adequate harvest in 40% of PMs.[75]
5. More recently, the use of plerixafor has demonstrated encouraging results. Plerixafor is a reversible inhibitor of HSPC adhesion to stromal cells by CXCR4 binding to stromal-derived factor-1. The best scheme for using plerixafor consists in combining it with mobilization by G-CSF. This treatment induces rates of successful CD34+ cell harvests in 70% of NHL, HD, and MM PMs.[76] Combining plerixafor with pegfilgrastim or with mobilizing chemotherapy seems safe and effective in PMs, but these results require confirmative data.[77] The main disadvantage of plerixafor is its cost, requiring the development of algorithms for the use of plerixafor in autologous HSPC mobilization.[78]

After mobilizing HSPCs from BM to PB, the apheresis teams have to harvest them from PB.

PB HSPC harvesting by apheresis

The purpose of apheresis sessions during treatment for NHL, HD, or MM is to harvest enough HSPCs to perform autologous transplantation in the patient. The most relevant number of HSPCs to be harvested is 4×10^6 CD34+ cells/kg BW for treating NHL patients and 4×10^6 CD34+ cells/kg BW per graft for treating MM patients by harvesting one or two grafts. In all diseases, the minimum number of HSPCs to be harvested is 2×10^6 CD34+/kg BW for each graft.

To perform allogeneic HSPC transplantations, apheresis sessions are performed in an human leukocyte antigen (HLA)-compatible donor after checking the absence of infectious, oncological, autoimmune, and vascular diseases.

The number of HSPCs to be harvested varies depending on the teams and nature of the transplantation (non-myelo, myelo-ablative, or haplo-identical), ie, from 4 to 10×10^6 CD34+ cells/kg recipients' BW.

A strong correlation was found between pre-apheresis PB CD34+ cell counts combined or not with the numbers of CD34+ cells harvested at mid-point and numbers of CD34+ cells harvested after the corresponding apheresis sessions.[79,80] The analysis of these pre-apheresis blood levels allows apheresis teams to adapt the blood volume processed and potentially perform large-volume leukapheresis.[81]

Apheresis techniques

Different apheresis devices have been developed to harvest PB HSPCs. All these techniques share a common process, ie, separating blood components in layers by centrifugation and harvesting blood-mobilized HSPCs in a particular layer associated with other blood cells. The principles of the main and current apheresis techniques are summarized in Table 5. Over the last 30 years, the COBE Spectra has been considered as the main apheresis device used to harvest PB HSPCs by most apheresis teams.[82,83] This technique was extensively used with the Manual Collection Protocol. Later, an automated version of COBE Spectra, named Auto-PBSC, was developed and operated with cyclical harvest of a mononuclear cell fraction.[84]

Studies have compared the performances of the devices (Table 6).[82,85–90] Indexes were developed to characterize the performances of the devices during harvesting. The main index is the collection efficiency (CE). Other indexes (platelets or hemoglobin losses, apheresis duration, contamination with nontargeted cells) were analyzed.[88–90] CEs were similar in both protocols (manual and Auto-PBSC) developed for COBE Spectra.[84] The platelet loss was lower with the Auto-PBSC than with the Manual COBE Spectra technique.[84] The comparison of the performances of Fenwal Amicus and COM.TEC showed identical CE.[85] Compared to the Haemonetics MCS+ or to the Baxter Amicus, a shorter apheresis duration and a better correlation between PB pre-apheresis CD34+ cell counts and numbers of CD34+ cells harvested were shown with COBE Spectra.[82,83]

The COBE Spectra technique is being replaced by a new technique, the Spectra Optia.[86] A comparison of the performances between COBE Spectra, Spectra Optia, and COM.TEC techniques showed higher CE in both Spectra Optia and COM.TEC techniques, but Spectra Optia sessions required longer durations.[87,88]

How to manage poor HSPC harvest by apheresis

The HSPC harvest depends on the pre-apheresis circulating CD34+ cell counts, performances of the apheresis techniques, and the blood volume processed. Each of these elements can be improved. After unsuccessful mobilization, the unfavorable situation can be quickly corrected by using plerixafor in a salvage administration.[65,91] The old devices gave the opportunity to improve the harvest manually, while new devices perform the task automatically. Another solution consists in processing more blood volumes during the apheresis sessions. In the past, apheresis sessions consisted of processing large volumes.[81] Such approach was safe, but a significant decrease in blood electrolyte concentration and platelets had to be prevented.[92] Studies considering the possibility to perform large-volume leukapheresis with the new devices should be carried out.

Table 5 Main current apheresis techniques used for hematopoietic stem and progenitor cell (HSPC) harvest

	COBE Spectra	Spectra Optia	Amicus	COM.TEC
Firm	TerumoBCT (Lakewood, Co)	TerumoBCT (Lakewood, Co)	Fenwall (Lake Zurich, IL)	Fresenius Healthcare (Bad Homburg, Germany)
Flow method	Continuous	Continuous	Continuous	Continuous
Blood separation	Continuous separation of blood components	Continuous blood separation monitored by automated interface management (AIM)	Continuous blood separation monitored by two sensors	Blood separation is performed during cycles whose features are individually determined
HSPC harvest	Continuous harvest of the mononuclear cells layer	Harvest in a collection chamber and line during cycles	Harvest in a collection chamber and line	Intermittent harvest at the end of each separation cycle
Operator adjustment	Continuous manual adjustment of the interface and the harvest	Interface and harvest automatically controlled with few adjustments	Interface and harvest automatically controlled	Adjusting the volumes of the separation cycle, buffy coat, and spillover

Note: A modified automatic COBE Spectra technique was also developed and named Auto-PBSC.

Table 6 Recent studies comparing the performances of apheresis devices for HSPC harvesting

Reference (year)	Goal	Number of procedures	Results
Altuntas et al[85] (2007)	Compare Amicus and COM.TEC performances	Amicus: 20 COM.TEC: 20	No difference in numbers of CD34+ cells harvested Higher decrease in PB platelets with COM.TEC
Reinhardt et al[86] (2011)	Evaluate performances of Spectra Optia and comparison with historical performances of COBE Spectra	Spectra Optia: 35 COBE Spectra: 401	Excellent usability of Spectra Optia CE2 superior with Spectra Optia
Wu et al[82] (2012)	Compare HSPC harvest using COBE Spectra, MCS+ Haemonetics, and Baxter Amicus	COBE Spectra: 99 MCS+: 81 Amicus: 38	Similar number of CD34+ cells harvested Better correlation of harvested/circulating HSPCs with COBE Spectra. Amicus collected less platelets
Brauninger et al[87] (2012)	Compare Spectra Optia and COBE Spectra performances	Spectra Optia: 50 COBE Spectra: 89	With Optia – CE1: 7.9% greater – Less platelets but more granulocytes in products
Flommersfeld et al[88] (2013)	Compare COM.TEC, COBE Spectra, and Spectra Optia	COM-TEC: 77 Spectra Optia: 52 COBE Spectra: 58	With Optia: – Higher CE – Longer duration
Ikeda et al[89] (2014)	Compare Spectra-MNC (manual) and Spectra Auto-PBSC performances	Spectra-Auto: 118 Spectra-MNC: 70	Correlation between circulating HSPCs and harvested HSPCs in Spectra – MNC Less reduction in PB platelets in Spectra-Auto
Cherqaoui et al[90] (2014)	Compare Spectra Optia and COBE Spectra performances in low-weight children	Spectra Optia: 8 COBE Spectra: 22	Similar CE Reduced platelet and Hb loss with Spectra Optia but higher duration

Note: Two collection efficiency (CE) indexes are described: CD34 CE1 (%) = absolute number of CD34+ cells harvested (×100%)/([pre-apheresis CD34+ blood levels + post-apheresis CD34+ blood levels]/2) × total processed volume; CD34 CE2 (%) = absolute number of CD34+ cells harvested (×100%)/pre-apheresis CD34+ blood levels × total processed volume.
Abbreviations: Hb, hemoglobin; HSPC, hematopoietic stem and progenitor cell; PB, peripheral blood.

How to ensure safety of donors

BM harvest

The safety of BM donors must be ensured during and after harvesting. Knowing donors' medical history and clinical evaluation enables medical teams to prevent occurrence of complications during general anesthesia. It is possible to harvest BM after local anesthesia associated or not with analgesia.[93] Decreases in blood red cell levels are linked to the volume of BM harvested and can be treated by iron supplementation or by autologous red cell transfusion. After the donation, donors usually complain of bone pain. The complications and quality of life in adult and pediatric BM donors must be evaluated.[94]

CB harvest

When harvesting CB, it is essential to ensure safety to both the mother and the infant. The CB harvest must be performed after the delivery. The safe management of obstetric delivery should never be compromised to facilitate CB harvest.[16] The umbilical CB clamping should not be performed too early after delivery to prevent deprivation in blood volume and hemodynamic disturbances in the infant.[95]

PB harvest

Ensuring safety is important for allogeneic donors of PB HSPCs. In this setting, three elements must be considered, ie, the immediate side effects after mobilization by G-CSF or new agents, side effects during apheresis, and long-term side-effects of the mobilizing agents. G-CSF stimulation induces pain (bone, muscle, headache) until the end of stimulation. Donors with higher WBC levels experience more fatigue; females experience headache, nausea, and fever; and higher G-CSF dosage is associated with bone pain.[96] G-CSF induces sometimes high WBC levels, which could generate vascular complications. Indeed, splenic rupture, which is a rare complication, has been observed in allogeneic and autologous donors.[97] When WBC is higher than 60,000/μL, a GSF dose adaptation can be proposed to prevent vascular complications.

The second element concerns the apheresis itself. In a minor proportion of donors (0.6%–20% depending on the apheresis center), a central venous catheter may be necessary.[98] Pain at the site of puncture occurs more frequently in donors with a central (58%) than peripheral vein access (38%).[99] The occurrence of acute side effects in large series of BM and PB HSPC harvests in donors has

been compared, which showed that peak levels of pain and toxicities were comparable in both harvesting methods.[100]

The incidence of complications (cancer, autoimmune disease, and thrombosis) was similar after BM and PB HSPC donation (0.99% vs 0.31%, respectively).[101] The long-term safety of G-CSF in donors is still being debated. G-CSF induces epigenetic and cytogenetic abnormalities, which persist for several months.[102] The donor immune response is disturbed during 6 months, with decrease in T-cell PB counts due to the apheresis, and also in interleukin (IL)-2 and IL-10 production due to G-CSF.[103] Long-term medical and biological follow-up are necessary to confirm the safety of G-CSF in donors.

When harvesting PB in patients, the intensity of the main symptoms or complications from G-CSF stimulation and apheresis can be confused with the symptoms from the chemotherapy-induced aplasia. Plerixafor can induce transient side effects (diarrhea, nausea, injection site erythema, headache, paresthesia). The long-term side effects of plerixafor should be studied in particular, if this treatment is to be used in routine or in donors.

Cell composition of graft and influence on HSPC transplantations

Clinical consequence of BM graft composition

BM contains HSPCs that are the main cell population to be transplanted, but other cells must be considered for hematopoietic transplantations.

In harvested BM, stromal cells and the marrow microenvironment are associated with HSPCs, and both are important to reconstitute BM in nononcological hematological diseases and diseases with marrow abnormalities. When the main aim is to obtain a graft-versus-leukemia (GVL) effect, PB and not BM HSPCs are preferentially harvested. The recent development of haplo-identical transplantations followed by posttransplantation cyclosphamide injection has reintroduced the interest for using BM.[104] BM was also used after solid-organ or composite-tissue transplantation to induce immune tolerance.[105]

Clinical consequence of CBU composition

The use of CBUs for allogeneic HSPC transplantation is associated with lower incidence of graft-versus-host-disease with slow immune and hematological recovery, inducing high incidence of opportunistic or severe viral infections. The transplantations of two CBUs decrease the incidence of these complications.[15] The numbers of HSPCs transplanted are associated with the outcome. The relation between lymphocyte composition in transplanted CB and outcome after transplantation is currently being studied.[106]

Clinical consequence of PB graft composition

During allogeneic transplantation, a PB graft is harvested when a GVL effect is aimed. Indeed, during apheresis, both HSPCs and lymphocytes (which participate in the GVL effect) are harvested in the same layer. This feature is used when harvesting allogeneic donor lymphocytes for infusion to reinforce the GVL effect. This principle has been developed in reduced intensity conditioning of allogeneic transplantations.

The PB autologous HSPC harvesting process is performed either in first-line or first-relapse poor-prognosis NHL, in HD, and in first-line treatment for MM or some solid tumors (neuroblastoma or brain tumors). The number of HSPCs transplanted is associated with a better outcome in patients treated for NHL and MM.[107,108] In addition to HSPCs, the PB harvests contain significant numbers of lymphocytes that influence the immune reconstitution after transplantation. The numbers of T and NK cells are higher in grafts after mobilization with plerixafor than after standard mobilization.[109] Studies should be carried out to determine whether autologous grafts mobilized with plerixafor are associated with a better outcome or to changes in immune reconstitution.

Conclusion

HSPC transplantations have become a referent treatment of severe hematological diseases and gives opportunities to obtain a long-term remission. The number of HSPCs infused during the transplantation is predictive of a better outcome in most types of diseases and hematopoietic transplantations. Therefore, as a first and main step in transplantations, the harvest must contain enough autologous or allogeneic HSPCs. Besides standard HSPC harvesting protocols, harvesting methods and techniques have been improved over the years. Studies also brought better knowledge of the factors associated with better harvests.

When considering BM transplantations with improved harvesting techniques, the latest studies have focused on the evaluation of the complications and quality of life of donors. On the other side, properties of stromal cells and their interaction with HSPCs have been characterized. Such knowledge has generated new indications for using BM transplantation to induce immune tolerance. The characterization of the

interactions between HSPCs and stromal cells has led to target the pathways involved in HSPC mobilization from BM to blood. Treatments have been developed to increase mobilization targeting these interactions. Plerixafor is the prototype of these new mobilizing agents. In the next few years, other new mobilizing agents targeting such interactions will be developed, but their cost will require the development of algorithms to use them.

The development of new mobilization agents has enabled teams to harvest autologous HSPCs in almost all patients. Autologous transplantation is therefore now possible for a majority of patients, inducing very low failure of HSPC harvests. Further studies should show whether such harvesting possibilities can change the prognosis of PM patients who need an autologous HSPC transplantation. The development of CBU transplantation and banks should improve the chances of performing successful allogeneic HSPC transplantations in patients without (HLA-) related or unrelated donors.

In conclusion, to increase the success of HSPC transplantations, it is necessary to continue improving the harvesting techniques, numbers of HSPCs, and clinical consequences in patients or donors, which emphasizes the major role of the teams performing HSPC harvesting. Indeed, this purpose can be achieved with further studies based on their work and carried out on a regular basis.

Disclosure

The authors report no conflicts of interest in this work.

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