Stem Cells Harvest from Volunteer Donors

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Background: Over the last several decades allogeneic hematopoietic cell transplantation (HCT) has emerged as an important therapeutic option for a number of malignant and non-malignant conditions. The collection of hematopoietic stem cells mobilized from the bone marrow into the bloodstream of healthy donors has now become a routine procedure throughout the world.
Materials and methods: A number of 86 procedures of hematopoietic stem cells (HSC) harvest and cryopreservation from 64 volunteer donors, 54 adults (28 women and 26 men) and 10 children (5 girls and 5 boys) with ages between 6–66 years (on ave-rage 30.5) were carried out in the Bone Marrow Transplant Center from Clinical Institute Fundeni, Bucharest.
Results and discussions: HSC mobilization was achieved for all the 64 volunteer donors by administration of Filgastrim, on an average 8.4 mcg/donor weight (limits: 5–16.64 mcg/donor weight), leukapheresis procedure being realized in day +5 of Filgastrim administration.
Conclusions: In conclusion, a healthy volunteer donor, will undergo in most cases 4 or 5 days of Filgastrim administration. The WBC and the number of CD34+ cells from the periphe-ral blood will be counted beginning with the 4th day. When the number of CD34+ cells from peripheral blood will reach a certain level (usually on the 4th or 5th day), the vo-lunteer donor will be sent to the apheresis unit for harvesting stem cells.

Keywords: stem cells, harvesting, volunteer donors

Introduction

Widely accepted cancer treatment strategies include chemotherapy and radiotherapy. The rationale for administration high-dose chemotherapy and/or radiation to patients with therapy-sensitive tumor is to reduce tumor burden. Delivery of these therapies with respect to higher drug doses and intensified schedule are often limited by organ toxicity (eg bone marrow, heart, lung) [1]. To overcome these dose limitations, hematopoietic stem cell transplantation, high-dose therapy supported by the infusion of hematopoietic stem cells, has evolved as a medical procedure to allow for administration of intense drug doses with tole-rable organ and hematopoietic toxicity [2].

Over the last several decades allogeneic hematopoietic cell transplantation (HCT) has emerged as an important therapeutic option for a number of malignant and non-malignant conditions. Besides the effect of high-dose chemotherapies and/or total body irradiation on malignancies the benefit of allogeneic HCT is due to the graft-versus-leukemia/tumor (GVL) immune reaction by transplanted donor immune cells. However, allogeneic HCT is limited by the immunologic recognition and destruction of host tissues, termed graft-versus-host-disease (GVHD). GVHD continues to be a major source of morbidity and mortality following allogeneic HCT which limits use of HCT for a broader spectrum of diseases and patients [3].

The collection of hematopoietic stem cells mobilized from the bone marrow into the bloodstream of healthy donors has now become a routine procedure throughout the world. Peripheral blood stem cells (PBSCs) mobilized by recombinant human granulocyte-colony stimulating factor (rhG-CSF) were usually used for allogeneic hematopoietic stem cell transplantations. The immediate side effects of rhG-CSF administration and PBSC collection have long been established; however, information as to possible long-term consequences has been limited up to now [4].

Transplantation of G-CSF-mobilized peripheral blood cells is our current standard practice in allogeneic transplantation.

Pluripotent stem cells express the cell surface marker Ag CD34. This marker is the indicator most frequently used in clinical practice to determine the extend and efficiency of peripheral blood stem cells collections.

Although not a complete measure of quantity and quality of collected cells, blood samples from collections are assayed to determine the number of CD34+ cells present. Once specific cell targets are achieved, cell collections are completed and stored for future use.

It is not known the minimum safe number of CD34+ cells needed for clinical engraftment of all lineages, as this may vary depending on the stem and progenitor cell subset composition in a given patient. However, it is known that a graft content of more than 5–10 × 10^6 CD34+cells/kg of body weight is safe, and, most important, only has a minor risk of engraftment failure [5].

Traditionally, HSC were harvested from the iliac crests under general anesthesia. Thereafter, mobilized PBSC have been increasingly used in both auto- and allo-HSCT. In the 1990s, unmanipulated CB cells collected and cryopreserved at birth have been used both in related and unrela-

ted HLA matched and mismatched allogeneic transplants in children, and more recently in adults [6,7].

The main differences between cell sources are:
Bone marrow:
- Collection under general anesthesia;
- Limited number of haematopoietic stem cells;
- Median number of nucleated cells: 2×10^8/kg;
- Median number of CD34+ cells: 2.8×10^6/kg;
- Median number of T-cells: 2.2×10^7/kg.

G-CSF mobilized PBSC:
- Easy collection;
- No requirement for general anesthesia;
- Side effects of G-CSF;
- High number of cells;
- Median number of nucleated cells: 9×10^8/kg;
- Median number of CD34+ cells: 7×10^6/kg;
- Median number of T-cells: 27×10^7/kg.

Cord blood:
- Easy and harmless collection;
- Immediate availability of cryopreserved units and low risk of transmissible diseases;
- Acceptable partial HLA mismatches;
- Number of cells is the limiting factor;
- Median number of nucleated cells: 0.3×10^8/kg;
- Median number of CD34+ cells: 0.2×10^6/kg;
- Median number of T-cells: 0.4×10^7/kg [8].

G-CSF (Filgastrim) is used for increasing cocentrations of circulating HSC. G-CSF is thought to stimulate HSC mobilization by decreasing SDF-1α gene expression and protein levels while increasing proteases that can cleave interactions between HSC and the bone marrow environment. The recommended dose of Filgastrim is 10 mcg/kg/day as a subcutaneous injection for 5 days. Data indicate that divided dose of G-CSF (eg 5 mcg/kg twice daily) are more efficacious than single dose-administration (eg 10 mcg/kg once daily) by producing a higher yield of CD34+ cells and the need for fewer apheresis procedures.

In 1995, 3 pivotal studies demonstrated the safety and feasibility of using G-CSF mobilized PB allografts. Patients experienced prompt engraftment with an incidence of GvHD similar to that of BM recipients. In addition, no serious short-term complication of G-CSF mobilized PB harvesting were observed in the donors [9,10].

Apheresis

Preparation for stem cell collections in an apheresis unit follows the pre-transplantation evaluation and catheter placement.

The risk for healty donors must be minimized. A careful inquiry before donation must be performed.

Donors can expect to undergo their first apheresis session in as little as 4 to 5 days.Established thresholds for apheresis initiation may vary across centres, but tipically range is around 20 CD34+ cells/microliter [11,12].

Donors are connected to the apheresis machine (COBE SPECTRA) by their catheter. One lumen is used to draw blood out of the patient into the machine. Here the blood is spun at high speed in a centrifugation chamber housed within the cell separator machine.The desired stem cells are collected during the entire procedure, either in cycles (auto PBSC procedure) or continuously (MNC procedure), and the remaining blood components are returned to the patient through the second lumen of their catheter. This second lumen additionally can be used to administer electrolyte supplements to the patient. Each apheresis session lasts 4–5 hours during which approximately 4 times the average human blood volume is processed. Collections can occur on a daily basis until the target CD34+ levels are achieved (usually 1 or 2 days) [13].

During MNC procedure, anticoagulated whole blood enters the inlet chamber through the inlet tube. As the blood flows through the channel, the system separates it into three layers: RBC on the outside, buffy coat containing WBC in the center, and platelet-rich plasma on the inside. The system establishes the RBC plasma interface during Quick Start. After Quick Start, the operator adjusts the plasma pump flow rate to hold the interface in a constant position. The system draws the MNC from the channel through the WBC collect tube, while the platelet-rich plasma exists through the plasma tube. The RBC exit through the RBC tube.

During Spectra AutoPBSC procedure, anticoagulated whole blood enters the first stage of the channel through the inlet tube. In the first stage, the system separates the RBC and WBC from the platelet-rich plasma. The RBC and granulocytes exit the channel through the RBC tube. Platelet- rich plasma flows over the dam into the second stage where the system concentrates the platelets in the plasma. The plasma exit through the collect tube to return to the donor, and the remaining plasma flows through the channel to the plasma tube. MNC accumulate above the layer of RBC. During the Harvest phase, the MNC flow over the dam into the second stage. Once the collect concentration monitor (CCM) detects cells in the collect line, the collect valve opens and the MNC flow to the collection bag. The Chase phase follows the Harvest phase during which plasma “chaces” the MNC in the collect line up to the collection bag. Concurrent collection of a specific plasma volume is optional. The system determines the plasma and collect pump flow rate based on the donor/ patient hematocrit, and maintains the interface postion. A small volume of plasma and RBC flow into the control tube to help maintain the interface.

Common apheresis complications: citrate toxicity (hypocalcemia, hypomagnesaemia, hypokalaemia, metabolic alkalosis), thrombocytopenia, hypovolemia, catheter malfunction, infection [14, 15].

Cryopreservation

The collected stem cell products are maintaining in liquid nitrogen at –196˚C until the time of patients transplantation. Quality testing is performed on collections to ascertain with microbes as well as to determine the number of viable cells available for transplantation [16].

Cryopreservation solutions:

- DMSO 10% + Albumine 4% or HES 6% for programmable freezing;
- DMSO 5% + HES 6% for freezing at –80˚C;
- DMSO 10% + NaCl + autoFFP/ACD.

DMSO maintains cells viability by preventing ice crystal formation within the cells during storage.

Reactions to DMSO:

- Common: nausea, vomiting, abdominal cramping, headache, garlic aftertaste;
- Rare: hypotension, rapid heart rate, shortness of breath, fever, neurologic complications [16].

Thawing

There are 2 possibilities of thawing: with flushing out or without flushing out.

The flushing out procedure must be done immediately after thawing, without any delay, for the purpose of limiting the toxicity of DMSO. This procedure consists of: spin over (2000 rpm), removal of the supernatant liquid and admixture to the remaining cells of a solution composed of 10% ACD + 2% Albumine.

The tawing procedure without flushing out: we out each frozen bag into a bath of water at 37˚C and we shake it with gentile movements, 2 minutes until it is completely defrosen [17].

Materials and methods

A number of 86 procedures of hematopoietic stem cells (HSC) harvest and cryopreservation from 64 volunteer donors, 54 adults (28 women and 26 men) and 10 children (5 girls and 5 boys) with ages between 6–66 years (on average 30.5) were carried out in the Bone Marrow Transplant Center from Clinical Institute Fundeni, Bucharest.

HSC mobilization from the bone marrow into the peripheral blood was realized by the subcutaneous administration of Neupogen (Filgastrim, G-CSF). We counted the WBC and the number of CD34+ cells from the peripheral blood, beginning the day +4 from the mobilization regimen.

HSC from peripheral blood were harvested by leukapheresis procedure with the help of discontinuous flow separators (Haemonetics MCS plus) and continuous flow separators (Cobe Spectra), autoPBSC procedure and MNC procedure, by treating several total blood volumes (SVL method – Standard Volume Leukapheresis and LVL method – Large Volume Leukapheresis).

A number of 24 procedures were carried out with Haemonetics separator (16 donors) and 62 procedures with Cobe Spectra separator (48 donors). ACD-A was the anticoagulant agent that we used with 1:9 ratio (Haemonetics separator) and 1:12 ratio respectively (Cobe Spectra separator).

HSC harvested were than combined with a cryopreservation solution (DMSO) at a final concentration of 10% (DMSO 10%).

The freezing procedure was realized with the help of nitrogen liquid programmable freezer MiniDigitCool.

The graft quality control was done as well before freezing procedure (native product) as after the freezing procedure (cyiotubes and cryocites bags). There have been done the following tests: total blood count, blood smear, total number of CD34+ cells, cells viability (with tripan blue), number of CFU-GM).

The HSC graft was thawed in a water bath at +37˚C by smooth movements, immediately followed by the infusion to the patient.

Results

HSC mobilization was achieved for all the 64 volunteer donors by administration of Filgastrim, on an average 8.4 mcg/donor weight (limits: 5–16.64 mcg/donor weight), leukapheresis procedure being realized in day +5 of Filgastrim administration.

We started leukapheresis procedure at a WBC level in peripheral blood of 45.9×10^9/L (limits 5.1–72.1×10^9/L) and a CD34+ cells level in peripheral blood of 101.5×10^6/L (limits 16.2–112.8×10^6/L).

The optimal dose of stem cells CD34+ was achieved at 45 donors by a single leukapheresis procedure and at 18 donors by two leukapheresis procedures.

There have been achieved 59 SVL procedures and 27 LVL procedures; we treated on average 2.98 total blood volumes (limits 1.8–4.2) in 331.4 minutes (limits 159–865 minutes).

The medium number of CD34+ cells harvested/procedure was 7.5×10^6/L (limits 0.29–52.1×10^6/L) and the medium number of CD34+ cells harvested/patient was 9.5×10^6/L (limits 1.3–52.1×10^6/L).

Cells viability obtained with the direct test was 97.6% (limits 87–100%) and cells viability after the mixing with DMSO solution was 71.4% (limits 30–100%).

In vitro testing of clonogenic capacity of progenitor cells showed on average 529.9×10^4 CFU-GM/body weight/sample (limits 34–3435×10^4).

The tests performed after the graft thawing showed the following results: cells viability 55.8% (limits 10–84%) in 71 collections; number of CD34+ cells 4.4×10^6/ patient body weight (limits 0.66–21.98) in 12 collections; number of CFU-GM: 98.5×10^4/patient body weight (limits 0–635) in 54 collections.

We performed allogeneic transplant with the source of stem cells from the peripheral blood in 50 patients; the graft that was administrated was formed on average by 5.6×10^6 CD34+ cells/patient body weight (limits 2.11–15.09×10^6/patient body weight).

Conclusions

In conclusion, a healthy volunteer donor, will undergo in most cases 4 or 5 days of Filgastrim administration. The WBC and the number of CD34+ cells from the peripheral blood will be counted beginning with the 4th day. When the number of CD34+ cells from peripheral blood will reach a certain level (usually on the 4th or 5th day), the volunteer donor will be sent to the apheresis unit for harvesting stem cells. 1 or 2 sessions of apheresis will be enough for collecting the total amount of stem cells for a graft. The apheresis session will take on average 4 hours (if it will be used MNC procedure) or 5 hours (if it will be used autoPBSC procedure).

HSC will be combined with a cryopreservation solution; then the graft will be stored in liquid nitrogen at –196˚C degree until the patient will be transplanted. When it will be needed, the graft will be thawed in a water at +37˚C by smooth movements, immediately followed by its infusion to the patient.

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