SYSMEX EDUCATIONAL ENHANCEMENT
AND DEVELOPMENT | AUGUST 2016

SEED HAEMATOLOGY

Haematopoietic stem cell transplantation – part 2

The first part of the SEED explained details about the stem cells and the immunology of stem cell transplantation. This second part focusses on the process of Haematopoietic Stem Cell Transplantation (HSCT) which is complex and involves a multidisciplinary approach and resource utilisation.

The stem cell transplant process can be summarised in distinct phases such as mobilisation, apheresis (collection), the preparation of product for storage, the infusion of the transplant, and engraftment and recovery. The entire process for allogeneic and autologous stem cell transplantations is depicted in Fig. 1.

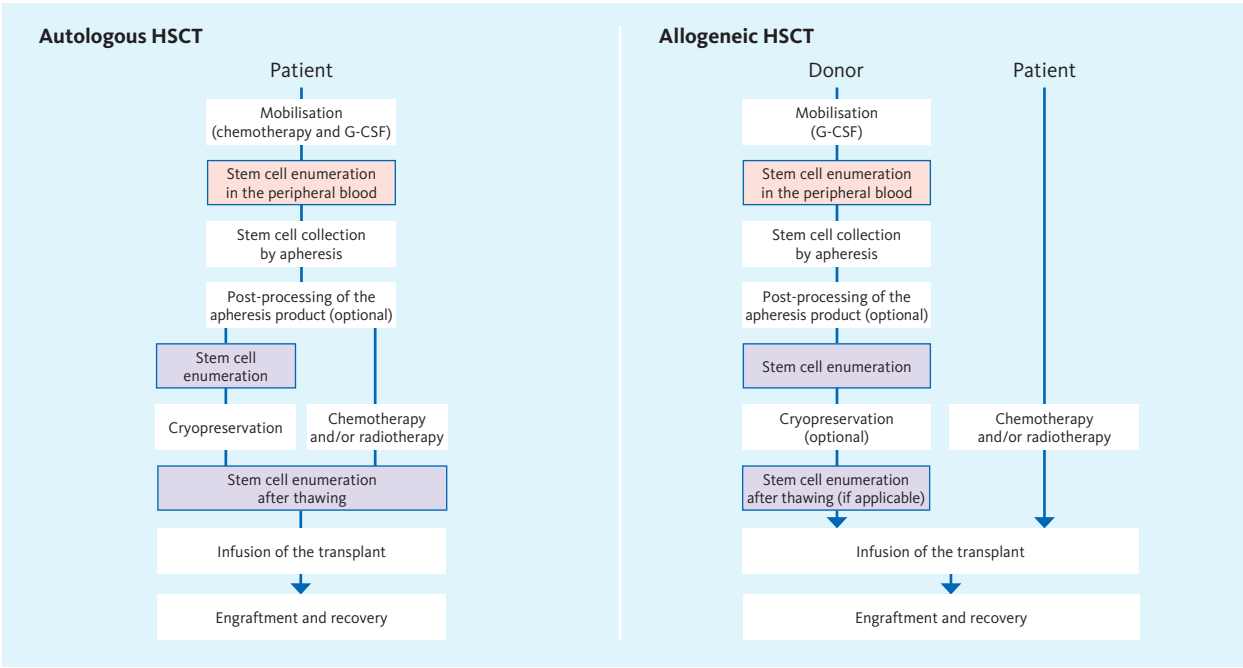

Fig. 1 *HSCT process for autologous and allogeneic stem cell transplantations*

Mobilisation of stem cells

Stem cells are present in peripheral blood in low numbers. In order to harvest enough stem cells for transplantation, they need to be moved from the bone marrow into the blood. This is achieved by administering recombinant human haematopoietic growth factors like granulocyte colony-stimulating factor (G-CSF) over a period of several days. G-CSF stimulates the division of stem cells in the marrow. Additionally, via a complicated gene and protein expression cascade, G-CSF leads to a decreased adhesion between stem cells and their bone marrow microenvironment. As a result, stem cells leave the marrow and enter the blood stream with the effect that their concentration in the blood increases by a factor of 10 – 100. The collection of these mobilised stem cells usually starts on day four or five after the administration of G-CSF.

In autologous transplantation, patients undergo additional chemotherapy prior to G-CSF administration and stem cell collection. Chemotherapy damages bone marrow stromal cells, which also leads to the mobilisation of stem cells into the blood stream, so chemotherapy and G-CSF act synergistically. However, if the chemotherapy regimen was too stringent, the mobilisation of stem cells in the patient may be poor and insufficient to start collection.

The concentration of stem cells in blood is measured prior to harvesting in all cases of autologous transplantation and – in most countries – also in allogeneic transplantation. It helps determine the optimal point in time to start collecting the stem cells. The optimal concentration of stem cells for successful engraftment is 5 – 10 x 10^6 CD34+ cells/kg of donor body weight, and the minimum concentration is 2 x 10^6 CD34+ cells/kg body weight. Established thresholds for initiation of collection may vary across countries and centres, but typically range from 5 – 20 CD34+ cells /µL.

Fig. 2 *Patient undergoing apheresis*

Apheresis procedure

Mobilised stem cells are collected from peripheral blood by the process of apheresis, which uses an instrument called ‘blood cell separator’.

Blood is accessed via large needles or catheters introduced into the veins of both arms (Fig. 2).

As blood is drawn from one arm, it is mixed with a citrate anticoagulant solution and pumped into the device. The blood cell separator is essentially a centrifuge, in which blood components are separated by centrifugal forces based on their density. Red blood cells – the component with the highest density – are pushed outwards to the wall of the centrifugal chamber. White blood cells form a buffy coat between the red blood cell layer and the plasma, which resides at the top. Platelets remain in the plasma. An aspiration needle in the centrifuge is adjusted in such a way that it aspirates the fraction of the white blood cells as this contains the mononuclear cells and is enriched in stem cells. This fraction is pumped into a sterile bag for further processing or storage. All other components are eventually pumped back into the donor’s blood stream via the other arm. This blood is supplemented with saline and albumins and warmed up to body temperature before being returned to the body. Citrate anticoagulant is non-toxic and is broken down in the liver within several hours (Fig. 3).

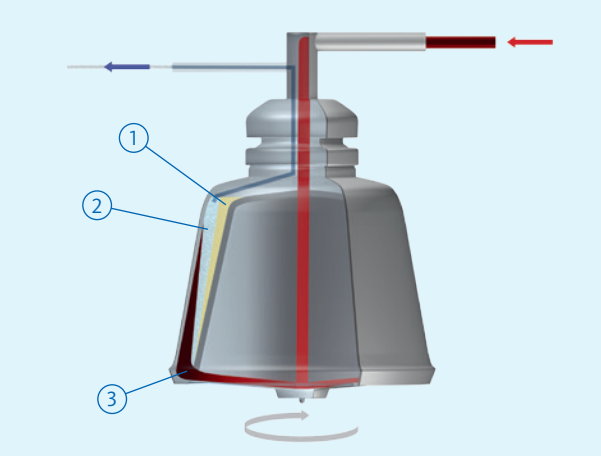

Fig. 3 *Principle of apheresis. 1: Plasma, 2: White blood cells, 3: Red blood cells*

An apheresis procedure is painless but requires several hours, usually four to five. In some cases where mobilisation was poor, several apheresis procedures may be required on consecutive days to obtain a sufficient amount of stem cells. Alternatively, a larger volume of blood may be processed compared to the standard apheresis procedure. Therefore, the terms 'SVL' (standard-volume leukapheresis) and 'LVL' (large-volume leukapheresis) are sometimes used. In the LVL more than 15 litres of blood, or more than 3 times the body's blood volume, are processed in one session. This can be achieved by extending the apheresis time and passing the blood through the apheresis system several times.

After the first round, when a single body's blood volume has passed through the separator, the concentration of stem cells in the donor's peripheral blood drops significantly and keeps falling after each consecutive round due to the removal of stem cells from the blood stream. On the other hand, stem cells continue to be mobilised from bone marrow into peripheral blood during apheresis, and up to 55 % of the stem cells in the final apheresis product represent those mobilised during the apheresis procedure itself. Both the drop in stem cell concentration and the mobilisation during apheresis vary substantially among individuals.

Once the desired amount of stem cells is obtained, the apheresis procedure may be stopped. Reaching minimum thresholds for CD34+ cell concentration is important as the cell dose positively correlates with engraftment outcome. In some cases apheresis is performed several times on consecutive days to obtain a sufficient amount of stem cells.

Enumeration of stem cells

Immune flow cytometry is the standard method for stem cell enumeration. Stem cells are counted from the peripheral blood of the donor during the mobilisation phase to assess whether the concentration of mobilised stem cells is sufficient to start apheresis, and later also from the apheresis product to evaluate the quality of the product.

Stem cell enumeration methods can be defined as dual-platform and single-platform methods.

With a dual-platform method, the total white blood cell count is first determined by an automated haematology analyser. Then a small portion of the blood (or apheresis product) is labelled with fluorochrome-conjugated anti-CD45 and anti-CD34 antibodies and subjected to flow cytometry. CD45 is a protein present on the surface of all white blood cells and CD34 is a stem cell surface marker. Following a special gating protocol, it is possible to determine the percentage of CD34+ cells in relation to the CD45+ cells. Knowing the absolute white blood cell count from the haematology analyser's results, the concentration of stem cells can also be calculated.

In a single-platform method, a small defined portion of the blood (or apheresis product) is labelled with a fluorochrome-conjugated anti-CD34 antibody and afterwards added to a tube containing a known amount of fluorescent microbeads. The ratio of CD34+ stem cells to the microbeads is determined by flow cytometry and the concentration of stem cells is calculated based on the known concentration of the microbeads. This method requires very accurate pipetting of the blood.

Several studies have compared the single-platform and dual-platform methods of stem cell enumeration; no significant differences have been found. However, the International Society for Hematotherapy and Graft Engineering (ISHAGE) recommends the use of the single-platform method.

Post-apheresis processing

Apheresis products may undergo pre-transplantation processing to improve the outcome of transplantation. So, in allogeneic transplants T-cells may be depleted by antibodies, reducing the risks of GvHD. One risk associated with autologous HSCT is contamination of the transplant with tumour cells, which would lead to reinfusion of the cells into the patient and increase the risk of relapse. When autologous transplantation is used in patients with CD34-negative tumours, CD34+ cell selection is used. This allows removing possible residual tumour cells from the transplant. During this procedure, anti-CD34 antibodies bound to magnetic beads are added to an apheresis product, after which the product is passed through a column with an activated magnetic field. All of the CD34-cells pass through the column, while CD34+ cells are retained by the column due to the magnetic beads that are attached to the anti-CD34 antibody. When the magnetic force in the column is deactivated, the CD34+ cells can be collected in a clean sterile bag. This method allows the production of a highly enriched CD34+ transplant with a cell purity of over 95%.

Engraftment and recovery

The last phase of HSCT is engraftment and recovery. During this critical time, the infused stem cells find their way back to the bone marrow microenvironment and repopulate the depleted marrow stores. Cytokines are administered after transplantation to enhance stem cell maturation and to re-establish blood cell components. The first sign of engraftment is the return of circulating white blood cells (WBC) to a sufficient level which is usually defined as an absolute neutrophil count (ANC) of >500 cells/µL for 3 consecutive days. This typically occurs 7 – 14 days after the stem cells have been infused into the patient. Increased platelet levels (absent of transfusion support) is another indicator of recovery, and occurs at a later time point, averaging 2 – 3 weeks following the transplant. More recent studies suggest also the use of immature reticulocyte fraction (IRF) and immature platelet fraction (IPF) as predictors of engraftment.

Until engraftment occurs, patients are at an increased risk of infections, so precautions must be taken to avoid exposure to microbial pathogens. Patients often require supportive care strategies and therapies, including administration of antiemetic's, pain medications, antibiotics, and nutrition support to ameliorate consequences following the high dose chemotherapy preparative regimen and the subsequent prolonged period of pancytopenia.

Compiled by
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Useful links

www.ebmt.org
The European Group for Blood and Marrow Transplantation

www.cibmtr.org
Center for International Blood and Marrow Transplant Research (CIBMTR)

www.asbmt.org
American Society for Blood and Marrow Transplantation (ASMBT)

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