

PEARLS OF LABORATORY MEDICINE

www.traineecouncil.org

TITLE: Peripheral Blood Stem Cell Collection

PRESENTER: Laura S Connelly-Smith

Slide 1:

Hello, my name is Laura Connelly-Smith. I am an Assistant Professor in Hematology and the Assistant Medical Director in Apheresis and Cellular Therapy at the University of Washington Medical Center and Seattle Cancer Care Alliance. Welcome to this Pearl of Laboratory Medicine on “**Peripheral Blood Stem Cell Collection.**”

Slide 2:

The purpose of peripheral blood stem cell (PBSC) collection is for the acquisition of hematopoietic stem or progenitor cells for the purpose of transplantation. Hematopoietic progenitor cells (HPCs) are responsible for the formation of blood cells. This presentation will cover the why, how and when PBSC collections are performed.

Slide 3:

HPC transplantation is a widely accepted treatment strategy for most hematological malignancies and for several non-hematological malignancies and non-malignant conditions. In several conditions hematopoietic progenitor cells transplantation (HPCT) is recognized as the standard of care with well-defined clinical evidence available in the form of high quality clinical trials and/or observational studies. HPCT includes Autologous and Allogeneic HPCT.

Slide 4:

In autologous transplantation reinfusion of the patient’s own stem cells allows for the timely recovery of the bone marrow (BM) following the provision of high dose therapy. Chemotherapy can be associated with significant toxicity but regimens do not include immunosuppression. The aim of allogeneic HPCT is to replace a diseased or nonfunctioning marrow with normal bone marrow from a healthy related or unrelated donor, such that the patients acquire a “new immune system”. Patients receive conditioning with chemotherapy and /or radiotherapy and regimens contain immunosuppression to prevent

rejection of the new graft as well as to prevent graft versus host disease (GVHD). In allogeneic transplantation, the healthy donor undergoes the HPC collection.

Slide 5:

CD34 antigen is a clinically important molecule expressed on the surface of, and, serves as a marker of the cell population containing HPCs. HPCs are a subset of CD34+ cells". At steady state CD34+ HPCs are rare in the peripheral circulation constituting 0.1% of circulating mononuclear cells. Their number is 10-100 times greater in the bone marrow. Studies have confirmed that CD34+ cells are mobilized into the peripheral blood (PB) and, when collected for transplantation, are similar to marrow-derived cells in their ability to fully reconstitute hematopoiesis after myeloablative conditioning. A minimum threshold of HPCs for transplantation has not been identified although 2×10^6 CD34+cells/kg is generally accepted as the minimum goal. With lower doses, there is increased risk of delayed or failed engraftment. Several studies have shown that higher doses of CD34+ cell infusions are associated with faster engraftment. There is some concern in Allo HPCT that sufficiently higher doses may be associated with GVHD. A target CD34+ cell dose between 4 and 5×10^6 CD34+cells/kg is deemed to be ideal based on available data.

Slide 6:

HPC's can be collected by different methods. Apheresis is the technique by which cells are removed by a machine from the blood. Following mobilization, the individual is connected to the apheresis machine by peripheral or central catheterization, blood removed from the body is separated by centrifugation and the buffy coat containing the HPCs is removed while the remainder the blood is returned to the patient. BM harvest involves multiple aspirations from the posterior superior iliac crests, usually in the OR setting. A total of up to 20ml/kg donor weight can be removed. Umbilical cord blood collection is performed by gravity using a bag system after term delivery with umbilical vein puncture with placenta in or ex utero. The preferred source of progenitor cells depends on donor availability, treatment or clinical trial protocol and patient preference.

Slide 7:

So what are the differences in PB and BM collections? Randomized trials of mobilized PB HPCT has translated into shorter duration of cytopenias, enhanced immune reconstitution with a reduction in infectious complications and overall reduced morbidity following transplantation. Individuals may require central venous access or may develop side effects of growth factor, chemotherapy or, anticoagulation used during the apheresis procedure. T-lymphocytes (CD3+ cells) are found in larger concentrations in a mobilized PB collection which is associated with a higher risk of chronic GVHD. Here Bensinger and colleagues compared the cellular content of mobilized PB to BM from related donors per kilogram of recipient weight. The table shows the PB grafts contain approximately 3, and 12 times the number of CD34+ cells, and CD3+ T cells, respectively that were present in the BM grafts. Most BM donors require general or spinal anesthesia. In BM collections, pain is common during recovery and can be present for the majority of donors for 1-2 weeks.

Slide 8:

PB stem cell collection is now the primary source of stem cells for HPCT transplantation. The Center for International Blood and Marrow Transplant Research Transplant Activity Report Covering 2010-2014 revealed a near 10 fold difference in PB as the progenitor cell source vs BM with the major difference being noted in autologous HPCT. The recovery of adequate HPCs from PB includes effective mobilization procedures and efficient apheresis techniques.

Slide 9:

Agents used to mobilize HPCs prior to collection include chemotherapy in patients with malignancy and where cytoreductive treatment is required. During the recovery phase after chemotherapy induced marrow aplasia, the HPCs are increased 20-25 fold. Here mobilization is enhanced by posttreatment administration of a recombinant cytokine. Other patients and all allogeneic donors are mobilized following cytokine alone. The most commonly used cytokines is recombinant human G-CSF or filgrastim. Another form of recombinant G-CSF (lenograstim) is not available in the United States but is used for HPC mobilization in Europe. GM-CSF (sargramostim) is seldom used for mobilization now but remains available as an alternate option. Over the last decade, a more novel agent, Plerixafor (Mozobil) has been indicated for patients that are poor mobilizers or who have previously failed to mobilize.

Slide 10:

HPCs reside within the bone marrow because of cytoadhesive interactions between membrane receptors and ligands expressed on microenvironmental stromal cells. Stromal cells produce the chemokine stem cell derived factor-1 alpha (SDF-1 α), an important signaling molecule involved in the proliferation, homing, and engraftment of stem cells. It is the loss of attachment to the stromal cells, along with the loss of SDF-1 α activity that favors the release of stem cells into the circulation. G-CSF decreases SDF-1 α gene expression and protein levels while increasing matrix metalloproteases by increasing the number of neutrophils. This can cleave interactions between HPCs and the bone marrow environment. For a given time in their development, stem cells express the chemokine receptor CXCR4 which is responsible for anchoring stem cells to the bone marrow microenvironment and binding to SDF-1 α . Blockade of this receptor with chemokine antagonist, Plerixafor increases circulating HPCs.

Slide 11:

This cartoon depicts an example of a typical cytokine mobilization and collection procedure. G-CSF is more commonly given at doses of 10mcg/kg/day. There is still limited data supporting higher doses. Apheresis is usually initiated at 96 to 120 hours after the start of G-CSF administration. For poor mobilizers or patients with previous mobilization failure, Plerixafor can be added 9-12 hours before HPC collection. By comparison, hematopoietic recovery and the day of maximal CD34+ cell mobilization after myelosuppressive chemotherapy are generally not predictable. Therefore, blood cell count parameters including PB CD34+ cell count are followed to determine the optimal time to start apheresis.

Slide 12:

Side effects of G-CSF occur frequently, but they are usually mild, transient and dependent on dose and schedule. Approximately 40-95% of apheresis donors experience bone and musculoskeletal pain which typically decreases after the GCSF is discontinued. Fatigue, headache, nausea are also common side effects. Mild transient splenomegaly occurs after 5 days of GCSF in most donors and probably

contributes towards the mild thrombocytopenia. Rare complications include marked splenomegaly and splenic rupture, severe thrombocytopenia and severe bleeding.

Slide 13:

Additional adverse events with Plerixafor may include gastrointestinal disorders and injection site reactions. Earlier randomized phase 3 studies demonstrated a higher proportion of multiple myeloma patients reaching a target of 6×10^6 CD34+ cells/kg with Plerixafor and G-CSF compared to G-CSF mobilized patients alone. The median number of days to reach $\geq 6 \times 10^6$ CD34+ cells/kg was one day for the Plerixafor group and four days for the placebo group. In another phase 3 RCT, a higher percentage of NHL patients managed to reach 5×10^6 CD34+ cells/kg using Plerixafor and G-CSF compared to patients mobilized with G-CSF alone. Cost is a limiting factor for the use of Plerixafor and the reason why many centers use it only in poor mobilizers or as a salvage therapy.

Slide 14:

For collection, there are several apheresis machines currently on the market. Donors are connected to apheresis blood cell separators. First whole blood is drawn into the apheresis machine to which anticoagulants, citrate and/or heparin are usually added. Blood is then separated by centrifugation which uses differences in specific gravity (density) to separate the cells. HPCs are found in the mononuclear (MNC) layer and are removed by collection channels/ ports during the entire procedure, either in cycles or continuously, and the remaining blood components are returned to the individual. Each apheresis session lasts approximately 2-6 hours during which 3- 6 x the individuals total blood volume, is processed. Collections can occur on a daily basis until the target CD34+ cell numbers are achieved.

Slide 15:

Adverse events related to the apheresis procedure primarily include pain and complications from venous access, hypotension and hypocalcemia from citrate (anticoagulant) toxicity. Citrate related complications include paresthesia's, light-headedness and nausea. A prospective study of 2408 NMDP donors showed 51% of donors having evidence of citrate toxicity. Citrate reactions can be managed by simply slowing down the apheresis process or citrate infusion rate. Infusing supplemental calcium has been required in some donors. Severe acute adverse events were uncommon and occur in less than 1% of donors during apheresis collections.

Slide 16:

Yu and colleagues have demonstrated a positive correlation for the number of circulating mobilized PB CD34+ cells just prior to collection, with, the final CD34+ cell content in the collection bag. This correlation is dependent on the collection efficiency (CE) of the procedure. CE is the percentage of cells that are removed from the circuit (into the collection bag) compared to the cells that go through the apheresis circuit. Factors that may influence the CE may include any interruption to the collection such as multiple machine alarms, venous access or return issues, donor ability to tolerate the procedure, and apheresis technician experience. It is now common to quantify circulating PB CD34+ cells/uL blood prior

to apheresis as a means of determining if a donor has mobilized well enough and to maximize collections based on these value using predictive formulae.

Slide 17:

Predictive algorithms have been developed and validated. They allow collection centers to determine what they might actually expect to collect on a given day. Here the predicted CD34+ cells/L of processed blood can be calculated using the PB CD34+ cell count just prior to apheresis, the recipient's weight as well as the collection efficiency. With extrapolation the algorithm can be used to determine the total blood volume that needs to be processed to collect a targeted CD34+ cell count/kg recipient weight. CE given as 30% in this example. These algorithms have now become standard practice in many centers worldwide. Their use allows for a reduction in overall apheresis time with increased donor and patient safety and allows the apheresis center to project expected cell yields and schedule procedures accordingly.

Slide 19: References

1. Majhail NS, Farnia SH, Carpenter PA et al. Indications for autologous and allogeneic hematopoietic cell transplantation: Guidelines from the American Society for Blood and Marrow Transplantation. *Biol Blood Marrow Transplant* 2015;21:1863-9.
2. Duong HK, Savani BN, Copelan E et al. Peripheral blood progenitor cell mobilization for autologous and allogeneic hematopoietic cell transplantation: guidelines from the American Society for Blood and Marrow Transplantation. *Biol Blood Marrow Transplant* 2014;20:1262-73.
3. Bensinger WI, Martin PJ, Storer B et al. Transplantation of bone marrow as compared with peripheral-blood cells from HLA-identical relatives in patients with hematologic cancers. *N Engl J Med* 2001;344:175-81.
4. https://bloodcell.transplant.hrsa.gov/research/transplant_data/transplant_activity_report/index.html
Accessed 20 August 2017.
5. Kronenwett R, Martin S, Haas R. The role of cytokines and adhesion molecules for mobilization of peripheral blood stem cells. *Stem Cells* 2000;18:320-30. Review,
6. Connelly-Smith L, Linenberger M. Chapter 28; 306-336. "Collection of Cellular Therapy Products by Apheresis". *Cellular Therapy: Principles, Methods and Regulations*. 2nd Edition AABB.
7. DiPersio JF, Stadtmauer EA, Nademanee A et al. Plerixafor and G-CSF versus placebo and G-CSF to mobilize hematopoietic stem cells for autologous stem cell transplantation in patients with multiple myeloma. *Blood* 2009; 113:5720-26.
8. DiPersio JF, Micallef IN, Stiff PJ et al. Phase III prospective randomized double-blind placebo-controlled trial of plerixafor plus granulocyte colony-stimulating factor compared with placebo plus granulocyte colony-stimulating factor for autologous stem-cell mobilization and transplantation for patients with non-Hodgkin's lymphoma. *J Clin Oncol* 2009;27:4767-73.
9. Pulsipher MA, Chitphakdithai P, Logan BR et al. Acute toxicities of unrelated bone marrow versus peripheral blood stem cell donation: results of a prospective trial from the National Marrow Donor Program. *Blood* 2013;121:197-206
10. Yu J, Leisenring W, Bensinger WI et al. The predictive value of white cell or CD34+ cell count in the peripheral blood for timing apheresis and maximizing yield. *Transfusion* 1999;39:442-450.

Slide 15: Disclosures

Nothing to disclose

Slide 16: Thank You from www.TraineeCouncil.org

Thank you for joining me on this Pearl of Laboratory Medicine on “**Peripheral Blood Stem Cell Collection.**”

