Leukopak 101: A Brief Review of Apheresis

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Introduction

Apheresis refers to the process by which blood is removed from a patient and separated into its constituent parts, allowing the removal of one specific component from the blood while the remainder is returned to the patient. Apheresis was first described over 100 years ago – by John Abel in 1914 – and has been used as a therapy for a number of different diseases, including sickle cell anemia and certain types of cancer (Korsack, 2016). In 1971 MD Anderson Cancer Center first used apheresis as a method for isolating peripheral blood stem cells (Korbling, 2011); subsequent breakthroughs in stem cell mobilization and cryopreservation have made apheresis the predominant method for peripheral blood stem cell collection. This paper will discuss the development of apheresis as a viable therapy as well as the current uses of apheresis in medicine.

Separation protocols

The main principle behind apheresis is the separation of donor blood into its component parts by either centrifugation or membrane filtration. Initial protocols relied on centrifugation to separate blood components by density, with erythrocytes at the bottom, overlaid by the Buffy Coat (comprised of granulocytes, lymphocytes, monocytes and platelets), and plasma as the top layer (Figure 1). Apheresis machines (Figure 2A) are characterized as either continuous flow (blood is withdrawn from one limb, centrifuged, and returned to the donor via another limb) or intermittent flow (blood is withdrawn, centrifuged, and the desired components returned to the patient by reversing the flow of the lumen line). Apheresis by centrifugation is the preferred method for removal of a specific cell type from the blood, and is also used for plasmapheresis (Figure 2B).

Separation by membrane filtration was developed in 1980 (Sueoka, 1997) and removes small molecules from the blood based on size exclusion. Because of this, it is the favored method for extracting plasma proteins from the blood. Extracted proteins can then be passed through subsequent membranes or over an affinity column to allow removal or further concentration of the protein of interest. Separation by membrane filtration can be used to remove autoantibodies, circulating immune complexes, soluble inflammatory factors, lipids such as LDL, and paraproteins (Kaplan, 2013).

A basic protocol for apheresis can be found in Appendix 1.

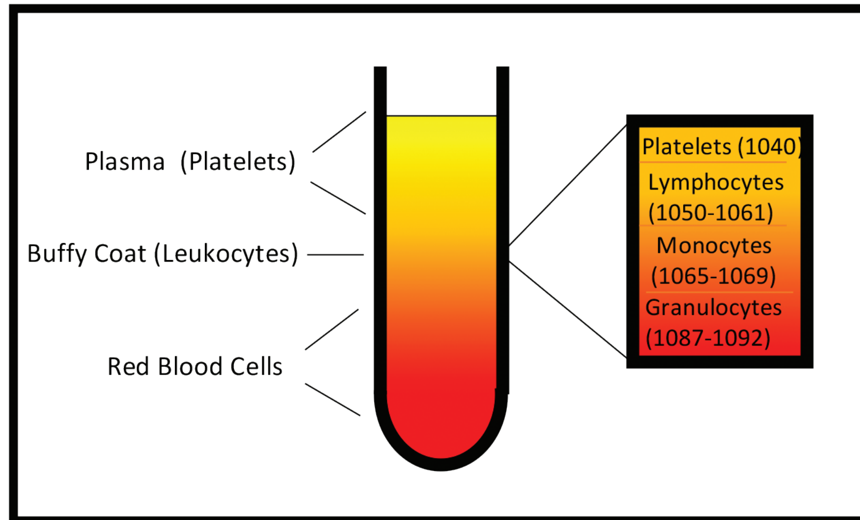


Figure 1: The result of separation of blood components by centrifugation. Plasma and platelets rest on top of the Buffy Coat, which overlays red blood cells. The desired density layer can be removed, while the rest of the blood is returned to the patient.

Therapeutic Apheresis

Apheresis as therapy was first used in 1952 to treat hyperviscosity due to multiple myeloma; in the 1960s it was used to treat chronic myelocytic leukemia (CML), since removal of the Buffy Coat layer reduced the total number of cancerous leukocytes in circulation (McLeod, 2010). Since its development, apheresis has gained popularity as a therapeutic treatment for a number of diseases that benefit from removal of one of the blood components (Korsak, 2015). Beginning in 2007 and updated every few years the American Society for Apheresis (ASFA) has published guidelines meant to help practitioners determine whether apheresis can act as an acceptable first line therapy for a variety of diseases (Szczepiorkowsk, 2010). The ASFA guidelines assign categories to each disease for which therapeutic apheresis has been attempted.

- Category I: Standard, first line therapy, based on randomized trials or a large number of published results
- Category II: Acceptable as supportive therapy; randomized trials occasionally show benefits.
- Category III: Suggestion of benefit, but evidence is insufficient.
- Category IV: No benefit seen in a randomized controlled trial, or anecdotal evidence reports adverse effects.

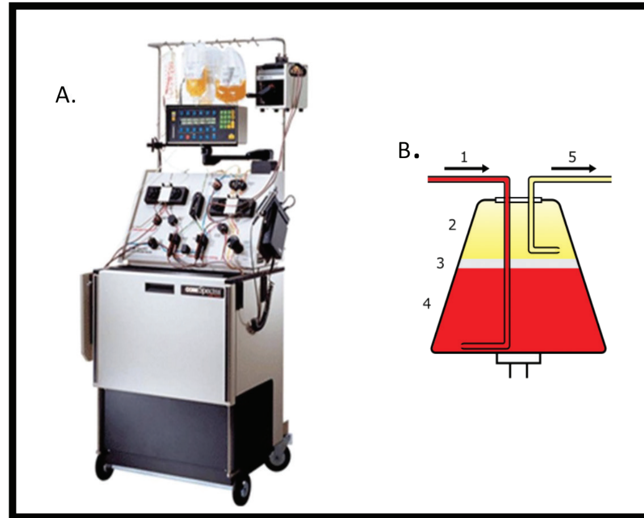


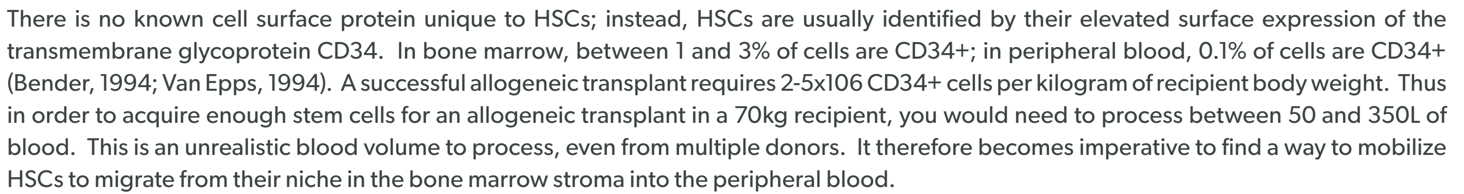
Figure 2: Apheresis machine and centrifuge. A. Cobe Spectra continuous flow apheresis machine (Terumo BCT). B. Cross section of the centrifuge in a continuous flow apheresis apparatus **(https://en.wikipedia.org/wiki/Apheresis#/media/File:Apheresis.svg).** Donor blood flows in at 1, and separates in the centrifuge into plasma (2), leukocytes (3) and erythrocytes (4). The desired blood components are then drawn out of the centrifuge (5) via a second line and either stored or returned to the donor.

Plasmapheresis refers to the removal of plasma and its replacement with saline solution, and is used to treat a variety of diseases caused by factors circulating in the plasma. Autoantibodies, circulating immune complexes, soluble inflammatory factors, lipids such as LDL, and paraproteins can all be removed from the patient via plasmapheresis (Kaplan, 2013). It is considered a Category I first line therapy in a number of renal diseases, including Goodpasture's Syndrome and thrombotic thrombocytopenic purpura (Korsack, 2016). Plasma exchange (PLEX) refers to the replacement of the patient's plasma with either albumin or purified donor plasma.

Cytophoresis refers to the removal of cells from the blood; this can take the form of **leukopheresis** (removal of excess white blood cells) in patients with certain forms of leukemia or other forms of hyperleukocytosis. Leukopheresis is also used to harvest peripheral blood stem cells (PBSCs) for therapeutic purposes. **Erythrocytapheresis** refers to the removal of red blood cells, and can be used as a Category I treatment for sickle cell anemia. Sickled cells may be sorted and replaced with normal RBCs from a donor (Swerdlow, 2006). Finally **thrombocytapheresis** is the removal of platelets, and is used to treat thrombocytosis (Adami 1993).

Peripheral Blood Stem Cell Collection

Hematopoietic stem cell (HSC) transplantation is an increasingly common therapy used to treat a number of cancers, including leukemia, lymphoma, multiple myeloma, and solid tumors such as neuroblastomas and Ewing's Sarcoma (Ali, 2015). It can also cure other types of diseases, such as sickle cell anemia, -thalassemia, Fanconi's Anemia and various immunodeficiency syndromes. A great deal of research has focused on the efficient collection of hematopoietic stem cells for use in transplantation. HSCs can be found in three locations: cord blood, bone marrow, and the peripheral blood. Of these three options, cord blood is easily obtained but has a low number of HSCs and its source does not allow multiple collections from the same donor. Collection of HSCs from the bone marrow is a surgical procedure requiring general anesthesia, but gives a large number of cells. Collection of PBSCs is relatively easy and repeatable, since it is not a surgical procedure, but peripheral blood contains few HSCs.



There is no known cell surface protein unique to HSCs; instead, HSCs are usually identified by their elevated surface expression of the transmembrane glycoprotein CD34. In bone marrow, between 1 and 3% of cells are CD34+; in peripheral blood, 0.1% of cells are CD34+ (Bender, 1994; Van Epps, 1994). A successful allogeneic transplant requires 2-5x106 CD34+ cells per kilogram of recipient body weight. Thus in order to acquire enough stem cells for an allogeneic transplant in a 70kg recipient, you would need to process between 50 and 350L of blood. This is an unrealistic blood volume to process, even from multiple donors. It therefore becomes imperative to find a way to mobilize HSCs to migrate from their niche in the bone marrow stroma into the peripheral blood.

Thus far, three different approaches have been used with success to mobilize stem cells. First, hematopoietic growth factors granulocyte colony stimulating factor (G-CSF) and granulocyte/macrophage colony stimulating factor (GM-CSF) have been used with success to increase the number of HSCs in the peripheral blood. Second, researchers have noted an in increase in PBSCs during the hematopoietic recovery phase after chemotherapy, making this a viable option for autologous stem cell transplants (Richman, 1976). Third, mobilization has been prompted using Plerixafor, a small molecule antagonist that reversibly inhibits the interaction between CXCR4 and stromal-derived factor 1 (SDF-1). Blocking the binding of SDF-1 allows HSCs to exit the bone marrow. The first two approaches offer higher collection numbers, since HSCs multiply faster after treatment and also migrate to the peripheral blood at a greater rate. Concurrent use of Plerixafor and G-CSF has shown increased mobilization of HSCs in patients with multiple myeloma and lymphoma compared to G-CSF alone (Flomenberg, 2005; Callandra, 2008).

Leukopak vs Buffy Coat: Use of Primary Human Leukocytes in Research

Apheresis allows the collection and concentration of leukocytes for use in a variety of research endeavors. Commercially available leukopaks contain enriched numbers of peripheral blood mononuclear cells (PBMCs) from healthy donors. Multiple blood volumes are processed from the same donor to generate a full leukopak, and the percentage of lymphocytes is significantly concentrated compared to whole blood. Leukopaks have several advantages over isolation of the Buffy Coat from whole blood – not only does apheresis give higher yields of mononuclear cells, it is also possible to minimize variability by performing more experiments from a single leukopak and returning to the same donor multiple times.

Summary

Apheresis is critically important in both therapy and research. As a therapy, the ability to remove a specific cell type or circulating protein (and replenish the same using donations from healthy individuals) has opened up new options in treating diseases ranging from Sickle Cell Anemia to various cancers. Apheresis combined with growth hormone- or small molecule-induced mobilization of PBSCs has provided the medical community with a non-invasive method of procuring HSCs needed for stem cell therapies. Enriching for a particular protein or cell type allows researchers to access large numbers of cells from a particular donor, thus reducing experimental costs and variability.

Appendix 1: A basic protocol for Apheresis.

Collection Procedure

- Mononuclear cells will be collected using a FDA-approved apheresis cell separator system, using continuous-flow centrifugation for separation of blood into different components. The apheresis cell separator system will be anticoagulated with a citrate-based anticoagulant, ACD-A.
- Donors will be made comfortable in an adjustable donor chair.
- Baseline vital signs (temperature, pulse, respiration, blood pressure) will be
- measured prior to the start of the procedure.
- The skin at the IV target sites will be cleansed with a 2% chlorhexadine/70%
- alcohol combination.
- Lidocaine 1% 0.2ml will be offered for intradermal injection at the target venipuncture site for anesthetic effect.
- An arteriovenous fistula needle will be inserted into a vein in the antecubital region of one of the donor's arms (venous access). An IV catheter will be introduced into a vein in the donor's opposite arm (venous return).
- Nurses will monitor the donor and apheresis cell separator continuously during the procedure.
- Approximately 3 blood volumes will be processed over approximately 196 minutes in a full-size leukapheresis donation. Approximately 1.5 blood volumes will be processed over approximately 100 minutes in a half-pak leukapheresis donation.
- The collection of cells will be performed at a collect rate range of 0.8 – 1.2 mL/min.
- Donor plasma will be collected concurrently for a total approximate product volume of 300ml in a full-size leukapheresis donation.
- Donor plasma will be collected concurrently for a total approximate product volume of 150 ml in a half-size leukapheresis donation.
- In addition to a peripheral whole blood sample drawn for CBC testing, up to 120 mL of peripheral whole blood may be drawn prior to and/or immediately after the collection procedure per researchers request.
- Donor will be monitored for signs of citrate toxicity by apheresis nurse.
- Signs and symptoms of hypocalcemia are caused by anti-coagulants such as citrate which block ionized calcium in the plasma during apheresis procedures. Mild reactions include oral and perioral paresthesias, paresthesias of fingers and toe, chills and muscle twitching. Moderate reactions include chest heaviness, or chest pain, widespread muscle cramping, nausea and vomiting. Severe citrate toxicity is characterized by frank tetany and life threatening arrhythmias.
- Donors who exhibit mild reactions to citrate will receive Calcium Gluconate, 10%, 100mg slow IV push over 5 minutes. Same dose may be repeated as needed every 20 minutes. Maximum total dose of Calcium Gluconate not to exceed 5 grams.
- Donors who experience moderate to severe signs of citrate toxicity will have WBC collections terminated immediately and a physician will be notified.
- Moderate to severe citrate symptoms will be treated witCalcium Gluconate, 10%, 100mg slow IV push over 5 minutes. Maximum total dose of Calcium Gluconate not to exceed 5
- Grams. Normal Saline fluid bolus 250-500 mL IV over 5-10 minutes may be given to support systolic blood pressure above 100 mm Hg. Normal Saline fluid bolus may be repeated x 1. Activation of 911 if unable to stabilize donor
- Upon completion of the collection, donor assessed to confirmed stable, needles removed, and donors given instructions for post-procedure care.

Potential Risks

The following risks from peripheral blood mononuclear cells collection are rare and/or transient:

- Citrate toxicity: muscle cramping, numbness, chills, tingling sensations, nausea or vomiting. Citrate toxicities are managed symptomatically with calcium gluconate 10% 10ml slow IV push every 20 minutes as needed.
- Minor bleeding and bruising at skin puncture site
- Vasovagal episode
- Syncope
- Allergic reaction
- Infection at venipuncture sites
- Loss of blood volume from machine malfunction
- Air embolus from machine malfunction

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