

Blood Product Modifications: Leukofiltration, Irradiation and Washing

1. Leukocyte Reduction

- Definitions and Standards:
 - Process also known as leukoreduction, or leukofiltration
 - Applicable AABB Standards, 25th ed.
 - Leukocyte-reduced RBCs
 - At least 85% of original RBCs
 - $< 5 \times 10^6$ WBCs in 95% of units tested
 - Leukocyte-reduced Platelet Concentrates:
 - At least 5.5×10^{10} platelets in 75% of units tested
 - $< 8.3 \times 10^5$ WBCs in 95% of units tested
 - $\text{pH} \geq 6.2$ in at least 90% of units tested
 - Leukocyte-reduced Apheresis Platelets:
 - At least 3.0×10^{11} platelets in 90% of units tested
 - $< 5.0 \times 10^6$ WBCs 95% of units tested
 - $\text{pH} \geq 6.2$ in at least 90% of units tested
- Methods
 - Filter: “**Fourth-generation**” filters remove 99.99% WBCs
 - Apheresis methods: most apheresis machines have built-in leukoreduction mechanisms
 - Less efficient methods of reducing WBC content
 - Washing, deglycerolizing after thawing a frozen unit, centrifugation
 - These methods do not meet requirement of $< 5.0 \times 10^6$ WBCs per unit of RBCs/apheresis platelets.
- Types of leukofiltration/leukoreduction
 - “Pre-storage”
 - Done within 24 hours of collection
 - May use inline filters at time of collection (apheresis) or post collection
 - “Pre-transfusion” leukoreduction/bedside leukoreduction
 - Done prior to transfusion
 - “Bedside” leukoreduction uses gravity-based filters at time of transfusion. Least desirable given variability in practice and absence of proficiency
 - Alternatively performed by transfusion service prior to issuing
- Benefits of leukoreduction
 - Prevention of alloimmunization to donor HLA antigens
 - Anti-HLA can mediate graft rejection and immune mediated destruction of platelets
 - Leukoreduced products are indicated for transplant recipients or patients who are likely platelet transfusion dependent
 - Prevention of febrile non-hemolytic transfusion reactions (FNHTR)
 - FNHTR mediated by WBCs or cytokines in donor unit

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- Indicated for patients who had FNHTRs
- Prevention of CMV transmission
 - CMV virus reside within WBC; leukoreduction reduces risks TT-CMV
 - Considered equivalent to products collected from CMV seronegative donors (“CMV-safe”)
- Prevention of immunosuppressive effects of transfusion (controversial)

2. Irradiation

- Goal of Irradiation
 - Prevention of TA-GVHD (Transfusion Associated Graft vs. Host Disease).
 - Causes DNA crosslinks, thereby preventing lymphocyte replication without significantly damaging red blood cell, platelet or granulocyte function
- What is TA-GVHD?
 - Transfused viable lymphocytes (CD4+ and CD8+) would attempt to mount an immune response against HLA incompatible host tissue
 - Normally, host lymphocytes counterattack and neutralize the response
 - Lack of host neutralization due to defective cellular immunity, or failure to recognize donor HLA molecule as foreign, may lead to transfusion-associated graft versus host disease (TA-GVHD) mediated by transfused lymphocytes
 - TA-GVHD:
 - Almost uniformly fatal without successful bone marrow transplant
 - Hallmark: bone marrow attacked leading to marrow fibrosis and aplasia /pancytopenia
 - Organ systems affected: Skin (rash), GI (diffuse mucositis/ diarrhea), liver (hepatitis, veno-occlusive disease)
- Who Is at Risk and Who Needs Irradiated Cellular Products?
 - Immune suppressed patients with deficient cellular immunity:
 - Congenital T-cell deficiencies syndromes (DiGeorge’s, SCID, Wiskott-Aldrich)
 - Stem cell/bone marrow transplantation
 - Note: Because many patients with diagnosis of hematological malignancies go on to receive stem cell transplants, they are also given irradiated products.
 - Intrauterine or exchange transfusions for premature neonate transfusions
 - Treatment with purine analogues, like fludarabine
 - Premature infants (weighing <1200 grams)
 - Irradiated products are NOT indicated for HIV/AIDS patients
 - HIV infection renders both host and donor lymphocytic response ineffective
 - Other less clear cut indications for irradiation:
 - Patient who are solid organ transplant recipients.
 - Although there are some reported cases of TA-GVHD in solid organ patients, the definitive source of the reactive T-cells is

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believed to be from lymphocytes in the transplanted organ and NOT from subsequent transfused blood components. Thus, irradiated blood components are only necessary for solid organ transplant recipients if they have some other indication such as concurrent bone marrow transplant or the use of purine analogues, like fludarabine, both which have been associated with TA-GVHD.

- Patients who are on “very immune suppressive” chemotherapy or irradiation
- Host not recognizing donor lymphocytes as “foreign”, but donor lymphocytes may recognize host as foreign
 - HLA-heterozygous recipient getting blood from a HLA-homozygous donor, and the homozygous HLA allele is shared
 - Receiving blood from a first-degree relative donor (**All directed donor units are irradiated.**)
 - Receiving “HLA-matched” units
 - Receiving a random unit, but this can still occur if the donor and the patient is in a HLA-homogeneous population
 - Interesting fact: In Japan, all products are irradiated.
- Methods
 - Dose of irradiation(AABB Standards):
 - Dose to the center of the irradiation field must be at least 2500 cGy (25 Gy).
 - Minimum delivered dose delivered to any other portion must be 1500 cGy.
 - No more than 5000 cGy should be delivered to the product.
 - Accomplished using cesium-137 or cobal-60 in self contained blood irradiators.
 - X-ray irradiator also available
 - Special labels (radiochromic film labels which change color upon being irradiated) are affixed to units to confirm irradiation of an adequate dosage
 - Process takes 5minutes or so
- Shelf Life of Irradiated Products
 - RBC products: **Shortened to 28 days after irradiation** (due to increase in K+ and free hemoglobin in supernatant of red cell units after irradiation) or until original expiration date, whichever comes first
 - Platelets: No change
 - Granulocytes: No change

3. Washing

- Goal: To remove plasma/supernatant in RBC/platelet products

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- Method
 - Uses semi-automated instrument, and 1 – 2 liters of saline to remove about 99% of plasma
 - Process takes approximately one hour at least, requires the full time attention of a technologist

 - Shelf Life of Washed Products due to “open system”
 - Red cells: 24 hours post-wash
 - Platelets: 4 hours post-wash

 - Drawbacks of Washing Products
 - Labor intensive, time consuming, causes potential delays to transfusion
 - Shortened shelf-life:
 - Washed RBC unit expires in 24hrs, platelets in 4 hrs, which means it might be logistically challenging to arrange transfusions within this time frame (especially if outpatient)
 - Wastage if unit is unused, or only a small aliquot is used
 - Quality of product
 - Cellular loss of both RBCs and platelets
 - RBCs more fragile and more susceptible to hemolysis
 - Platelet functions adversely affected
- Consider requests for washing carefully!**

- Indications for Washing
 - IgA Deficiency (classic example)
 - Some IgA deficient patients develop IgE anti-IgA; exposure to normal plasma (which contains IgA) leads to anaphylactic transfusion reaction
 - Washing requires higher volume of saline (3L+) to remove as much plasma as possible.
 - Alternative is to use products from severely (no detectable levels of IgA) IgA deficient donors
 - Neonatal Alloimmune Thrombocytopenic Purpura (NAIT)
 - Severe congenital thrombocytopenia usually due to maternal anti-PLA1 (HPA-1A), directed against high incidence platelet antigen expressed on fetal platelets
 - HPA-1A antigen negative platelets are very rare
 - Maternal platelets lack the offending antigen. If washed, also will also lack anti-HPA-1A and can then be transfused to the baby
 - Other Possible Indications for Washing and Potential Alternatives
 - Severe allergic reactions to transfusion
 - Some donors repeated exhibit severe allergic reactions to plasma containing products

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- Even though allergen may never be identified, a trial with washed products is reasonable
- RBC unit for patients “sensitive” to hyperkalemia
 - RBC unit accumulates K⁺ during storage:
 - Patients who may develop hyperkalemia are those who: receive large volumes of products, received older RBCs, those with pre-existing renal/liver disease, and neonates
 - Most adult patients tolerate single units of RBCs without problem. K⁺ content in each unit (even older units) is not excessive
 - Alternatives to washing:
 - Offer fresh units (e.g: < 10 days old)
 - Volume reduction: unit is spun down, 2/3 plasma removed and replaced with saline. Process less laborious and time consuming
- Paroxysmal Nocturnal Hemoglobinuria
 - Caused by loss of complement decaying factors on RBCs (loss of CD55 and CD 59), therefore increased complement mediated hemolysis
 - Thought is that transfusing plasma may add fuel to the fire, as more complement proteins are given to the patient
 - Fear may be unwarranted, because patient’s own complement proteins are much more significant in amount
 - If patient has tolerated platelet or plasma transfusions well, then washing RBCs is clearly unnecessary