

Purely Platelets

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- I. Topics for discussion:
 - A. Platelet physiology and metabolism
 - B. Collection and storage
 - C. Indications and contraindications
 - D. Complications
 - E. Future directions
- II. Physiology and Metabolism
 - A. Origin
 1. Derived from megakaryocytes
 - a) Megakaryocytes are multinucleate cells derived from hematopoietic stem cells
 - b) Megas respond to growth factor (thrombopoietin; see below) by increasing in size and forming cytoplasmic projections called “proplatelets”
 - c) Proplatelets “bud” off to become platelets
 - d) Steady state production: 30,000-60,000/ μ l/day
 - e) New research: Platelets may sometimes “bud off” new platelets AFTER leaving the marrow, especially in thrombocytopenic states; implication unclear
 2. Thrombopoietin (TPO)
 - a) Hepatic-derived growth factor
 - b) Body responds to decreased platelet counts by increasing circulating TPO, which stimulates megakaryocytes as above
 - c) Synthetic forms available (see later)
 - B. Structure
 1. Size: 1-4 μ m
 2. Shape:
 - a) At rest, discoid
 - b) When activated, take on one of two shapes
 - (1) Spider-like, with central “body” and filopod-like projections
 - (2) Flattened with central elevation (“fried-egg” shape)
 3. Surface:
 - a) Glycoprotein-associated antigens forming platelet-specific antigens
 - (1) Human platelet antigens (“HPA” antigens)
 - (2) These antigens are present in association with glycoproteins on platelet surface such as glycoprotein Ia, Ib, IIb, IIIa, and CD109
 - (a) Those structural glycoproteins have functional capabilities and often work as dimers
 - i) GP Ib/IX: von Willebrand’s factor receptor
 - ii) GP IIb/IIIa: Fibrinogen receptor
 - (3) HPAs are numerous (more than 20), and antibodies against them may be associated with specific disease states
 - (a) Refractoriness to platelet transfusion
 - (b) Post-transfusion purpura
 - (c) Neonatal alloimmune thrombocytopenia (NAIT)

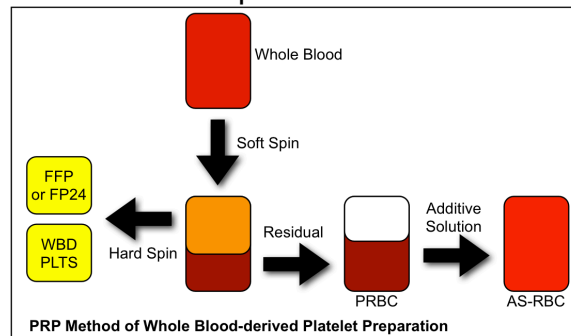
- b) Glycoproteins forming red cell blood group antigens
 - (1) ABH antigens present in abundance
 - (a) A antigens in a group A person generally far outweigh B antigens in a group B person
 - (b) A₂ individuals usually have NO A antigen on platelets
 - (c) Occasional A or B persons have an extraordinarily high level of A or B antigen; these patients are called “high expressers”
 - i) These platelets may be cleared from circulation faster by group O recipients
 - (2) Lewis, I, i, and P antigens (ABO-related) are also present on platelets, but are thought to be clinically insignificant
 - (3) Rh antigens are completely absent
 - (4) Duffy, Kell, Kidd, Lutheran antigens are also absent
 - c) HLA antigens
 - (1) Class I antigens (HLA-A, HLA-B, HLA-C)
 - (a) Despite HLA-C being present in similar quantities to -A and -B, HLA-C is thought to have little clinical significance in most cases
 - (2) Quantitatively more HLA in whole blood from platelets than WBCs
4. Cytoplasm
- a) Dense body contents:
 - (1) ADP and serotonin (for activating platelets)
 - (2) Calcium (coagulation system substrate)
 - b) Alpha granule contents:
 - (1) vWF, fibrinogen (for binding and aggregating)
 - (2) Various coagulation factors (especially V, but also XI, XII)
 - (3) Inhibitors of coagulation (Protein S, platelet factor 4, B-thromboglobulins)
 - (4) Platelet derived growth factor (for wound healing)
 - c) Alpha granules are more numerous than dense bodies
- C. Lifespan
- 1. Approximately 10 days
 - 2. Majority of platelets are free in the circulation for this time frame (approximately 1/3 sequestered in normal liver/spleen)
 - 3. Decreasing platelet counts lead to decreased lifespan
 - a) Platelet circulating life begins to decrease sharply when count drops below 50,000/ μ l; more on this later
- D. Function and interactions
- 1. Baseline hemostatic function
 - a) Maintains vascular integrity by assisting in “plugging holes” in endothelial spaces
 - b) Requires approximately 7100 platelets/ μ l/day to complete this task
 - 2. Role in challenges to hemostasis
 - a) Formation of “platelet plug” at a wound surface is the first step on the road to coagulation
 - b) Adhesion:
 - (1) Normal conditions: Platelets do not stick to endothelium

- (2) With damage to endothelial surface, von Willebrand factor (vWF) binds to subendothelial collagen, then binds to platelets at GPIb/IX receptor
- (3) Platelets are “activated” by this interaction, which stimulates release of additional vWF and fibrinogen from alpha granules and serotonin and ADP from dense bodies
- c) Aggregation
 - (1) Platelets change shape from discoid to spider-like, with prominent pseudopod formation for interaction with other platelets and the raw surface
 - (2) Increased expression of GP IIb/IIIa on platelet surface, with resultant fibrinogen binding and bridging between platelets
 - (3) Coagulation cascade activation and resultant fibrin clot formation
 - (4) In addition, thromboxane A₂ secretion from activated platelets recruits other platelets and induces vasoconstriction
3. Platelets also act in thrombosis (in contrast to hemostasis), but the pathway is different and will not be discussed
4. Consequences of deficiency
 - a) Thrombocytopenic bleeding is typically mucocutaneous (petechiae, epistaxis, etc)
 - b) Decreased numbers of platelets (or decreased function) also may lead to increased surgical bleeding
 - c) Worst-case scenario for platelet-related bleeding: Intracranial hemorrhage
- E. Metabolism
 1. Despite not having a nucleus, platelets are quite metabolically active
 2. Energy required for shape change, release of contents of granules and dense bodies
 3. Oxygen metabolism (Kreb’s cycle, oxidative phosphorylation) is the best source of ATP
 4. Decreased oxygen supply leads to increased anaerobic metabolism (glycolysis), decreased pH, and subsequent platelet shape changes
 - a) Dependence on glycolysis is what we are trying to avoid with modern storage solutions and containers
- III. Collection, Processing, Modifications, and Storage
 - A. Whole blood-derived (WBD) platelets (“platelets”)
 1. Platelets derived from a single whole blood donation
 - a) A single donation provides only about enough platelets for 1/4 to 1/6 of a “dose” to a patient
 - b) As a result, virtually all WBD platelets are *pooled* prior to transfusion
 2. Commonly known as “random donor” platelets
 - a) Misnomer: This implies that apheresis platelets (see later) are NOT random, when in most cases, they are not specifically produced for a specific patient
 3. Formerly most common used platelet product in the U.S., but in the minority now

4. Methods of preparation

a) Platelet-rich plasma (PRP) method

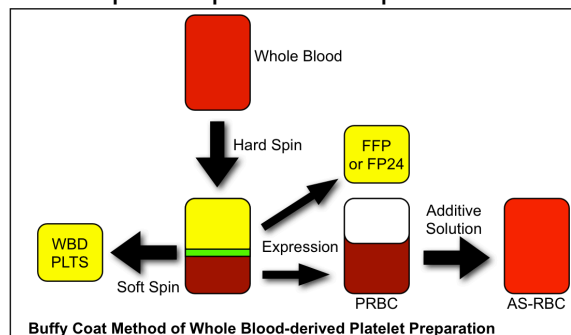
- (1) U.S. method; see figure below
- (2) “Soft” spin: Separates blood into platelet-rich plasma (top) and “packed” red blood cells (bottom); PRP is expressed off and undergoes...
- (3) “Hard” spin: Platelets are “pelleted” at the bottom of the satellite bag and most of the plasma is removed



- (4) Please note: Most U.S. blood centers in 2010 do NOT do this, choosing to rely on apheresis to supply their platelets (see later)

b) Buffy coat (BC) method

- (1) Used in Europe and Canada; see figure below
- (2) “Hard” spin: Separates the blood into a plasma layer (top), “buffy coat” layer (middle), and red cell layer (bottom); plasma is expressed off the top and red cells off the bottom
- (3) “Soft” spin: Separates the platelets and residual WBCs and RBCs



c) Comparison of methods of WBD platelet preparation

- (1) BC method appears to “activate” platelets somewhat less, likely because the “hard spin” is cushioned by red cells on the bottom of the bag (while the hard spin pellets the platelets against the bottom of the bag in the PRP method)
- (2) BC platelet preparation leaves behind MORE PLASMA for component preparation or sale
- (3) BC platelets are thought to contain fewer WBCs than PRP (though leukoreduction would make this irrelevant)
- (4) Relative platelet counts similar
- (5) Clinical response appears similar

5. Storage
 - a) WBD platelets (non-pooled): Up to 5 days at 20-24 C, with continuous gentle agitation
 - (1) Agitation has been shown to decrease activation of platelets and lead to greater post-transfusion viability
 - (2) Platelet-specific storage “rotators” either rotate side-to-side or in tumbling motion
 - b) WBD platelets (pooled, open system): 4 hours after pooling
 - c) WBD platelets (pooled, FDA-approved closed system such as Pall Acrodose™): Up to 5 days at 20-24 C, with continuous gentle agitation (see below)
 - d) Anticoagulant/preservative solution for WBD: Generally CPD (most common) or CPDA-1; whatever was used to collect whole blood
6. Quality testing
 - a) AABB Standard 5.7.5.17 and 5.7.5.18
 - (1) WBD platelets must contain at least **5.5 x 10¹⁰** platelets in **90%** of units tested
 - (a) Not generally a problem, as these units average 8 x 10¹⁰ PLTs per bag
 - (2) **90%** must also have a pH of **6.2** or greater at the end of shelf life
 - (3) If leukoreduced, the proportion of platelets needing the above platelet count drops to **75%** (the pH requirement does not change) AND the residual WBC count must be less than **8.3 x 10⁵** in **95%** of units tested
 - b) FDA requirements (21 CFR 640.24 c and d)
 - (1) Similar for platelet count, though the 5.5 x 10¹⁰ PLT requirement is only for **75%** of units tested
 - (2) FDA does not specify a percentage threshold for pH ≥6.2
7. Pooled WBD platelets
 - a) This method, commonly used in Europe, is relatively new in the U.S.
 - (1) Introduced by Pall in the US in 2005
 - b) Up to six units of ABO-identical WBD platelets are pooled in sterile conditions
 - c) Product approved by FDA for storage up to 5 days at 20-24 C
 - d) After pooling and 24 hour wait, product can be tested for bacterial contamination like apheresis units
 - e) Potential lower-cost alternative to apheresis platelets
- B. Apheresis-derived (AD) platelets (“apheresis platelets”, “platelets, pheresis”)
 1. Platelets derived from a single apheresis procedure
 - a) Apheresis is an automated process where blood is taken from the body and separated into components in a closed system
 - b) The desired product (or products) is harvested; the rest is given back to the donor
 - c) Far more efficient platelet collection method than whole blood donation
 - (1) One procedure commonly gives enough platelets for two recipients, occasionally enough for three

2. Commonly known as “single-donor” platelets
3. Major manufacturers and their platelet collection equipment
 - a) CaridianBCT (formerly Cobe/Gambro)
 - (1) Trima and Trima Accel
 - (2) Spectra
 - b) Fenwal
 - (1) Amicus
 - c) Haemonetics
 - (1) MCS+ 9000
4. Donor requirements
 - a) Must meet same history and physical requirements as whole blood donors
 - b) No anti-platelet medications for donor
 - (1) ASA, other anti-inflammatories, clopidogrel, ticlopidine
 - c) May donate as often as every 48 hours but not more than twice per week, and only up to 24 times in a 12-month period
 - (1) This requirement may be waived if the donor meets a specific need for a patient and the medical director approves
 - (2) Centers required to track annual red cell loss and ensure that the combination of loss from apheresis and whole blood donations does not exceed the amount a donor would lose by donating whole blood alone
5. Comparison to WBD platelets
 - a) No significant differences in clinical/hemostatic effect
 - b) No significant differences in post-transfusion platelet count
 - c) Less preparation time (no pooling required) in transfusion service for AD platelets (less true now that WBD pooling may be done in blood centers)
 - d) AD platelets are generally far more expensive than WBD platelets (about \$500 per dose, exclusive of other charges if necessary for modifications below)
 - e) So, why use AD platelets?
 - (1) Traditional belief:
 - (a) Decreased foreign donor exposure leads to decreased formation of anti-HLA antibodies
 - (b) Debunked thoroughly by large study in 1990's (*Trial to Reduce Alloimmunization to Platelets*; TRAP)
 - i) WBD vs AD platelets; no difference in HLA immunization if products are leukocyte reduced
 - (2) Currently valid reasons for use of AD platelets:
 - (a) Fewer donor exposures is desirable for infectious disease purposes
 - i) i.e., less risk of infection with fewer donor exposures
 - ii) Hard to argue with that, but current exceptionally good infectious disease screening makes this less of an issue
 - iii) Future possibility of pathogen reduction may lessen this issue even further (see later)

- (b) Lower risk of bacterial contamination in AD platelets
 - i) True when comparing AD platelets to WBD platelets that are pooled immediately prior to transfusion; probably not true compared to pre-pooled WBD platelets
 - (c) Specific patient circumstances requiring platelets from a specific donor or donors
 - i) HLA-matched or crossmatched platelets
 - ii) Antigen-negative platelets for HLA antibodies and platelet antibodies (Neonatal Alloimmune Thrombocytopenia)
 - 6. Current U.S. status
 - a) Roughly 80% of all platelets transfused in the U.S. are apheresis-derived
 - 7. Storage
 - a) Like WBD platelets, up to 5 days at 20-24 C, with continuous gentle agitation
 - b) Anticoagulant/preservative solution: ACD-A (acid citrate dextrose)
 - 8. Quality testing
 - a) AABB Standard 5.7.5.20 and 5.7.5.21
 - (1) AD platelets must contain at least **3.0 x 10¹¹** platelets in **90%** of units tested
 - (a) Not generally a problem, as these units average 4 x 10¹¹ PLTs per bag
 - (2) **90%** must also have a pH of **6.2** or greater at the end of shelf life
 - (3) If leukoreduced (in contrast to WBD PLTs), the proportion of platelets needing the above platelet count and pH does not change AND the residual WBC count must be less than **5.0 x 10⁶** in **95%** of units tested
 - b) FDA requirements
 - (1) As with WBD platelets, the count requirement is only for 75% of units tested
 - (2) No pH threshold percentage specified
- C. ABO and Rh Issues
 - 1. ABO
 - a) ABO-mismatched platelets are given commonly, despite studies that show increased complications and refractoriness historically with mismatched transfusions
 - (1) Major mismatch (example: O recipient, A donor)
 - (a) Decreased response due to faster clearing (except with A2 donors)
 - (2) Minor mismatch (example: A recipient, O donor)
 - (a) Not a problem except in donors with high-titer antibodies
 - i) More common in group A donors, seen also in O and B
 - ii) "High-titer" not clearly defined (some use 1:64, some 1:128)
 - iii) Caution with low-volume recipients
 - (3) Both major and minor mismatch (example: A recipient, B donor)
 - (a) Same considerations as above

- b) While the above considerations are commonly accepted, the presence of soluble A or B antigens in either donor or recipient plasma can lead to immune complex formation, which may have harmful effects
 - c) In general, ABO-matched is best, but mismatched can be used when necessary
2. Rh
- a) No D antigens on platelets; potential for Rh issues comes from contaminating RBCs in unit
 - (1) In general, WBD-PLTS have more RBCs than AD-PLTS
 - (2) If given to childbearing potential female, RhIG should be given
 - (a) May use IV forms
 - (3) Some use formulas (1 vial per 7 AD-PLTS or 30 WBD-PLTS), but in general, protection still in place if demonstrable anti-D present
- D. Potential modifications to AD and WBD platelets
1. Pooling
 - a) See above for discussion
 - b) Formerly required for WBD platelets; now may be pooled in blood center
 2. Leukocyte reduction
 - a) Removing donor white blood cells reduces:
 - (1) Recipient immunization to HLA antigens (HLA alloimmunization)
 - (2) Transmission of cytomegalovirus
 - (3) Febrile nonhemolytic transfusion reactions
 - b) Leukoreduction *may* also reduce:
 - (1) Incidence of prion disease transmission (e.g., variant Creutzfeldt-Jakob Disease)
 - (2) Recipient immunosuppression after transfusion (**T**ransfusion-**R**elated **I**mmunomodulation, a.k.a. "TRIM")
 - c) AD platelets: Apheresis equipment generally performs leukocyte reduction as part of collection process ("process leukoreduction")
 - d) WBD platelets: Leukocyte reduction done with filters either near time of collection or time of pooling
 3. Irradiation
 - a) Specific doses of irradiation deactivate donor lymphocytes
 - (1) Usually gamma irradiation, dose 2500 cGy ("centigray") in US
 - (2) Deactivation of lymphs prevents **T**ransfusion-**A**ssociated **G**raft **v**s. **H**ost **D**isease (TA-GVHD), a nearly universally fatal complication of transfusion to immunocompromised patients
 - (a) TA-GVHD results when transfused lymphs attack host tissues without an appropriate neutralizing response from the host
 - (3) HLA-matched products and products from blood relatives are also at risk for TA-GVHD, so they should also be irradiated
 - (4) Platelets may be irradiated at any time during storage, and doing so does not change the expiration date
 4. Volume reduction
 - a) Removal of a portion of the plasma associated with platelets may be desirable

- b) Uncommonly necessary, but two scenarios may necessitate:
 - (1) Neonates, both for volume reasons and for removal of unwanted antibodies and/or electrolytes (potassium)
 - (2) Highly volume sensitive adult patients
 - c) Product may be centrifuged and excess plasma removed
 - d) Platelets are lost during this process, sometimes as much as 20%
 - e) Resultant product may be stored in syringes for a short time until transfusion with minimal loss of function or metabolic activity
5. Washing
- a) Most commonly done for patients with risk of anaphylactic response to transfused plasma proteins
 - (1) IgA most common
 - b) Also done to remove unwanted antibodies (ABO incompatibility) or electrolytes in neonatal transfusions
 - c) Results in substantial platelet loss (up to 1/3)
 - d) Shelf life is 4 hours after washing
6. Freezing
- a) Freezing platelets is uncommonly done and not well regulated
 - b) When done, DMSO is used as cryopreservative
 - c) Poor recovery of previously frozen platelets (33% in-vivo)
- E. Storage conditions
1. Bags
- a) Modern platelet storage bags are gas-permeable
 - b) Oxygen transport is essential for oxidative metabolism
2. Three enemies of platelet storage
- a) **Bacterial contamination**
 - (1) Typically thought to be the main risk of platelet transfusion
 - (2) Warm storage temperature is nice environment for gram + bacterial growth
 - b) **Activation**
 - (1) Activated platelets do not circulate/survive as long as unactivated (see discussion above)
 - (2) Agitation leads to less activation
 - c) **Anaerobic metabolism**
 - (1) Increased glycolysis due to insufficient oxygen supply produces lactic acid
 - (a) This may happen with things like too many platelets in a bag, too little plasma in a bag, bag malfunction
 - (2) Resultant declining pH leads to rapid loss of platelet viability
 - (a) Platelets essentially nonviable below pH 6.2 or above pH 8.4
 - (3) Platelet QC: pH of ≥ 6.2 required at end of storage life
3. Temperature and agitation
- a) See above for storage conditions
 - b) Cold storage of platelets
 - (1) Described, and thought to lessen bacterial growth

- (2) Unfortunately, cold storage leads to irreversible shape change from discoid to spherical, with resultant loss of viability

F. Bacterial detection

1. Requirements

- a) Currently, all platelet units must be checked for contamination (AABB Standard 5.1.5.1)
- b) This is easier accomplished with apheresis platelets and pre-storage pooled WBD platelets, though Verax approval has changed things (see “Point of issue testing” below)
 - (1) WBD-platelets have traditionally used surrogate methods (see below), though it is absolutely possible to culture them, too
- c) Interim standard 5.1.5.1.1 (effective January 2011) requires methods to be FDA-approved or have equivalence to FDA-approved methods

2. Culture-based methods

- a) All require a sample to be taken from the bag after 24 hours, since these methods DON'T WORK if less time has passed!
 - (1) Product may be transfused while waiting for results
 - (2) Unfortunately, may still be insensitive (some estimates as low as 15-22% sensitivity!)
- b) Two currently approved methods:
 - (1) BacT-ALERT system
 - (2) Pall enhanced Bacterial Detection System (eBDS)

3. Point of issue testing

- a) Verax Platelet PGD test approved by FDA in late 2009
- b) Designed for use in the transfusion service, and designed to combat the problem noted above with culture-negative but truly contaminated units
- c) PGD = “Pan Genera Detection”, which looks for the presence of antigens present on the surface of both gram-positive and -negative bacteria
- d) Can be done on leukoreduced apheresis platelets or pooled whole blood-derived platelets (either leukoreduced or non-leukoreduced)
- e) Early testing: More sensitive than culture-based tests

4. Surrogate methods

- a) “Swirling”
 - (1) Platelet units containing normal “discoid” platelets show a shimmering swirl appearance when examined under room light
 - (2) Shape change (as happens with activation or low pH) leads to loss of swirling
 - (3) Bacterial contamination (with resultant decline in pH) is just one reason platelets may stop swirling
 - (4) Really VERY subjective and nonsensitive, but VERY widely used
 - (5) Check it out at http://www.youtube.com/watch?v=U4bgD_i5lnE
- b) pH meters (dipsticks)
 - (1) Infection may lead to increased anaerobic metabolism and resultant decline in pH

- (2) Nonsensitive (about as sensitive as swirling for contamination detection)
- c) Glucose
 - (1) Declining glucose levels may correlated with contamination
 - (2) Insensitive
- d) Gram stain
 - (1) Considerably more sensitive than either swirling or dipsticks, but more cumbersome

IV. Indications and Contraindications for Platelet Transfusion

Thrombocytopenia Not Bleeding	Thrombocytopathy Not Bleeding
Thrombocytopenia Bleeding	Thrombocytopathy Bleeding

A. General

1. Thrombocytopenia vs. thrombocytopathy
 - a) Decreased platelet *count* (quantitative problem) is established as a risk for spontaneous bleeding, as well as for bleeding during a procedure
 - (1) The above statement is not disputed by anyone
 - (2) The problem is that the actual at-risk threshold has almost certainly been overestimated by clinicians for years
 - (3) Ongoing studies attempt to better define the threshold
 - b) Decreased platelet *function* (qualitative problem) also puts a patient at theoretical risk for spontaneous hemorrhage or procedure-related bleeding
 - (1) In general, this occurs far less often than thrombocytopenia (some report less than 20% of platelet transfusions)
 - (2) Platelet transfusions are indicated far less often than in thrombocytopenia
 - (3) Problems may be hereditary or acquired
 - (a) Hereditary
 - i) Glanzmann's thrombasthenia
 - ii) Bernard-Soulier Disease
 - (b) Acquired
 - i) Uremia
 - ii) Procedure-related (cardiac bypass)
 - iii) Disease-related (hematologic disorders such as myelodysplasia)
 - iv) Drug-related
 - (1) Very common with modern pharmaceuticals for stroke prevention and other clot prevention
 - (2) Aspirin, platelet-inhibiting drugs like clopidogrel (Plavix) and ticlopidine (Ticlid)

2. Prophylaxis vs. therapeutic
 - a) “My patient might bleed” vs. “My patient is bleeding”
 - b) Majority of platelet use (both appropriate and inappropriate) occurs in prophylactic settings
 - c) Current research efforts largely focused on the prophylactic transfusion and whether or not it is indicated
 - d) Some reports suggest moving to a “therapeutic-only” strategy (i.e., only transfuse platelets when a patient is bleeding)
 3. History of thresholds
 - a) 20,000 platelets/ μ l count has long been considered a “trigger threshold” for platelet transfusion for many clinicians
 - b) Based on very old studies that did show some increase in risk for spontaneous gross hemorrhage at 20K platelet count (Gaydos et al, *NEJM* 1962).
 - (1) This study was observational and was done before aspirin’s effect on platelets was known
 - (2) Patients lacked modern medical care and methods to detect hemorrhage
 - (3) Authors did NOT conclude to use 20K as trigger for transfusion
 - (4) Despite this, the 20K “trigger” was passed down without question for many years
 - c) Current studies (noted below) debunk this “trigger”
- B. Specific acceptable indications
1. Thrombocytopenia
 - a) Prophylactic (non-bleeding patients)
 - (1) Main concern: Spontaneous hemorrhage leading to death, especially spontaneous intracranial hemorrhage
 - (a) Formerly, the most common reason for leukemia patients to die
 - (b) Today, this is really rare (infection is more common cause of death for leukemia patients)
 - (2) Basic facts
 - (a) Platelet counts are not linear when very low, and counts reported from lab instruments may not be completely accurate
 - (b) The basic daily loss of platelets (at steady state) from the circulation may be as much as 40,000/ μ l (7100/ μ L/day for vascular integrity, the rest due to senescence)
 - (c) Platelets disappear from circulation at a faster rate when platelet count is low
 - i) In thrombocytopenic patients, a higher proportion of the platelets are performing routine hemostatic functions, so an individual platelet may only circulate for TWO days
 - ii) This should make it obvious that if a patient is not producing their own platelets and they have a very low count, their only real option is platelet transfusion
 - (3) When considering transfusion, consider complicating factors
 - (a) Fever (especially in association with infection)

- (b) Sepsis
- (c) Major surgery
- (d) Thrombocytopathy
- (4) **INDICATION:**
 - (a) Most current data suggests that a patient is not at significantly increased risk of bleeding until their platelet count is approximately **5000/ μ L**
 - i) However, most use **10,000/ μ L** as threshold for prophylactic transfusion
 - ii) Most commonly seen in oncology patients undergoing chemotherapy and/or stem cell transplant patients
 - (b) For fever or sepsis, most use threshold of either 10,000 or 20,000
 - (c) For major surgery, most use threshold of 50,000
 - (d) For patients at high risk of fatal or highly morbid complications as a result of potential bleeding, many use a higher prophylactic threshold of 100,000/ μ L
 - i) Intracranial surgery patients
 - ii) Ophthalmologic surgery patients
- (5) Controversy surrounds the prophylactic use of platelets in procedures like lumbar puncture, liver biopsy, and endoscopy
 - (a) Common threshold is 50,000, but randomized studies are not abundant
 - (b) Many recommend therapeutic approach instead (transfuse if bleeding)
 - (c) Problem is that pre-procedure count is not predictive of bleeding
- (6) Dose of platelets to use
 - (a) Recent study ("PLADO" study, *NEJM* 2010; see references) showed no significant bleeding difference when thrombocytopenic (below 10K) patients got either low, medium, or high doses of platelets
 - i) "Medium" dose corresponded to "normal" platelet dose
 - ii) Low dose group received fewer total platelets but more actual transfusions
 - iii) High dose group received more total platelets but fewer actual transfusions
 - iv) Conclusion: Giving lower doses more often may help conserve resources
- (7) Additional possible benefit of lower thresholds
 - (a) Thrombopoietin (TPO) levels increase when platelet count is low, to stimulate additional production
 - (b) TPO is absorbed by transfused platelets, which may decrease available TPO levels
 - (c) Allowing decreased platelet counts before transfusion may stimulate increased endogenous production and resultant shorter periods of thrombocytopenia

b) Therapeutic

- (1) In general, a platelet transfusion given for a patient who is “bleeding” and thrombocytopenic
 - (a) Bleeding definition is most commonly by WHO grade in published studies
 - (b) Most define a “bleeding” patient as WHO grade 2 or greater

WHO Grade	Manifestation
0	No bleeding
1	Minor bleeding (petechiae, ecchymoses, blood in secretions, vaginal spotting)
2	Gross hemorrhage not requiring RBC transfusion (epistaxis, hematemesis, hematuria)
3	Gross hemorrhage requiring 1+ RBCs per day
4	Life-threatening hemorrhage (bleeding causing hemodynamic compromise or into vital organ, like head/lungs/heart)

- (2) A bleeding patient uses currently circulating platelets at an accelerated rate, in an attempt to stop the bleeding
- (3) However, this does not mean that giving additional platelets to these patients will automatically stop their bleeding (one study only showed about 20% of patients with decreased WHO grade after platelet transfusion, while over 60% didn't change at all)
 - (a) Ironically, the bleeding most impacted by platelet transfusion in thrombocytopenic patients is grade 1 or 2, which by definition is not that serious anyway!
- (4) **INDICATION:**
 - (a) **50,000/ μ L** is the most common platelet transfusion threshold for bleeding thrombocytopenic patients, despite the lack of definitive data
 - (b) Patients bleeding into heads, eyes, or lungs are often transfused at a threshold of 100,000/ μ L

2. Thrombocytopenia

a) Prophylactic

- (1) A non-bleeding patient with thrombocytopenia, in general, should not get a platelet transfusion
 - (a) This includes congenital as well as acquired thrombocytopenia
- (2) Obvious exceptions:
 - (a) Associated severe thrombocytopenia
 - (b) Documented history of significant bleeding at a particular platelet count
- (3) Possible exception:
 - (a) Emergency cardiac surgery in patient taking an antiplatelet drug such as clopidogrel (Plavix)

- b) Therapeutic
 - (1) In general, bleeding patients with thrombocytopathy are transfused on an as-needed basis, without regard to the platelet count
 - (2) Effectiveness is gauged by cessation of bleeding
 - (3) This is seen most commonly with drug-related thrombocytopathic bleeding in cardiac surgery, uremic thrombocytopathic bleeding, and cardiac bypass-related thrombocytopathic bleeding
 - (a) Drug-related bleeding
 - i) Most common thrombocytopathic bleeding, often in association with cardiac surgery
 - (1) Most common scenario we encounter: Patient on a platelet-inhibiting drug that needs emergent surgery
 - ii) Clopidogrel (Plavix) and aspirin most common
 - iii) Clopidogrel blocks ADP receptor on platelets; effect persists for at least 5 days after drug stopped
 - (1) No drug antagonist therapy available
 - (2) Proven association with increased bleeding during CABG, as well as with increased transfusion needs and need for re-exploration due to bleeding
 - (3) Well-defined and studied recommendations for platelet transfusion (as in, how much?) are lacking
 - (4) Platelet transfusions are indicated for emergency surgical and post-surgical bleeding; judge clinically
 - (5) Intra-operative monitoring such as thromboelastography (TEG) may assist in the decision
 - iv) Aspirin blocks cyclo-oxygenase pathway irreversibly; effect persists for life of that platelet
 - (1) Mixed results in studies; majority show no increased bleeding in cardiac surgery after ASA
 - (2) Treat postoperative bleeding with platelet transfusion only after consideration of other factors
 - (3) Exact amount to transfuse not well-defined; judge clinically
 - (b) Cardiac bypass-related bleeding
 - i) 18% of blood products transfused in the US are associated with cardiac surgery!
 - ii) Multifactorial hemostatic defect, including contributions from hypothermia, dilution, and platelet issues
 - iii) Platelets are decreased both quantitatively and qualitatively
 - (1) Dilution and sequestration account for quantitative issues
 - (2) Hypothermia, activation, adhesion, and aggregation in bypass apparatus help account for qualitative issues
 - iv) If patient is NOT on anti-platelet medication, prophylactic platelet transfusion is not indicated

- v) If advanced intra-operative point of care testing (such as thromboelastograph {TEG} is not available, recommendation is to transfuse with active bleeding and counts less than 50,000/ μ l
 - (c) Uremic bleeding
 - i) Platelet dysfunction with multiple causes
 - ii) Platelet transfusion is NOT primary therapy, but rather for use in emergencies only
 - iii) Primary therapy:
 - (1) Increase hematocrit: Pushes platelets to periphery of vessels and may help clots form
 - (2) DDAVP: Increases von Willebrand factor (vWF) and increases platelet adhesion
 - (3) Conjugated estrogens: May decrease nitric oxide and/or directly increase endothelial function
 - (4) Cryoprecipitate before platelets (for vWF)
- C. Contraindications
1. Thrombotic thrombocytopenic purpura (TTP)
 - a) Defect of ADAMTS13 protein, leads to increased large vWF molecules that induce thrombosis and microangiopathic hemolytic anemia
 - b) Adding platelets can worsen situation by increasing microthrombi
 - c) Platelets only used in life-threatening bleeding
 2. Heparin-associated thrombocytopenia (HAT)
 - a) Similar rationale to TTP
 3. Immune thrombocytopenic purpura (ITP)
 - a) Platelets are ineffective due to same factors that cause ITP
 4. Post-transfusion purpura (PTP)
 - a) Most often antibody-induced, with patient antibody vs. common platelet antigen
 - b) Again, adding more (most likely) antigen-positive platelets may worsen situation
 5. Uremic thrombocytopenia
 - a) See discussion above
 6. Disseminated Intravascular Coagulation (DIC)
 - a) Controversial; some advocate use in forms of DIC where consumption results in thrombocytopenia and/or bleeding
- V. Complications
- A. Platelet refractoriness
1. Defined as a lack of adequate response to platelet transfusion
 - a) Can evaluate response with standard tools such as corrected count increment (CCI) and/or percent platelet recovery (PPR)
 - (1) CCI is not just quantitative; it factors in the increase in count with the amount of platelets given and approximate blood volume
 - (2) Decreased CCI (≤ 7500) at 1 hour usually means immune refractoriness

- (3) Decreased CCI at later than one hour implies consumption of platelets
 - b) Seen historically in as many as 70% of multiply transfused patients with thrombocytopenia (before leukoreduction)
 2. Multiple potential causes
 - a) Immune refractoriness
 - (1) Antibodies to class I HLA antigens
 - (2) Antibodies to platelet specific antigens
 - (3) ABO incompatibility
 - b) Non-immune refractoriness
 - (1) Fever/infection
 - (2) Splenomegaly
 - (3) DIC (consumption)
 - (4) Medications (e.g., amphotericin, vancomycin, heparin)
 - (5) Dilution in massive transfusion
 3. Basic strategy: Rule out non-immune causes (more common) before moving to interventions for immune causes
 - a) This means that you should investigate before attempting to provide HLA-matched or crossmatched platelets
 - b) Consider this simple approach:
 - (1) Evaluate clinically for non-immune causes
 - (2) Check pre- and post-transfusion platelet counts to ensure patient is really not responding (use CCI to be sure)
 - (3) If truly not responding, consider closer ABO matched platelets and/or fresher (48 hours or so from collection if possible) platelets
 - (4) Perform screening test to see if anti-HLA or anti-platelet antibodies are present
 - (5) If antibodies ARE present, only THEN move to specific products (HLA-matched or crossmatched)
 4. HLA-based selection vs. crossmatching
 - a) HLA methods are traditional and include “matching” of antigens (which may not be very good based on the patient’s HLA type) or selection of antigens that are *compatible* with the patient’s antibodies (antibody specificity prediction)
 - b) Crossmatching uses samples of patient serum reacting against samples from platelets already on the shelf, and choosing the most compatible
 - c) Neither method is perfect; crossmatching tends to be faster
 - d) Current/future trend: Using programs like HLAMatchmaker to find compatible donors based on antigen morphology; more sophisticated
- B. Transfusion reactions
1. Hemolytic reactions (HTR)
 - a) Incompatible plasma, mistransfusion (wrong product to wrong patient)
 - b) “Out-of-group” (ABO-mismatched) transfusions commonly given to adults, but children are at MUCH greater risk due to smaller blood volume

- c) All clinical manifestations (including fatality) can occur with platelet transfusion-related HTRs
 - 2. Febrile transfusion reactions
 - a) Interaction with transfused white cells and/or their products (cytokines and other breakdown products) leads to recipient fever
 - b) Important primarily for recipient comfort and ruling out hemolysis
 - c) Pre-storage leukocyte reduction decreases incidence
 - 3. Transfusion-related acute lung injury (TRALI)
 - a) Acute lung injury within 6 hours of transfusion; associated with the most fatalities reported to FDA currently
 - b) Recent US and European shift to near male-only plasma has decreased amount of TRALI reported (males have fewer anti-HLA antibodies)
 - c) Requiring platelet donors to be all-male is MUCH more difficult
 - d) Some centers are testing female donors for anti-HLA antibodies
 - e) Remember: Not all TRALI is antibody-caused! Substances that accumulate in the plasma associated with platelet products (various cytokines, CD40 ligand) also contribute to TRALI
 - 4. Post-transfusion purpura
 - a) Recipient with antibody against platelet specific antigen, most commonly HPA-1a, from prior pregnancy or transfusion
 - b) Severe thrombocytopenia following exposure to antigen-positive platelets via transfusion
 - c) Treated successfully with intravenous immune globulin (IVIg)
- VI. Possible future directions
 - A. Pathogen inactivation/reduction technology (PI/PR)
 - 1. Despite our very good infectious disease screening, small risks remain
 - 2. In addition, new agents emerge as potential problems almost constantly
 - a) Can we develop new screening tests?
 - b) If developed, do we use those tests on all donated blood?
 - c) Can we afford to add more and more tests (with associated costs) to a product that already costs essentially as much to produce as we can sell it for?
 - 3. PI uses a combination of an agent that binds to nucleic acids (such as riboflavin or a substance called a “psoralen”) with subsequent ultraviolet illumination (to form irreversible bridges) to deactivate everything with genetic material
 - a) This deactivation includes viruses, bacteria, and white blood cells
 - b) Red blood cells (no nuclei), platelets (no nuclei), plasma components should not be affected
 - 4. This process is easier with platelets and plasma, but is being studied with whole blood as well
 - 5. Added benefit: May eliminate need for gamma irradiation and leukocyte reduction
 - B. Platelet additive solutions (PAS)
 - 1. In use outside of the US for years
 - a) Only one PAS approved for US use at this time (Intersol)

- b) Approved in late 2009, not widely used as of this writing
 - 2. Replacement of majority of plasma with PAS
 - a) This allows for several possibilities:
 - (1) More controlled metabolic environment
 - (2) Smaller amount of potentially incompatible ABO and other antibodies
 - (3) Smaller amount of plasma in general, which MAY reduce the risk of TRALI in immunized donors
 - C. Growth factors
 - 1. Thrombopoietin has been synthesized
 - 2. Studies show some benefit for those who are thrombocytopenic due to chemotherapy for non-hematologic malignancies
 - 3. For those whose marrow is wiped out (myeloablative treatment for hematologic cancers), thrombopoietin has minimal effect
 - D. Lyophilized platelets
 - 1. Frozen and dehydrated platelets
 - 2. If viable strategy, would help overcome storage issues and short shelf life issues
 - 3. Mixed results in studies, however
 - E. Platelet rich plasma “therapeutic” products
 - 1. Noted in mainstream press
 - 2. Autologous platelet rich plasma is prepared and is injected into an injury site
 - 3. Conclusive benefit not proven
- VII. Summary
- A. We’ve come a long way with our understanding of platelet physiology
 - B. We haven’t come far enough in our use of platelet transfusions
 - C. Economic pressures and new technology will likely make platelet transfusion look substantially different in the near future

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