

Chapter 4

HEMOSTASIS AND BLOOD TRANSFUSION

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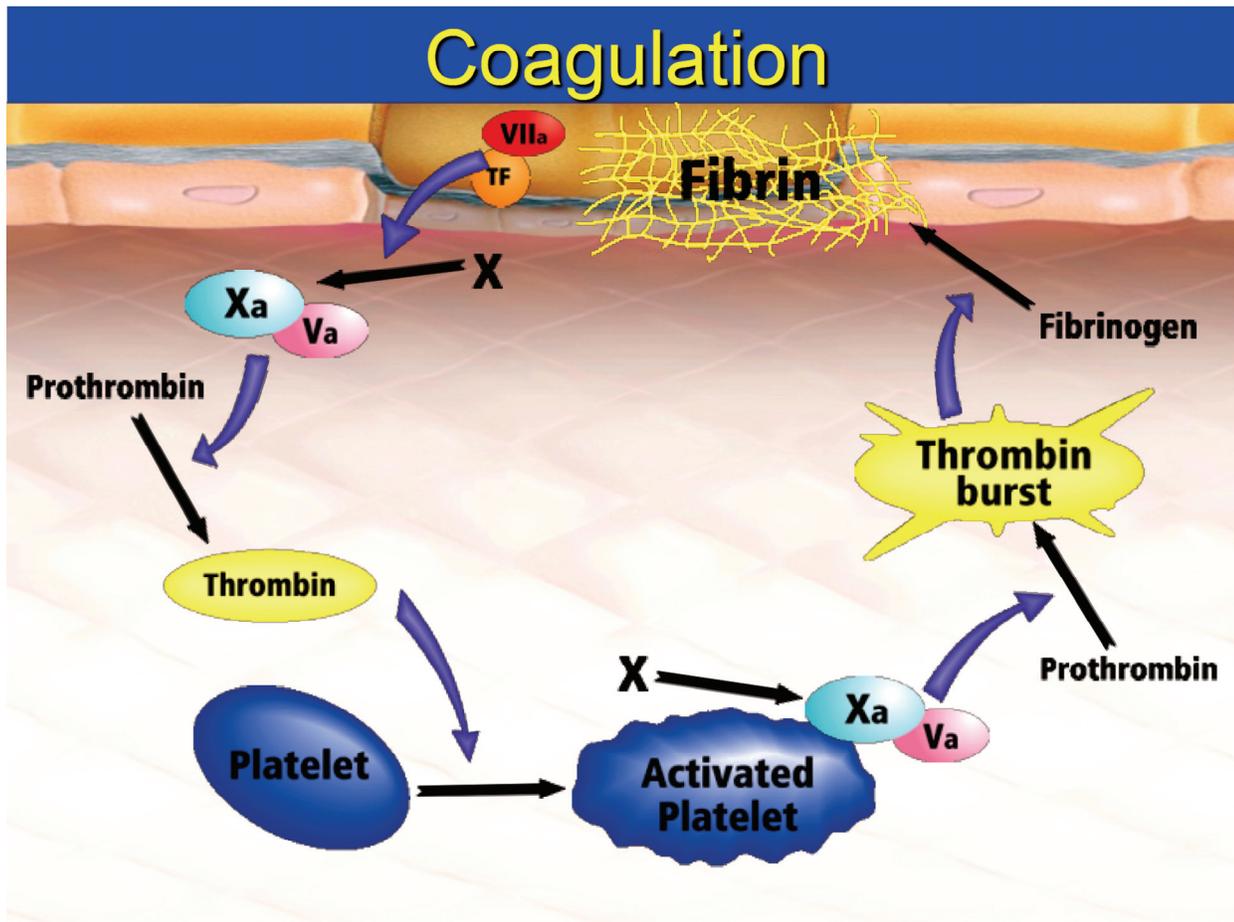
The infusion of blood and blood products from one individual to another to stop or prevent bleeding, provide adequate oxygen delivery, and to prevent death from hemorrhage saves millions of lives. Nevertheless, the practice of Transfusion Medicine remains in its infancy. In the 20th century, blood transfusion became a relatively safe and common practice, despite the relative absence of transfusion guidelines based on randomized, prospective controlled trials. Most transfusion practices are based on convention and convenience. The understanding of hemostasis, safe transfusion practices, appropriate testing of blood and blood products, as well as the consequences of transfusion continues to unfold. An understanding of these principles is very important for anesthetists who transfuse about half of the blood transfused in hospitals. Without this knowledge, transfusion practices often result in poor outcomes.

This chapter will discuss the current understanding of hemostasis, laboratory tests that assist in the decision of which components or products to transfuse and when to transfuse. Current pediatric transfusion practices, and the laboratory collection, preparation, and testing of blood and blood components are included.

HEMOSTASIS

Hemostasis is now understood to be a complex system of checks and balances designed to prevent abnormal clotting and uncontrolled bleeding. Coagulation is a cell-based event initiated on the surface of endothelial cells, in the subendothelium, and on platelets (**Figure 4-1**).

Figure 4-1: Coagulation



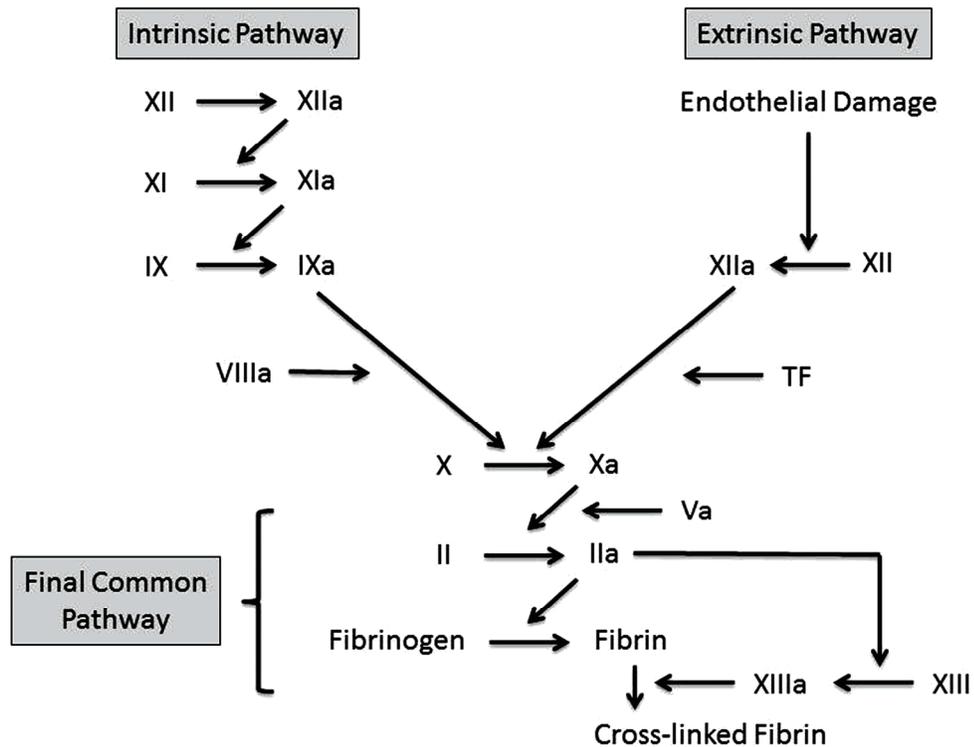
Overview of coagulation: Coagulation is activated when endothelium is disrupted and blood contacts tissue factor (TF). TF activates factor VII in turn activates factor X, which in combination with factor V, forms the prothrombinase complex. Further amplification occurs with platelet surface interaction resulting in a thrombin burst, which catalyzes formation of fibrin.

Following endovascular injury, control and termination of vascular bleeding consists of two sequential responses: primary hemostasis and secondary hemostasis. Primary hemostasis has four overlapping phases 1) vasoconstriction at the site of endovascular injury 2) von Willibrand factor (vWF) mediated platelet adhesion to exposed underlying tissue factor 3) platelet activation 4) platelet aggregation. Secondary hemostasis is the activation of serine proteases and their cofactors on endothelial and platelet surface phospholipids, which culminates in the formation of cross-linked fibrin and stabilizes the platelet plug.

Coagulation Cascade

In the 1960s, coagulation appeared to be the result of a serial stepwise activation (cascade) of either an intrinsic (intravascular) or extrinsic (extravascular) system of coagulation factors (**Table 4-1**) leading to a common pathway ending in the formation of fibrin (**Figure 4-2**). The prothrombin time (PT) assessed adequacy of the extrinsic system, partial prothrombin time (PTT) assessed the intrinsic system and the thrombin time (TT) the common coagulation pathway. A stable clot formed with the contribution of platelets activated and trapped at a bleeding site. These laboratory tests, in addition to the hemoglobin/hematocrit and platelet count continue to be useful in determining the need for transfusion of blood and blood products.

Figure 4-2: Intrinsic and Extrinsic Pathways



Classic coagulation cascade depicting the intrinsic (left), extrinsic (right), and final common pathway: Refer to Table 4-1 for the names of coagulation factors.

Table 4-1: Coagulation Factors

Coagulation Factors	
Number	Name
I	Fibrinogen
II	Prothrombin
III	Tissue Factor
IV	Calcium
V	Labile factor/proaccelerin
VII	Stable factor/proconvertin
VIII	Antihemophilic factor
IX	Christmas factor
X	Stuart Factor
XI	Plasma thromboplastin antecedent
XII	Hageman factor
XIII	Fibrin stabilizing factor

Coagulation Tests

Prothrombin time evaluates the extrinsic pathway of the coagulation cascade as well as the common pathway (factors I, II, V, VII, X) (**Figure 4-2**). Due to differing reagents used in laboratories to measure PT causing varying normal reference ranges, the *international normalized ratio (INR)* was developed to standardize results. It is a ratio of the patient's PT to a normal control sample raised to the international sensitivity index, a value assigned to tissue factor used to activate clotting by the manufacturer. Most often, PT is used to monitor therapeutic levels of warfarin that are being given to prevent clotting. PT may also be prolonged in patients who have liver disease, vitamin K deficiency, disseminated intravascular coagulation (DIC), or factor I, II, V, or X deficiency.

Partial Thromboplastin Time, or activated partial thromboplastin time (aPTT), assesses the intrinsic pathway and the common pathway (factors I, II, V, X, VIII, IX, XI, XII) (**Figure 4-2**). PTT is often used to monitor therapeutic levels of unfractionated heparin. Low molecular weight heparin (LMWH) must be monitored by anti-Xa levels. Mild factor deficiency may result in a normal PTT. Prolonged PTT may not be observed until factor levels are 40% of normal. Von Willebrand disease, liver disease, DIC, factor I, II, V, VIII (Hemophilia A), IX (Hemophilia B), X, XI deficiencies may prolong PTT. Lupus anticoagulant (LA), a non-specific inhibitor, may prolong PTT as well. The LA-sensitive PTT or dilute Russell viper venom test should be done if LA is suspected. Mixing studies may also be used to differentiate between factor deficiency and non-specific inhibitors by mixing normal plasma with the patient's prolonged PTT sample. If mixing results in a normalized PTT, factor deficiency may be diagnosed. If the results remain prolonged, a non-specific inhibitor may be suspected, and further studies may be indicated.

Thrombin time or thrombin clotting time (TCT), is used to evaluate the coagulation cascade at the level of fibrinogen by measuring the time to clot after thrombin is added to plasma. The test is sensitive to heparin, fibrinolytics, and antifibrinolytics. Prolonged TCT times may be due to the effects of heparin, fibrinogen dysfunction or deficiency, fibrin degradation products, or factor XIII deficiency. Methylene blue, which neutralizes heparin, can be added to the assay to determine whether heparin contamination of the specimen is the cause of a prolonged TT. A functional fibrinogen assay has largely replaced this test. Malnutrition, liver disease, DIC, and fibrinolysis may contribute to low fibrinogen values. Fibrinogen is an acute phase reactant and elevated levels are non-specific.

Blood to be tested for PT, PTT, and TT is collected in a tube containing buffered sodium citrate to allow chelation of calcium and to maintain proper plasma pH. The tube is then centrifuged to remove platelet phospholipids. Standard amounts of calcium and phospholipid are added back to the plasma for testing at 37° C. Under-filled anticoagulated tubes will result in excess anticoagulant, prolonging the results of these tests. The ideal ratio of blood to anticoagulant is 9:1.

Activated clotting time (ACT), or *activated coagulation time*, is commonly used to monitor the effects of anticoagulants during procedures that require anticoagulation, such as cardiopulmonary bypass, extracorporeal membrane oxygenation, or other vascular surgeries. Most frequently, the anticoagulant that is monitored is unfractionated heparin, but it may also be used to monitor other anticoagulants, such as direct thrombin inhibitors. Fresh whole blood is added to a tube containing surface activators. The activator may be celite, glass, or kaolin. These activators initiate coagulation through the intrinsic pathway to form clot after the sample is warmed. Results are measured in seconds. ACT is a fully automated point of care test that results in obtaining more rapid results than PTT. PTT is insufficient for monitoring the effects of heparin because the PTT is overly sensitive to high doses of heparin that cause coagulation to remain incomplete. Factors that may affect the ACT results include temperature, thrombocytopenia or other qualitative platelet abnormalities, platelet inhibitors, anticoagulants, factor deficiency, hemodilution, and antithrombin deficiency. A baseline ACT is commonly obtained prior to anticoagulation. After heparinization, another ACT is obtained to ensure adequate anticoagulation for the procedure. A target ACT of 480 seconds is commonly used prior to initiation of cardiopulmonary bypass. Subsequent ACTs are obtained to determine the need for additional heparin to maintain the desired ACT level, depending on the procedure. ACT does not correlate with heparin levels. These measurements are less precise than PTT and large variability exists between patients and their response to heparin.

Platelet count is included as part of complete blood counts. Thrombocytopenia is defined as a platelet count $<150 \times 10^3/\mu\text{L}$. Patients with platelet counts $>50 \times 10^3/\mu\text{L}$ are generally asymptomatic, unless undergoing surgical procedures that have hemorrhage risk or if there is

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significant trauma. At counts of $<10 \times 10^3/\mu\text{L}$, the risk of spontaneous bleeding is increased; petechiae and spontaneous bruising occur. Platelet count $<5 \times 10^3/\mu\text{L}$ is considered a hematological emergency, given the risk of severe spontaneous hemorrhage (intracranial, mucosal, gastrointestinal). The etiology of thrombocytopenia can be divided into increased platelet consumption, decreased platelet production, and platelet sequestration in the spleen. Increased platelet consumption may be immune mediated (idiopathic, autoimmune, drug induced), platelet activation (DIC, hemolytic uremic syndrome, thrombotic thrombocytopenic purpura, necrotizing enterocolitis), or mechanical destruction. Decreased platelet production may result from infection, cyanotic congenital heart disease, bone marrow failure, malnutrition, or impaired thrombopoiesis. Platelet sequestration may occur in liver disease, von Willebrand disease (Type 2B, platelet-type), and hypersplenism related to malaria or other diseases.

Bleeding time (BT) is a classic test used to determine functional platelet deficiency and disorders in primary hemostasis. Currently, this test is rarely used because more reliable, comprehensive, and less invasive tests are available. The principles of bleeding time involve a controlled cutaneous puncture on the forearm and observation of the time required for bleeding to stop, measured in 30 second intervals. In the Ivy method, a blood pressure cuff is placed on the upper arm and inflated to 40mmHg. A standard incision of 1mm deep and 1cm long is made on the hairless ventral side of the arm. Blood is blotted away every 30 seconds. Normal bleeding time is less than 9 minutes. Von Willebrand disease, Glanzmann's thromboasthenia, Bernard-Soulier disease, connective tissue disorders, thrombocytopenia, medications affecting platelet function, liver failure, uremia, and hypofibrinogenemia may prolonged bleeding time. Bleeding time is not performed when the platelet count is less than $100 \times 10^3/\mu\text{L}$ and not recommended in children under the age of three years or children unable to cooperate. This test is primarily used as a screening test and is not sufficient for diagnosing specific conditions.

Platelet aggregation studies are used to determine the presence of intrinsic platelet abnormalities by examining the platelet clumping response to platelet agonists. Whole blood is collected in a citrated blood tube and centrifuged to obtain platelet rich plasma, which is then placed in a cuvette. Whole blood may also be used in a variant method. Continuous light transmission aggregometry is performed by placing the cuvette between a light source and photocell where transmission is detected. After a platelet aggregate is added, platelets begin to clump and more light is passed through the sample, which is sensed by the photocell. Most agents that induce platelet aggregation (collagen, epinephrine, and thrombin) act through effects on the adenosine diphosphate (ADP) normally released by platelets upon activation.

Decreased collagen and epinephrine aggregation occur in samples from patients taking aspirin and anti-inflammatory agents. Abnormal thrombin aggregation occurs in patients with some intrinsic platelet defects (Bernard-Soulier disease, storage pool defects). The addition of exogenous ADP causes direct platelet aggregation. Patients with Glanzmann's thrombasthenia

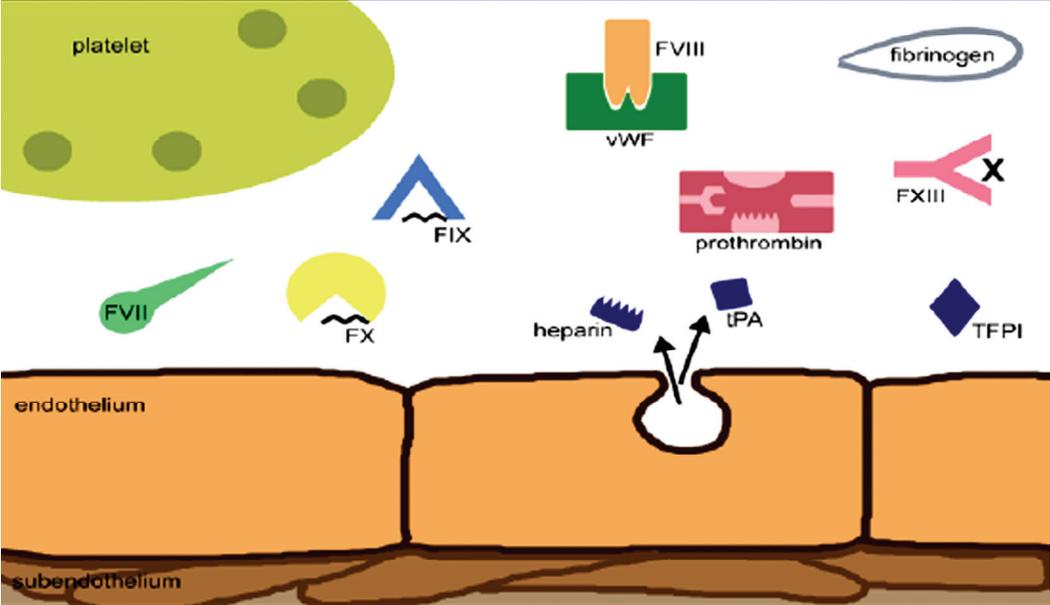
fail to aggregate their platelets when ADP is added. Patients with von Willibrand's disease have normal platelets and consequently show aggregation with the addition of ristocetin. This test is sensitive to platelet count, fat content of blood, temperature, pH, fibrinogen content, and anticoagulants. Patients with uremia or severe liver disease may develop complex bleeding disorders, which can include platelet dysfunction.

Platelet function assay (PFA) is a screening test that measures platelet adhesion and aggregation in primary hemostasis. It depends on platelet function, number, von Willebrand factor, and hematocrit. Anticoagulated whole blood is passed through a membrane that contains collagen and epinephrine or ADP at a rate that simulates small capillaries. The platelets adhere to the membrane and gradually occlude the membrane. The time to complete occlusion is referred to as the closure time. This test has a high *negative* predictive value of platelet dysfunction if the collagen/epinephrine membrane closure time is normal. If this value is prolonged, a collagen/ADP test is automatically performed. If this closure time is normal, an aspirin-induced platelet dysfunction may be inferred. If both values are prolonged, anemia, thrombocytopenia, or other platelet function defects are present, such as von Willebrand disease, renal failure, release defect, Bernard-Soulier disease, or Glanzmann thrombathenia; further work up should be pursued.

Thromboelastography (TEG) is a method of evaluating the viscoelastic properties of blood coagulation. Whole blood is placed in a cup. A metal pin oscillates in the cup as clot formation is initiated. The instrument rotates the cup and as fibrin and platelet aggregates form, it begins to adhere with the cup. The torsion wire connected to the pin measures clot strength over time. Lysis of the clot causes a decrease in clot strength. *Rotational thromboelastometry (ROTEM)* is another version in which there is a rotating sensor shaft and oscillating pin with an optical sensor.

Figure 4-3a: Tissue-Based Coagulation

Normal Endothelium:
 Procoagulants (FVII, FVIII, FIX, FX, FXIII, prothrombin), fibrinogen, and platelets circulate in their inactive forms.
 Anticoagulants (TFPI, heparin, and tPA) prevent endothelial spontaneous thrombus formation.
 Tin-Na Kan, MD



Vascular Injury: TF exposed to circulating FVII forms TF:VII complex.
 TF:VII activates FIX and FX. FIXa binds to platelets.
 FXa activates FV forming prothrombinase complex which converts localized, small amounts of prothrombin to thrombin.
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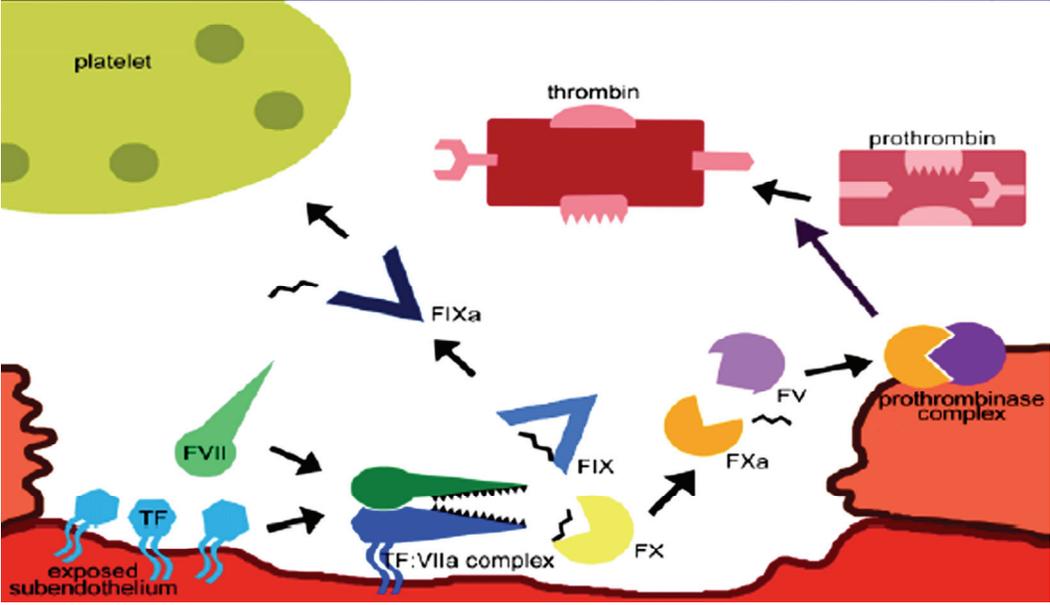
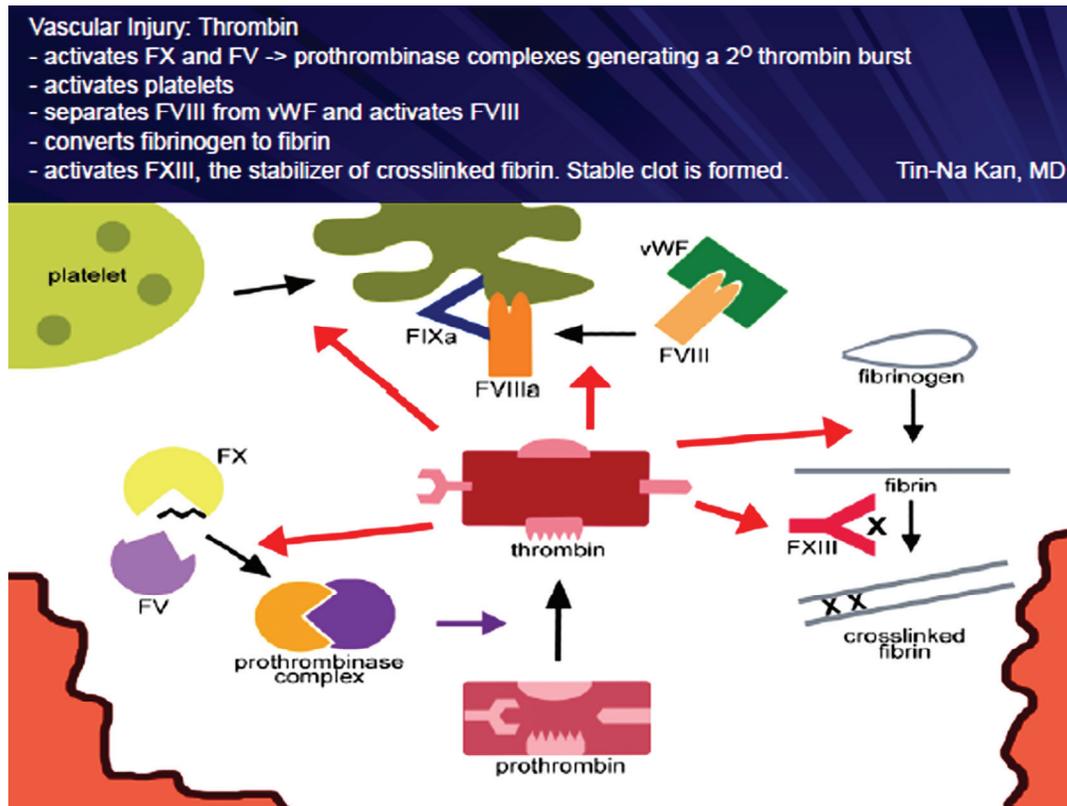


Figure 4-3b: Tissue-Based Coagulation



See text above figures for details. vWF, von Willebrand factor; TFPI, tissue factor pathway inhibitor; tPA, tissue plasminogen activator.

The physiologic response to bleeding is initiated with endothelial damage and intravascular exposure of tissue factor. Vessel wall-platelet interactions cause vasoconstriction and exposure of *tissue factor*, a transmembrane protein, to the intravascular environment. Platelets aggregate at the site of injury and are activated, releasing cytoplasmic granules with the formation of an unstable primary plug. Coagulation then proceeds with three overlapping stages resulting in the formation of fibrin plug: tissue factor initiated activation of coagulation factors, amplification of activated coagulation factors, and thrombin propagation on activated platelet surfaces.

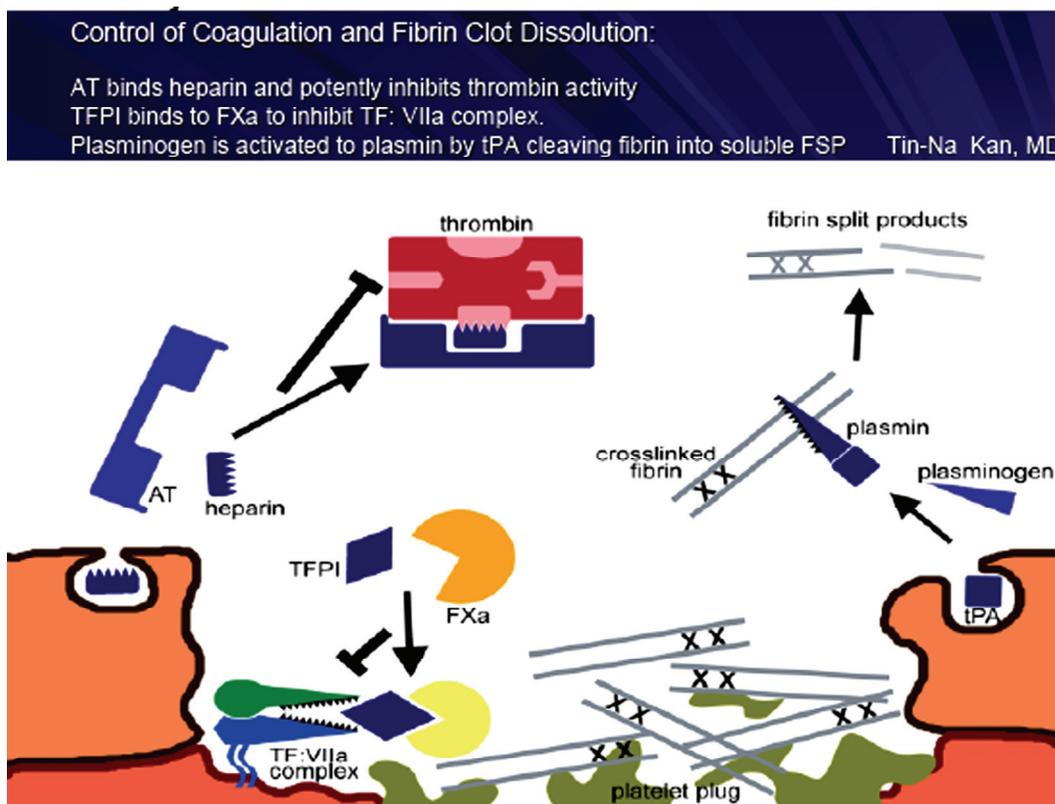
Intravascular surface exposure of tissue factor activates small amounts of local factor VII (FVIIa) to which it attaches (**Figure 4-3a**). The TF-FVIIa complex activates factor X (FXa) and factor IX (FIXa). FXa activates and complexes with factor V (FVa). Prothrombinase complex (FXa-FVa) catalyzes the conversion of small amounts of prothrombin to thrombin (initiation phase). This thrombin activates local platelets that change membrane configuration and release cytoplasmic granules (ADP and serotonin, vWF and factor V), causing changes in platelet shape and membrane expression of surface receptors. Thrombin, through a positive feedback mechanism, activates additional FV, FVIII, FXI and platelets (amplification phase). The propagation phase proceeds with FIXa binding to FVIIIa (the tenase complex). Tenase activates additional FX (FXa) which again

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complexes with FVa forming large amounts of prothrombinase complex and consequently large amounts of thrombin (*thrombin burst*) (**Figure 4-3b**). Secondary hemostasis proceeds on the platelet surfaces with binding of fibrinogen to platelet surface receptors, leading to thrombin cleavage of fibrinogen to fibrin. Large numbers of platelets are entrapped and activated forming an unstable fibrin monomer plug. Activated factor XIII cross-links and stabilizes fibrin monomers, which are irreversibly polymerized. Thrombin also activates FXIII and thrombin-activatable inhibitor, which participate in stable clot formation. Thrombin participates in its own down-regulation by complexing with thrombomodulin, which activates protein C and its cofactor protein S, inactivating FV and FVIII. Circulating thrombin is inactivated by antithrombin.

Fibrinolysis

Figure 4-4: Fibrinolysis



Refer to text above figure for details. TFPI, tissue factor pathway inhibitor; tPA, tissue plasminogen activator.

Normally, plasmin circulates as *plasminogen* (the inactive form). Endothelial cells secrete plasminogen activator inhibitor type 1 to inhibit the activation of plasminogen. Endothelial injury initiates secretion of tissue plasminogen activator generating plasmin by stimulating the cleavage of plasminogen (**Figure 4-4**). Plasmin degrades cross-linked fibrin to soluble D-dimers and fibrin degradation products, both of which inhibit thrombin. Since tissue plasminogen activator also binds to fibrin, the generation of plasmin remains a localized event. Circulating plasmin, by comparison, is regulated by a more powerful (100x) inhibitor, α 2-antiplasmin. Fibrinolysis is also contained in surgery and trauma by release of acute phase reactants that limit fibrinolysis.

Developmental Hemostasis

Procoagulant and anticoagulant proteins form in utero. Most of these proteins reach normal adult levels by six months after birth. Some reach adult levels during adolescence. These differences can complicate the correct diagnosis of coagulation disorders in early childhood and can affect monitoring of anticoagulation treatment. Differences in levels of coagulation proteins, as compared with adult levels, may not be important in hemostasis. Some coagulant proteins, such as TF and thrombomodulin, are important in angiogenesis, inflammation, and wound healing.

Coagulation factors do not cross the placenta. The fetal liver begins production of coagulation factors at five weeks gestation, with measurable, albeit low, plasma levels at 20 weeks gestation. Fetal forms of protein C and fibrinogen change to the adult forms at birth. The only procoagulant proteins in the normal adult range at birth are fibrinogen, FV, and FVIII. Vitamin K dependent factors (II, VII, IX, and X) and contact factors (XI, XII, prekallikrein) are at 50% of normal adult values. By six months of age, values overlap with the normal adult range values, but the average of most factor levels is 20% lower than average adult levels until adolescence. FVIII levels are normal at birth, and levels of vWF are elevated until three months of age.

Low levels of *vitamin K dependent factors* and *contact factors*, FV, FVIII and fibrinogen between 19 and 30 weeks gestation result in prolonged values for PT, PTT and TT.

The *anticoagulants* antithrombin (AT) and heparin cofactor II (HCII) are at 50% of normal adult values at birth, normalizing to adult levels by three months of age. Proteins C and S are less than 50% of normal adult values at birth and reach adult levels by six months of age. On the other hand, anticoagulant α -2 macroglobulin values are higher than adult levels at birth and twice the adult levels at six months of age, not normalizing to adult levels until the third decade.

Developmental Hematopoiesis

Blood cell formation (hematopoiesis) is an ongoing self-renewing process. The life span of mature blood cells is short. The average circulating life span for mature red cells is 110 – 120 days, for neutrophils 5 – 6 days, and for platelets 5 – 9 days. Some memory lymphocyte cells survive for

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years, but the majority of circulating lymphocytes survive for weeks. Hematopoietic stem cells replenish precursor and progenitor blood cells over a person's lifetime.

Initially, hematopoietic stem cells appear in the embryonic yolk sac at four weeks gestation; progenitor cells then migrate to the fetal liver for expansion and maturation. Stem cells are later produced in the aorta-gonad-mesonephros, major blood vessels, and the placenta. Fetal liver blood progenitor cells migrate to the thymus, spleen, and then to the bone marrow, where they mature under the influence of multiple hematopoietic growth and transcription factors. Extra embryonic hematopoiesis stops by 10 – 12 weeks gestation. From 20 – 24 weeks gestation, hematopoiesis occurs, primarily in the fetal liver. Bone marrow hematopoiesis gradually increases in the second trimester of pregnancy as liver hematopoiesis diminishes.

Red cell hemoglobin production evolves through a variety of forms, starting with the embryonic forms (Gower-1, Gower-2, Hb Portland) to fetal hemoglobin, and finally progressing to the adult forms (Hbg A and Hbg A2) during infancy. The majority of hemoglobin, a tetrameric protein containing four iron-containing units and four globin (protein) chains, will ultimately mature in composition to two α and two β chains manufactured in the bone marrow. Embryonic and fetal hemoglobin have higher oxygen affinity than hemoglobin A. This higher affinity for hemoglobin facilitates transplacental oxygen delivery.

Thrombopoiesis

Megakaryocyte progenitors (burst forming units and the more mature colony forming units) ultimately give rise to bone marrow megakaryocyte under the influence of thrombopoietin. Megakaryocytes undergo duplication without cell division (endoreplication). As a megakaryocyte matures, cytoplasmic pseudopods are formed. These pseudopods initially have thick membranes, which thin with maturity. When mature the cytoplasmic pseudopod fragments are released as platelets. Generally, circulating large platelets are the result of early megakaryocyte platelet release in response to thrombocytopenia.

TRANSFUSION

Blood is transfused based on blood type. The distribution of blood types varies worldwide. Red blood cells have surface antigens to which antibody may form. There are more than 400 known red cell antigens and 30 of these antigens are of major importance. Transfusion of donor red cell antigens to a recipient who does not have the antigen may elicit an antibody response. Each antigen is controlled by one gene. One gene is inherited from each parent. Antigenic determinants of a red cell group are produced by alleles at a single gene locus or at a locus so closely linked that crossing over is extremely rare. For any antigen of a group, a single allele is present at a locus, excluding other antigens. Antigens on red cells are usually determined by phenotype after mixing red cells with antigen specific commercial antisera and observing

hemagglutination. The number of antigenic determinants per erythrocyte and the ability to elicit an immune response varies from antigen to antigen.

ABO and Rhesus (Rh) Red Cell Antigen Groups

The two most significant red cell antigen systems are ABO and the Rh blood group. These two antigenic groups are the most immunogenic and generally illicit the most frequent and strongest antibody response in antigen negative individuals exposed to antigen. Transfusion of incompatible blood into a sensitized individual (previously exposure to the antigen with the production of IgG antibody) may result in antibody formation and a subsequent hemolytic transfusion reaction.

The *ABO blood group* consists of blood types A, B, AB and O and all individuals belong to one of these blood groups. Groups A and B represent carbohydrate antigens bound to surface glycoprotein and to some extent to membrane lipid on red cell and endothelial surfaces controlled by transferase enzymes conjugating either N-acetylglucosamine (A) or galactose (B) to a terminal fructose on the stem carbohydrate added by the H gene coding for a fucosyl transferase. The absence of H gene (hh) is rare and phenotypically termed O_h or Bombay type which results in high titer anti-H and no addition of either A or B carbohydrate despite the presence of A or B genes. Group O indicates the absence of both A and B genes. Consequently, neither the A nor B carbohydrate is present on erythrocytes or endothelial cells. AB individuals have both antigens. There are subgroups of A and B antigens, most of which are rare, with differences between subgroups quantitative, i.e., in the number of surface antigenic sites. Weak variants of group A may be difficult to detect with routine antisera. Antibody to group A and B are “naturally” occurring, meaning that they are thought to be stimulated by normal intestinal flora bacteria and plant and food proteins rather than by transfusion. A and B antibodies develop in the absence of antigen during the first 3-6 months of life, peak at age 5 – 10 years, and decrease with age and some immunodeficiency states.

Second in importance is the *Rh blood group*. Anti-Rh antibody is the leading cause of hemolytic disease of the newborn and an important cause of hemolytic transfusion reactions. There are more than 40 antigens in this group. Antigens are inherited as a gene complex set with three products, one codominant set from each parent. There are two nomenclatures for the Rh system: Fisher-Race and Weiner. The Fisher-Race system is now in more common use. In that system, there are five commonly produced antigens: C, c, E, e, D (no d has been identified). The other antigens in this system are minor variants. Individuals who are Rh negative (Rh⁻) are those who do not have D. Individuals who are Rh⁺ have the D antigen. Rh⁻ individuals are uncommon in most of the world but are present in roughly 15% of Caucasians. People with weaker forms of D (fewer antigens per red cell membrane) are designated as D^u. Transfusion of Rh⁻ individuals with D^u positive erythrocytes can cause sensitization.

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Other Important Red Cell Antigen Groups

Of the remaining red cell antigens and groups, the four red cell groups most often implicated in hemolytic transfusion reactions are Kell, Duffy, Kidd and MNS. These reactions are generally delayed hemolytic reactions, often detected by the absence of an expected increase in hemoglobin/hematocrit after transfusion. For a red cell antigen to stimulate antibody production following transfusion, the antigen must be both immunogenic and prevalent. If an antigen is highly immunogenic but is low incidence, it is unlikely to be transfused. Hemolytic antibodies are IgG antibodies and react at 37°C. Hemolysis is rare with IgM (cold reacting) antibody.

Kell system antigens are frequently associated with delayed hemolytic transfusion reactions. Originally described as K and k (more frequent) allelic pairs, now there are two additional allelic pairs and several variants identified. Kell antigens are highly immunogenic. One-in-20 recipients transfused with K⁺ blood develop antibody. Antibodies to Kell cause hemolytic transfusion reactions, hemolytic disease of the newborn, and at times hemolytic anemia. The McLeod phenotype lack Kx, a precursor to Kell antigen, results in depressed expression of k. Those with the McLeod phenotype have erythrocyte and neuromuscular abnormalities. This phenotype has been associated with chronic granulomatous disease. *Duffy system antigens* (Fy^a, Fy^b) are codominant with Fy^a more frequently implicated in delayed hemolytic reactions. The Fy^{a-b-} phenotype, more common in people of African origin, provides resistance to *Plasmodium vivax* (not *P. falciparum*) infection as Duffy antigens facilitate erythrocyte entry receptors for the parasite. The *Kidd system* comprises four genetic phenotypes: JK^{a+b-}, JK^{a-b+}, JK^{a+b+}, and JK^{a-b-} (found in some Pacific Island populations).

Pre-transfusion Testing of Red Cells

“Type and Screen”: testing for both blood type and preformed red cell antibody in the blood recipient

Blood Type

The ABO and Rh type of all potential blood recipients must be completed prior to non-emergent transfusion of blood. This is accomplished by mixing recipient red cells with commercial anti-A, anti-B and anti-D. Red cell clumping indicates the presence of antigen on the red cell surface. The blood type is confirmed by mixing commercial A, B or D red cells with recipient serum or plasma (back typing). The likelihood of a hemolytic transfusion reaction transfusion with type specific blood is approximately 1:1000 units administered.

Antibody Screen

The recipient’s serum or plasma is screened for antibodies, other than A, B or D, by mixing recipient serum with a set of three commercial O red cells containing the most common red cell

antigens. Antigen-antibody complexes form following either hemolysis or agglutination. The screen can be completed with the indirect antiglobulin test (IDAT) looking for agglutination. Should one of the screening cells react, an expanded red cell antigen panel is used to identify the antibody, delaying the availability of cross-matched blood. In laboratories without three cell antigen panels and expanded antibody identification panels, an alternative strategy is to test both donor and recipient red cells using commercial anti-sera with a single common red cell antibody (more stable than red cells) such as Kell, Duffy, Kidd antibody to lower the possibility of antibody sensitization.

Crossmatch

Red cells from the unit to be transfused are mixed and incubated with the intended recipient’s serum or plasma prior to transfusing a unit of packed red cells. The method for accomplishing the cross-match depends upon the resources available and recipient circumstance. In the least complicated form, the cross-match can be made at the bedside using a clean glass or ceramic plate. In environments with more abundant resources, red cells are washed and incubated with various reagents, which promote interaction between antigen and antibody and fixation of complement.

Blood intended for use in the crossmatch should be collected no longer than three days prior to the intended transfusion unless it is certain the patient has not been pregnant or transfused within the preceding three months. The crossmatch methodology varies with circumstance and available resources. In an emergency, donor type O negative packed red cells or donor packed cells known to be of the recipient’s blood type (“type specific”) can be transfused and the crossmatch performed retrospectively. The most simple, but least sensitive crossmatch, consists of mixing donor segment red cells with recipient serum/plasma on a clean non-reactive surface and observing agglutination. The routine tube crossmatch employs mixing donor cells in saline with recipient serum/plasma and recording hemolysis or agglutination at room temperature, after incubation at 37°C and after addition of antihuman globulin. Various reagents (albumin, low ionic strength saline, polyethylene glycol, etc.) can be added to enhance reactions.

Table 4-2: ABO Compatibility

Donor Red Cells	Recipient Blood Type
O (universal donor)	O, A, B, AB
A	A, AB (B and O have anti-A)
B	B, AB (A and O have anti-B)
AB	AB (A, B, O have anti-A or B or both)

Generally, donor red cells of the same type as the recipient are transfused. However, it is not necessary for donor red cells to be of the same blood type as the recipient provided that the blood types are compatible with one another. For example, blood type O which has neither A nor

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B antigen can be transfused as packed red cells to any recipient. For this reason, O red cells are considered to be from “*universal*” donors as long as the crossmatch is non-reactive for other red cell antibodies. Recipients who are type A can receive either A or O red cells. Type B recipients can receive either B or O blood and AB recipients can receive either O or AB red cells that are crossmatch compatible (**Table 4-2**).

Compatibility testing for infants less than four months of age

Infant red cells must be tested for ABO and Rh type pretransfusion. Serum/plasma from either the infant or the mother may be used to detect unexpected antibodies and for crossmatch. Infant serum/plasma need not be tested for antibody when O cells are to be transfused. In the absence of an unexpected antibody, donor red cells need not be crossmatched. Repeat infant testing can be eliminated during any single hospital admission.

Check Specimens, Immediate-Spin, and Computer Generated Crossmatch

The vast majority of ABO compatible packed red cell units are crossmatch compatible, provided the patient has not been pregnant or transfused previously. The majority of hemolytic transfusion reactions are due to ABO incompatible transfusions resulting from clerical errors, such as recipient blood samples labeled with the incorrect patient name or misidentification of the recipient at the time of transfusion.

Many transfusion services require two separate ABO type tests prior to releasing blood or blood components from the Blood Bank. One sample collected at the time of the “Type and Screen” and a second consisting either of a record from previous ABO testing in the same Blood Bank or the testing of a second sample (i.e. the “check specimen”). This procedure markedly reduces the number of major hemolytic transfusion reactions.

Transfusion Services can employ an “*immediate-spin*” crossmatch for recipients who do not have previous or current clinically significant antibodies. Saline suspended washed donor segment red cells are mixed with recipient serum/plasma at room temperature and the tube centrifuged immediately. This method is employed to detect ABO incompatibility.

A *computer-generated crossmatch* is an electronically generated match between the recipient and the red cell unit and/or other blood component that matches the ABO and Rh of inventory donor blood and the confirmed recipient blood type. Recipients without clinically significant antibodies by testing or history and without recent transfusion are suitable for computer-generated crossmatch. However, a number of rigorous on site requirements for computer system validation are required. This practice is increasingly adopted by large blood services with sophisticated laboratory and transfusion service computer programs.

Table 4-3: Transfusion Reactions

Transfusion Reactions		
<u>RBC</u>	<u>WBC</u>	<u>Plasma Proteins</u>
Immediate (intravascular)	Febrile	Allergic
Delayed (extravasvular)	TRALI (transfusion, other)	Anaphylactic (IgA, other)
	GVHD	
	Alloimmunization	

Although increasingly safe, reactions to blood transfusions continue to occur. Each transfusion must to be monitored during and following infusion. It is important that vital signs be obtained and recorded before transfusion, at 30-minute intervals during transfusion and 30 minutes after transfusion. Reactions to transfusion must to be reported to the transfusion service and reviewed by the hospital Transfusion Committee.

Transfusion reactions are either immune-mediated or non-immune mediated. Reactions can be due to recipient antibody to donor red cells, reactions to white cells, platelets or plasma. Preventable reactions are sepsis due to blood component bacterial contamination (most frequently platelets) and fluid overload (transfusion associated cardiac overload or TACO). Sepsis and TACO are the most common adverse outcomes of transfusion.

Red Cell Reactions

Hemolytic reactions following transfusion of red cells can be immediate or delayed. Immediate reactions are intravascular and are due to IgG antibody to donor red cells. These reactions are fatal in 10% of ABO incompatible transfusions and generally occur following large volume infusion of incompatible blood as a result of clerical error. Clerical error is the consequence of mislabeling the blood specimen used for crossmatch with the wrong patient name or not matching the name on the unit of cells or blood to be transfused with the recipient at the time the transfusion is initiated. Immediate hemolytic transfusion reactions occur in less than 0.02% of red cell transfusions. Fever, chills, and burning at the infusion site herald a reaction. Hypotension, dyspnea, sometimes back or joint pain, and then hematuria follow. These reactions can proceed to cardiovascular collapse, disseminated intravascular coagulation and renal failure. The transfusion should be stopped at the first sign of a hemolytic transfusion. The remainder of the red cell unit and a blood and urine sample should be sent to the laboratory for testing. The unit will be examined for signs of bacterial contamination, a repeat crossmatch, and a *direct Coombs test* will be performed. If a hemolytic transfusion reaction has occurred, recipient antibody will coat transfused donor cells. Anti-Ig is added to recipient red cells. Following incubation, red cell agglutination occurs in a positive direct Coombs test. The recipient blood sample can be tested

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for haptoglobin, which will be cleared from the blood with hemolysis, and the patient's urine will be positive for free hemoglobin.

Delayed hemolytic transfusion reactions are extravascular, occurring in the liver and spleen. These reactions occur 3-10 days after transfusion. A delayed hemolytic transfusion reaction is suspect in blood product recipients with or without fever in which the appropriate increase in hemoglobin is not observed following transfusion. This reaction is detected with a positive direct Coombs test and elevated bilirubin levels. They are the consequence of primary immunization to red cell antigens or are due to an anamnestic response in a previously sensitized recipient with undetectable antibody levels at the time of pre-transfusion testing. These reactions are thought to occur once in every 6,000 red cell transfusions but are often undetected.

Inadvertently overheating or freezing donor blood or the shear stress caused by rapid transfusion of donor red cells through small-bore needles can artificially produce red cell destruction. This has led to hyperkalemia, lack of predicted hematocrit elevation, and hematuria.

Leukocyte Reactions

There are several types of leukocyte reactions and reactions to leukocyte produced cytokines within the donor unit after collection. The frequency of adverse leukocyte reactions and the transmission of leukocyte associated viruses, such as cytomegalovirus, declines markedly when blood is leukoreduced (leukocytes are removed) at the time of collection or at the time of transfusion. The most common leukocyte reaction is febrile non-hemolytic transfusion reaction (FNHTR), occurring in 1-2% of non-leukoreduced red cells and platelet transfusions. These reactions are characterized by fever ($\geq 1^\circ\text{C}$) 30 – 60 minutes following transfusion and often accompanied by chills. The reactions are generally self-limiting with fevers of 38 - 39°C and must be distinguished from the fever associated with a hemolytic transfusion reaction. Bacterially contaminated blood components commonly present with high fever ($\geq 40^\circ\text{C}$) and rigors. Only one in eight recipients will have a repeat febrile reaction during the following transfusion. These reactions most commonly occur in multiply transfused recipients and in multiparous women who were leukocyte sensitized during past pregnancies. Recipients with recurrent febrile reactions can benefit from pre-treatment with antipyretic drugs.

Transfusion associated acute lung injury (TRALI) is a rapid onset pulmonary edema of generally 24 – 48 hours duration. It is uncommon for TRALI to be fatal. High-titer anti-leukocyte antibody, usually in the donor plasma to recipient leukocytes, cause leukocyte aggregates that are filtered in the lungs and lodge in pulmonary capillaries. Complement activation results in endothelial damage, flow of intravascular fluid into alveoli, and pulmonary edema. Recipient leukocyte antibodies to donor leukocytes can also cause TRALI. All plasma containing blood components have been implicated in TRALI, but fresh frozen plasma is most frequently associated with it. For that reason, there has been a shift towards only using male plasma for production of fresh frozen

plasma, reducing the incidence of TRALI in both Europe and the United States. Female blood donors who have been pregnant can make white cell antibodies and HLA antibodies to white cells of their fetus. Females with a history of multiple pregnancies have been most frequently implicated in cases of TRALI.

Transfusion associated graft versus host disease (TA-GVHD) is a rapidly fatal form of GVHD following transfusion of HLA haplo-compatible lymphocytes between first-degree relatives. Designated donations between first-degree relatives are gamma irradiated (25 gray, Gy, to the central portion of the blood bag and no less than 15 Gy to the outer portions), inhibiting T lymphocyte blastogenesis and engraftment. Irradiation damages red cell membranes with consequent increases in plasma potassium over time and decreases post-transfusion cell viability. For this reason, red cell units expire at their original outdate or 28 days from the time of irradiation, whichever comes first. Blood is generally irradiated immediately prior to transfusion. Platelets expiration dates are not affected since platelets are relatively resistant to irradiation. TA-GVHD can also occur in some immunocompromised patients triggering pre-transfusion irradiation. Blood transfused during intrauterine transfusion or transfused to infants ≤ 1500 grams and to severely immunocompromised children (congenital cellular immunodeficiency disorders, children undergoing bone marrow or progenitor cell transplant, treatment for hematologic malignancies, Hodgkin's disease or cancer patients undergoing intense chemotherapy) is irradiated to prevent GVHD.

Cytomegalovirus (CMV) seronegative children at risk of severe CMV infection should be transfused with CMV minimal risk blood. Latent CMV infection of leukocytes follows primary infection with periods of reactivation. CMV infection in healthy individuals is of little consequence, but infection can be serious and even fatal in the immunocompromised. Transfusion associated CMV is most often transmitted by viremic acutely infected otherwise healthy, asymptomatic blood donors prior to specific CMV antibody formation. Although not perfect, leukoreduced blood ($<5 \times 10^6$ WBC) or CMV seronegative blood rarely transmits CMV. Previously frozen blood components do not transmit CMV. CMV reduced risk blood is indicated for recipients of intrauterine transfusions, premature infants <1500 gms, and for seronegative children with congenital immunodeficiency disorders, AIDS, and for bone marrow, progenitor cell, and organ allograft recipients, as well as for cancer patients undergoing intense chemotherapy.

Allergic Reactions

Allergic reactions are the most frequent type of transfusion reaction, occurring in 1-3% of recipients of plasma containing components. Allergic reactions are more common with platelets (3.7%) than with red blood cells (0.15%). Most reactions consist of hives, itching, and local erythema. These reactions can, however, proceed to bronchospasm and rarely to anaphylaxis. When an allergic reaction is detected, the transfusion should be stopped until the severity of the

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reaction is determined. Mild reactions respond to antihistamines. The transfusion can be continued when the hives abate. Reduced plasma or washed red cells/platelets can be transfused in patients with recurrent allergic reactions uncontrolled with antihistamines. While washed components are more effective in preventing reactions than reduced plasma volume components, the volume of transfused cells is also reduced and the quality of platelets may not be the same. There is no evidence that pretreatment with prophylactic antihistamine prevents allergic reactions.

Plasma proteins are often implicated as the cause of allergic reactions and rarely cause anaphylaxis. These reactions were first described when normal donor blood was transfused into IgA deficient patients with anti-IgA and later in haptoglobin deficient patients making haptoglobin specific antibody. It is now also understood to occur in patients with subclass and allotype variations who have been transfused and formed antibodies. There are racial differences in the incidence of IgA and haptoglobin deficiency. The incidence of haptoglobin deficiency is 1-in-1,000 – 1-in-4,000 in East and Southeast Asians but very uncommon in Africans, Europeans and western or eastern Asians. On the other hand, the incidence of IgA deficiency in Europeans is 1-in-2,500 and 1-in-30,000 in the Japanese. Also described are anaphylactic reactions to complement C4 in deficient individuals and to Factor IX in hemophiliacs with Factor IX inhibitors.

Chemically induced anaphylaxis is less common and is described as caused by methylene blue added to fresh frozen plasma for viral inactivation. Also reported is anaphylaxis precipitated by donor derived food allergens, such as peanuts, transferred to a sensitized individual. Subpathways to anaphylaxis, not evolving IgG or IgE, are described. *Allergen-independent mechanisms* caused by biological response modifiers (BRMS), such as inflammatory cytokines and chemokines accumulating in blood components during storage, can cause allergic reactions. Passive transfer of sensitization has been described from donor-derived allergy, in one case to cephalothin and in another to peanuts.

Two laboratory tests are used to determine occurrence of an allergic reaction. Elevated levels of serum or *plasma tryptase* indicate mast cells activation and degranulation in allergic and in the more severe form of anaphylactic reactions. Secondly, there is interest in applying the *basophil activation test (BAT)* to transfusion medicine to detect allergic reactions. In this test, patient's blood is incubated with the allergen in question, tested for upregulation of the basophil activation markers, CD63 and CD203c, using flow cytometry.

Alloimmunization

Recipient antibody can be formed following transfusion of red cells, white cells, and platelets. Red cell antibodies complicate the ability to provide crossmatch compatible blood, and they add additional costs and delay the delivery of blood. White cell antibodies are a cause of TRALI. HLA and platelet specific antibodies in platelet recipients rapidly destroy transfused platelets leaving

some patients vulnerable to fatal hemorrhage. Factors that determine antibody formation are poorly understood. Antigenic differences between donors and recipient, number of transfusions, genetic predisposition, and an underlying inflammatory state may be of importance.

Antibodies to red cell antigens are not common in the general population and are estimated to occur in between 0.5 – 1.5% of individuals. These antibodies are formed during pregnancy or following transfusion of red cells. When sensitive methods for antibody detection are used, 8.4% of patients receiving a mean of three RBC units form alloantibodies. In contrast, individuals with diseases such as sickle cell disease (SCD) or some forms of thalassemia, who may require lifelong RBC transfusion, develop RBC alloantibody at a much higher rate (18 – 76%) and face difficulty and delay in finding compatible blood. Several strategies to limit the prevalence of RBC alloimmunization in these patients are in use. In addition to ABO and D matching, limited RBC phenotype matching and transfusion with blood from C, E and K similar donors result in 5 – 14.5% of patients forming alloantibody. Antibody to C, E and K comprise two thirds of the antibodies found in SCD. Extended RBC matching beyond C, E and K antigens can be even more successful with 7% of transfused patients forming alloantibody.

Antibodies to human leukocyte antigens (HLA) are responsible for a number of different transfusion reactions: FNHTRs, TRALI, and TA-GVHD (see above). Refractoriness to random platelet transfusions occurs in 30-50% of platelet dependent patients. Immune mediated platelet refractoriness is primarily due to HLA class I antibodies and occasionally due to anti-platelet specific antibodies or high titer ABO antibody. Non-immune factors may contribute to a lack of an adequate platelet increase following transfusion, such as poorly stored platelets, sepsis, disseminated intravascular coagulation, and certain drugs, such as amphotericin B and ciprofloxacin. Leukoreduction of donated blood, best done at the time of blood collection (but can also be done prior to transfusion) decreases alloimmunization and adverse leukocyte reactions.

Neonatal alloimmune thrombocytopenia (NAIT), although not a transfusion reaction, is the result to maternal alloimmunization against paternally inherited platelet antigen that is similar to red cell antibody seen in *hemolytic disease of the newborn (HDFN)*. Contrary to HDFN, NAIT can occur with the first pregnancy. IgG antibody is transplacentally transferred to the fetus with destruction of fetal platelets. Antibody is most often (80%) directed against platelet antigen-1a (HPA-1a). In Asian populations, HPA-1a is very rare and anti-HPA-4b is the most common cause of NAIT. About 20% of infants born to women with anti-HPA-1a will have severe thrombocytopenia and clinically manifest signs of bleeding, such as petechiae, purpura, or mucocutaneous bleeding. The risk of intracranial hemorrhage is 1 – 14% in severely affected infants. Maternal antibody clears from the infant in 1-3 weeks. NAIT can be treated in utero by giving IVIG and dexamethasone to the mother; it can be treated after birth with IVIG and/or platelet transfusion to the infant.

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Immunomodulation by viable white cells from blood transfusion is poorly understood and the mechanism causing it is unknown. Blood transfusion prior to renal transplantation decreases allograft rejection and has a beneficial effect in women with recurrent abortion. Although controversial, blood transfusion is reported to decrease survival in patients with cancer and to increase infections in patients undergoing major abdominal, cardiac, and orthopedic surgery.

Transfusion Transmitted Infection

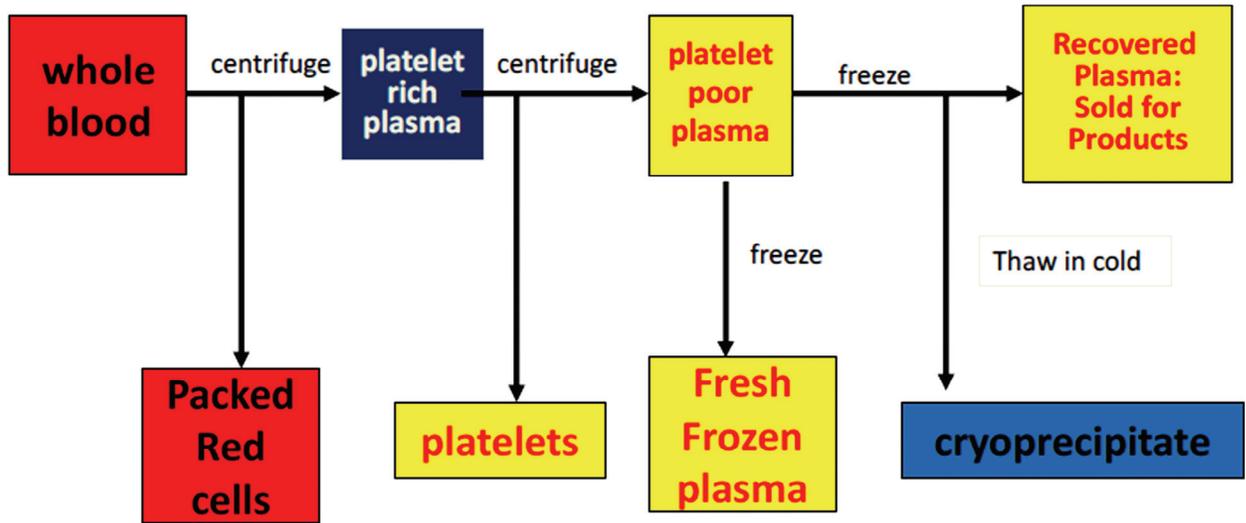
Great strides have been made in improving the safety of the blood transfusion. The risk of acquiring an infectious agent remains a constant threat to transfusion recipients and requires ongoing vigilance by practitioners. The risk of infection may vary by season for some organisms, such as West Nile virus, babesiosis and dengue, by the geographic prevalence for other microorganisms such as HIV, HTLV, hepatitis viruses and Chagas disease, or by methods of component collection, processing and storage, such as the majority of bacterial contamination. Progressive interventions have improved the safety of the blood supply beginning with testing for syphilis in late 1930, collection and storage of blood in integral system plastic containers, cold storage of components other than platelets, and improved donor screening and application of an expanding number tests to the blood collected. The delayed response to hepatitis and HIV transmission via blood and blood derivatives rightfully resulted in a hypersensitivity to disease transmission by blood transfusion. At this point, however, the cost of applying an increasing number of tests balanced with the decreasing benefits of those tests has been called into question.

Clearly, resources need to be balanced with risks. At a minimum, the World Health Organization (WHO) recommends all blood donations be screened for HIV-1 and HIV-2 antigen-antibody or antibody alone, hepatitis B surface antigen, hepatitis C antigen-antibody, or antibody alone and for syphilis (*T pallidum* antibody). Also, blood donations should be collected from volunteer, non-paid donors, preferably from repeat donors who are less likely to transmit infection. Other screening tests for infection, such as malaria, Chagas disease, or HTLV, should be based on local epidemiological evidence.

The United States has developed a number of different screening strategies, depending on the transmissible agent and the risk of transmission. A sample from each blood donation is pooled in batches and tested for HIV 1/2 and hepatitis B and hepatitis C viral nucleic acid. Individual nucleic acid testing for West Nile virus, a seasonal infection, is triggered based on the positivity of pool testing. Specific antibody testing is employed on each donation for HTLV-I/II, hepatitis B core, hepatitis C, HIV-1/2 and syphilis. Antigen testing is utilized for hepatitis B surface antigen. Geographic exposure, using donor history, triggers temporary donor deferral for malaria but may be indefinite as is the case for variant Creutzfeldt-Jacob disease. Once-in-a-lifetime donor testing is utilized for *T. cruzi* and sometimes for cytomegalovirus antibody.

Figure 4-5: Blood Component Therapy

Blood Component Therapy



Overview of the blood fractionation process

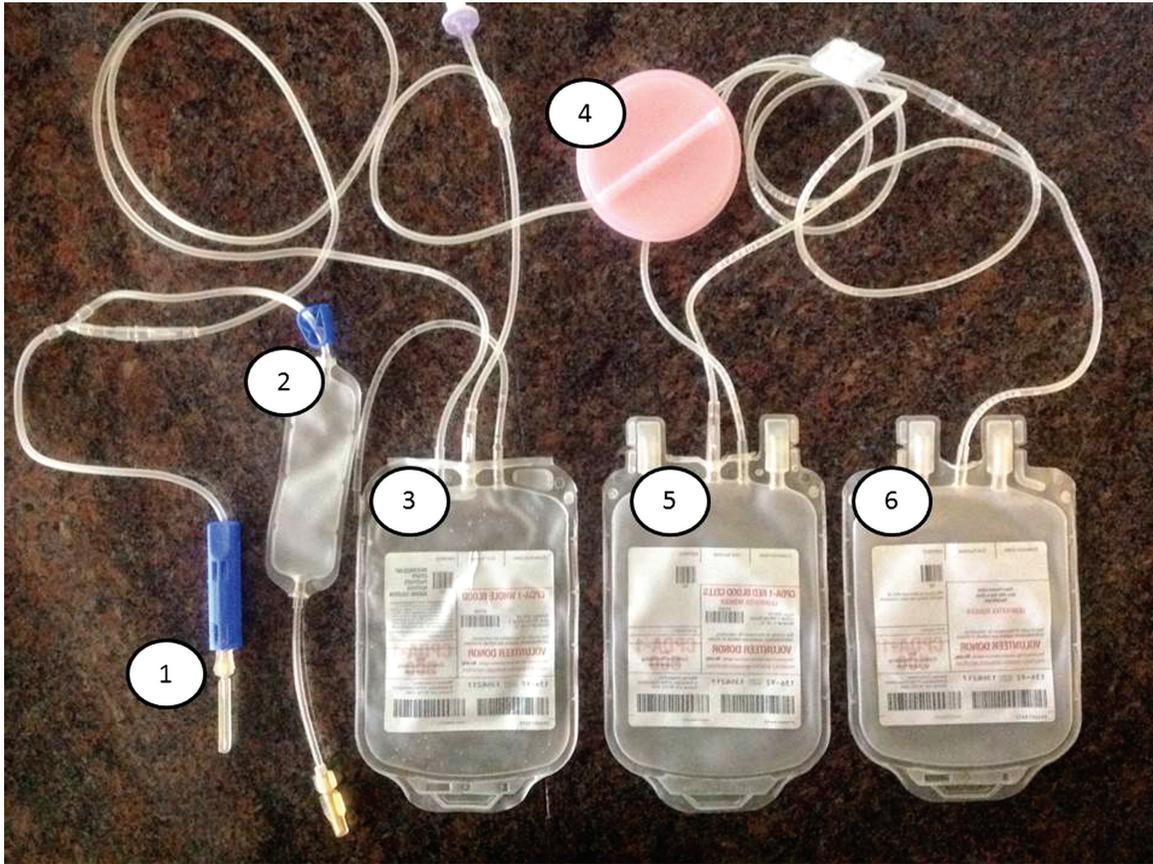
Blood Collection and Transfusion

Using sterile technique, whole blood is collected in a closed system with a hollow bore needle leading to a sterile plastic collection bag that contains a fixed amount of preservative. Collection systems with a variable number of attached satellite bags are available, depending on the planned number and type of derivative blood components to be produced (**Picture 4-1a**). Generally, the main collection bag is a 500 ml capacity bag with a suitable amount of preservative, which necessitates a donor weight of at least 110 lbs., since no more than 10% of the donor's blood volume may be collected at one time. Smaller capacity collection bag systems (such as 250 ml or 350 ml) with suitably reduced amounts of preservative are available for donors who weigh less than 110 lbs.

Each whole blood unit can be transfused as whole blood or fractionated. A single whole blood unit can be fractionated into packed red cells, a platelet concentrate and either a unit of fresh frozen plasma or cryoprecipitate. Using the proper collection system, cryoprecipitate is produced using a refrigerated slow thaw technique, centrifuging, and then discarding or selling the supernatant (recovered plasma).

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Picture 4-1a: A Collection System for Red Cells, Fresh Frozen Plasma, or cPyoprecipitate



1. *Hollow bore 16 gauge collection needle*
2. *In-line 10 ml bag for infectious disease testing*
3. *Main collection bag with CPDA-1 (whole blood)*
4. *In-line white cell filter*
5. *Satellite bag for leukoreduced packed red cells*
6. *Satellite bag for leukoreduced fresh frozen plasma*

The use of whole blood transfusions are discouraged by blood services able to fractionate blood into components because refrigerated blood loses platelet activity within 24-48 hours and coagulation factors begin to decline. After one week, 50% of factor V and VIII is lost in refrigerated plasma. Packed red blood cells (pRBCs) are suspended in thawed fresh frozen plasma (FFP) when needed for exchange transfusions.

Red Blood Cells

After whole blood is centrifuged, the platelet rich plasma layer is expressed into a pilot bag, leaving pRBCs in the primary collection bag (**Figure 4-5**). The tubing is sealed and the pRBCs refrigerated. RBCs may also be obtained by apheresis. RBCs should be stored at 1^oC. Shelf life depends on the preservatives and additive solutions. Citrate-phosphate-dextrose (CPD) and citrate-phosphate-dextrose-dextrose (CP2D) used as anticoagulants allow blood storage for 21 days (70% viability in the recipient at 24 hours). Citrate-phosphate-dextrose-adenine (CPDA) can extend shelf life to 35 days. Adenine saline (AS) solutions may further increase shelf life to 42 days. Coolers used to transport RBCs should maintain a temperature of 1-10^oC. RBCs should only be transfused with 0.9% normal saline or plasmalyte. Lactated Ringers (LR) and hypotonic solutions, such as D5W and 0.45% normal saline (NS), should not be used as carrier fluids for transfusions. All red cell transfusions must be filtered with a minimum filter size of 170 – 200 microns.

Low volume red cell transfusions for intrauterine and repeated low volume neonatal transfusions present unique difficulties. The red cells to be transfused are usually O⁻, CMV negative, and irradiated. Often only 10 -15 ml of red cells are required. Exposure to multiple donors is not desirable and sterility of the unit must be maintained for each transfusion. Several strategies are in use. Large institutions with multiple neonates requiring transfusion employ four to six aliquot bag collection units attached to the pRBC unit. If the satellite packed red cell unit is entered using a spike attached to the aliquot bags (**Picture 4-1b**), then the unit expires in 24 hours. If, however, the red cell unit is attached using a sterile docking device (**Picture 4-1c**), then the aliquots expire on the original red cell expiration date. More than one child is assigned to a single donor unit. Other hospitals sterilely draw blood from the pRBC unit, using a syringe. They filter the blood either at the time of withdrawal or at the time of transfusion in the neonatal intensive care unit. In the past, “walking donors” were used, but now the risk of infection using this technique is considered too high. However, in some low-income countries walking donor donations are still used as a source of blood, especially in an emergency. This blood has higher levels of coagulation factors and platelets.

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Picture 4-1b and 4-1c: Aliquot Bags for Small Volume (Infant) Red Cell Transfusions



Picture 5-1b

1. *Packed red cell satellite bag*
2. *Spike attached to four aliquot bags*

Picture 5-1c

Sterile docking device

Red cells are transfused to increase oxygen-carrying capacity in patients with anemia due to infection (malaria, dengue fever) with or without severe malnutrition, congenital hemoglobin disorders (sickle cell disease, thalassemia, other hemoglobinopathies), iron deficiency, or acute blood loss. Red cell transfusion should be avoided in non-emergent situations when other appropriate therapies are available, such as oral iron for iron deficiency. The child's hemoglobin/hematocrit and signs/symptoms of anemia and the rapidity of blood loss guide the appropriateness of red cell transfusion. Generally, in otherwise healthy children, acute loss of more than a 15 - 20% of their blood volume is needed before transfusion is initiated. Usually a hemoglobin (Hgb) of 6-7g/dl is well tolerated. Pallor, hypotension, tachycardia, and mental status guide the need for transfusion in the absence of a reliable measure of hemoglobin. Children with cyanotic cardiovascular disease may require a higher hemoglobin concentration. Studies in pediatric intensive care units support a transfusion trigger of 7g/dl in stable critically ill children, including children with non-cyanotic cardiac malformations. This restrictive transfusion trigger has also been repeated in pediatric patients with sepsis and postoperative general and cardiac surgery settings with similar results. A higher threshold of 9g/dl for children with cyanotic heart malformations is indicated. Children with chronic anemia, such as the hemolytic anemia of malaria, can tolerate an Hgb of 4.5 – 5g/dl or lower before transfusion is necessary.

Hemoglobin triggers for transfusion in neonatal ICU depend on age and clinical status of the patient (need for respiratory support or mechanical ventilation, hemodynamic instability and vasopressor requirements, presence of active bleeding, cardiac abnormalities). Most published guidelines are based on expert opinion and not clinical studies. Risks and benefits should be weighed before transfusion.

Low potassium or washed PRBC units may be indicated in patients who require ECMO, cardiopulmonary bypass (CPB), or who have poor renal function, until their hyperkalemia resolves. Washing is a process using 0.9% normal saline to remove unwanted plasma proteins and electrolytes. Washed RBCs may also be considered in pediatric patients with an unexplained severe or anaphylactic response to prior transfusion. The shelf life for RBCs after washing is 24 hours. Washing damages the cells. As many as one third of the red cells are lost during the washing process.

Hemoglobin/hematocrit transfusion increments are difficult to determine and several methods are used. Three common methods are:

- 1) 10-15ml of red cells/kg body weight are thought to increase the hemoglobin by 3gm/dl
- 2) A formula for *maximum allowable blood loss (MABL)* can be used to guide transfusion.

Volume of pRBC transfused = Total Blood Volume x (desired Hgb – actual Hgb)/
pRBC Hgb.

Total Blood Volume (TBV): pre-term neonates: 90 – 100ml/kg; full-term neonates:
80 - 90ml/kg; 6mos – 2 years: 80ml/kg; >2 years: 70ml/kg

- 3) Volume of pRBCs to be transfused = 4.8 x weight (kg) x desired rise in Hgb (gm/dl)

Platelets

Whole blood derived platelet concentrates are prepared from expressed platelet rich plasma that is not cooled below 20° C and is processed within eight hours of blood collection (**Figure 4-5**). The platelet rich plasma pilot bag is centrifuged and the surface platelets expressed into a second pilot bag. Platelets from multiple sources can be pooled at the blood center prior to transfusion. Apheresis platelets are obtained from one donor, limiting donor exposures, the possibility of alloimmunization, and infectious disease transmission, at an added expense. One pheresis unit is equivalent to six single donor platelet concentrates. Platelet pheresis is often divided in half (3x 10¹¹ platelets) for pediatric platelet transfusion. Sterility while obtaining and preparing platelets is vital, given the high risk of bacterial contamination. Platelets may only be stored for five days at 20-24°C, and they must be constantly agitated.

Prophylactic platelet transfusions can be considered for stable premature infants at a platelet count of <30,000 and at <50,000 for more unstable premature infants. Term infants <4 months old should be prophylactically transfused at <20,000 and at <10,000 if a term infants is older than four months. Patients who are scheduled for procedures having a risk for bleeding should be transfused when platelet counts are <50,000. Traditional dosages of platelets are approximately 10-15 ml/kg in the neonatal population with the expectation of a 6 x 10³ increase in platelet count. Cross match and ABO compatibility are normally not needed prior to platelet transfusion, but transfusing platelets for patients with the same ABO type may have advantages. Lack of response to platelet transfusion may indicate refractoriness. HLA-matched or cross-matched

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platelets may be used in this situation. Residual RBCs may also remain in platelet units, especially when using whole blood derived platelets.

In actively bleeding patients, platelet transfusions may be indicated when counts are $<50,000$. Platelets can be transfused regardless of count in actively bleeding patients with a qualitative platelet abnormality. In more specialized situations, such as diffuse bleeding related to CPB or ECMO, platelet transfusion may be required if counts are $<100,000$.

Platelets are issued as the most compatible blood type available but are not crossmatched. The more compatible the blood type, the longer the platelets will circulate in the patient's blood. Platelets concentrates can be "volume reduced" to remove the majority of the plasma for patients in whom volume overload is problematic. Platelets can be "washed" to remove leukocytes and cytokines accumulated during storage as well as chemokines to decrease FNHTR where leukodepletion is not available. However, platelets are lost with washing, and the concentrate expires 24 hours after washing. Platelets must be filtered prior to transfusion.

Fresh Frozen Plasma

Fresh frozen plasma (FFP) can be obtained by apheresis or from whole blood after platelets are removed from platelet rich plasma (**Figure 4-5**). Following this process, the plasma is frozen at -18°C within 8 hours and may be stored in this state for one year. Once needed for transfusion, FFP is warmed at $30-37^{\circ}\text{C}$. This thawed plasma is stored at $1-6^{\circ}\text{C}$ for up to 24 hours. If not transfused within 24 hours, it may be refrozen and thawed at a later date for use (FFP-24).

FFP is transfused to provide coagulation factors in bleeding patients when the PT/PTT is 1.5 times greater than the mid-point of the normal range. This indicates that less than 30% of the normal amount of a coagulation factor is present, which is the point at which bleeding can occur in the face of a normal level of platelets. The International Normalized Ratio (INR), developed to standardize PT measurements within laboratories using tissue thromboplastin of variable strength, approximates this point within the usual clinical range for the PT. Importantly, the INR of FFP itself is approximately 1.2-1.5.

FFP transfusion may be considered when INR is greater than 1.5 to 2.0 in a non-bleeding patient scheduled for an invasive procedure or when $\text{INR} >1.5$ in diffusely bleeding patients. FFP is also indicated in patients who have a warfarin overdose and will undergo major surgery or an invasive procedure. Other indications include patients with TTP undergoing transfusion or plasma exchange, protein C, protein S, AT deficiencies with heparin resistance, C1 esterase inhibitor or other single factor deficiency (with the exception of Factor IX - where it is relatively ineffective) when no product is available and a patient is bleeding. FFP may also be used to treat bleeding secondary to vitamin K deficiency. FFP should not be used for volume expansion or for ITP. FFP must be filtered with a large pore filter (150-170 micron). The dosing is approximately 10-15ml/kg in pediatrics.

Cryoprecipitate

Cryoprecipitate is a source of factor VIII, fibrinogen, von Willebrand factor (vWF), factor XIII, and fibronectin. It is quality controlled to contain at least 80 IU (international units) of factor VIII and >150mg fibrinogen per concentrate. It is the milky white precipitate that forms when FFP is thawed at 4°C and remains in small volume following centrifugation and removal of the surface plasma (now termed recovered plasma). It is commonly pooled from multiple units prior to storage. Cryoprecipitate may be stored for one year at -18°C. Once thawed at 30-37°C, it may be stored for six hours at 20-24°C.

First developed as a source of factor VIII and used in pools for small children who could not tolerate the volume load of FFP, it is used in the operating room as a source of fibrinogen where fibrinogen concentrate is not available or affordable. The amount of fibrinogen in one concentrate is roughly equivalent to the amount of fibrinogen contained in the one unit of FFP from which it is made. Cryoprecipitate is indicated when fibrinogen levels are <100mg/dl and an invasive procedure is scheduled, or for patients with qualitative fibrinogen disorders experiencing diffuse bleeding or undergoing a scheduled procedure with the potential for blood loss. It may also be used in von Willebrand disease or factor VIII deficiency when there is active bleeding; it may also be used for scheduled procedures when the patient is unresponsive to DDAVP or factor concentrates. Cryoprecipitate may be transfused without compatibility testing, though testing may be the preferred method by some practitioners. Cryoprecipitate is dosed at one concentrate per 10 kg body weight in pediatrics.

Resuscitation of Acute Surgical Bleeding and Trauma

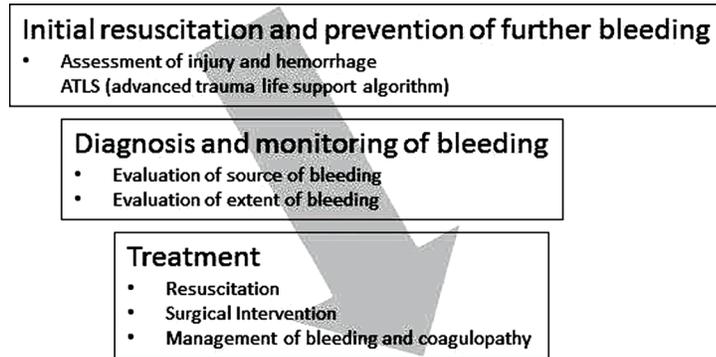
Massive bleeding resulting from trauma or acute, active surgical bleeding may produce shock via hypovolemia and decreased cardiac output, with the end result being decreased oxygen delivery to end organs. Metabolic acidosis, inflammation, and signs of hypovolemia and hypoperfusion often accompany this presentation. Hypothermia is common during active resuscitation, especially in trauma situations, from environmental exposure, use of unwarmed intravenous (IV) fluid, and disruption of thermoregulatory mechanisms. Acute coagulopathy related to shock, hypoperfusion, and hemodilution follow. Combined, acidosis, hypothermia, and coagulopathy form what is known as the “lethal triad” or “triad of death,” and thus need to be corrected rapidly and aggressively. The primary treatment of hemorrhage is surgical hemostasis and fluid resuscitation.

With initial management of bleeding (Figure 4-6), large bore IV access is obtained and crystalloid is infused in acutely unstable patients while avoiding hemodilution, which may further decrease oxygen carrying capacity, oxygen delivery, and coagulation factors. Blood samples should be sent for type, screen, and crossmatch when time allows. If the patient is in emergent need of blood, O negative blood should be transfused without testing. If O negative blood is not available, type

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specific blood may be transfused in emergent scenarios. Crossmatched blood should always be transfused first when available. Refer to the above *hemoglobin/hematocrit transfusion increments*.

Figure 4-6: General Algorithm for Management of Acute Bleeding Following Trauma



General algorithm for evaluation and treatment of trauma patient: Each step is part of a continuum, which may be performed simultaneously during resuscitation.

Massive Transfusion Protocols

In some institutions, massive transfusion protocols (MTP) exist and may be activated when faced with active and massive bleeding. Massive transfusion has been defined many ways in the literature, but a common definition in adults is transfusion of 10 units in 24 hours or replacement of one blood volume. In pediatrics, it has been defined as transfusion of greater than 40 ml/kg in the first 24 hours. The principles of MTP involve clinician activation. Type and screen and a second confirmatory specimen are usually required. Emergent uncrossmatched O negative blood is immediately released. MTP “packs” made up of crossmatched PRBCs, FFP, and platelets are then created. Cryoprecipitate is sometimes included as well. In some pediatric protocols, these packs are based on weight. The blood bank stays in close contact with the clinical team in order to determine the ongoing need to provide “packs” of blood products. The protocol is discontinued at the clinician’s discretion.

Transfusion of PRBCs, FFP, and platelets in proportions similar to whole blood is used to prevent dilutional coagulopathy. The ideal ratio of PRBC to FFP is controversial. Commonly ratios of 1:1- to-1: 2.4 are used and have been shown to improve outcomes in retrospective studies on adults. Pediatric massive transfusion data is more limited and further studies need to be done for validation in this patient population. Calcium repletion is needed due to citrate in the storage bags. Ideally regular laboratory studies, including blood gasses, complete blood counts, potassium, calcium, PT/PTT, fibrinogen, and thromboelastography, should be obtained to guide transfusion therapy.

Leukoreduction

Most cellular components are leukoreduced in the United States prior to transfusion. Universal leukoreduction is the rule in Europe. Leukoreduction decreases the incidence of FNHTR, CMV transmission to patients at increased risk, and alloimmunization to leukocyte antigens. The concentration of leukocyte content in whole blood is on the order of 10^9 per adult collection. Approximately 90% of leukocytes are fractionated with RBCs. The recommended goal of leukoreduction is that each component contains $<5 \times 10^6$ residual WBCs per collection. The recommended residual WBC content for platelets is $<8.3 \times 10^5$ per unpooled unit. FFP does not require leukoreduction, as the residual WBC content is below the recommended limit. This is accomplished primarily through filtration but also by cell adsorption to the filter. Filtration should occur through a closed system that involves an in-line filter and sterile tubing. All blood products should be leukocyte reduced within five days of collection and prior to storage to ensure quality control and standardization of this process. It is possible to leukoreduce at the bedside if an appropriate filter is available, but this has been associated with the occurrence of hypotension. Filters for platelets are not interchangeable with filters intended for RBC use, due to differences in design. Modern filtration systems have effective pore sizes <10 microns. All blood products should be infused through at least, a large pore filter (170-260 micron).

Limiting exposures

It is important to limit donor exposures in the pediatric population, if possible. One strategy includes blood collections from a limited pool of known, screened donors that are available when transfusions are needed for a specific patient. Another strategy is to divide one adult component unit into smaller aliquots in a sterile fashion.

Irradiated Components

It is also important to note that leukocyte depleted products cannot be used to prevent graft versus host disease. Irradiated components are needed. Candidates for irradiated products include infants <6 months old, all pediatric patients with malignancies, neonatal exchange transfusions, recipients of components from first degree relatives, myelosuppressive therapy, candidates for marrow or peripheral blood stem cell transplants, and congenital immunodeficiency syndromes. Irradiation adds to component cost and alters red cell metabolism, causing accelerated potassium loss, increased free plasma hemoglobin, and shortened red cell survival.

IgA Deficiency

Special considerations for IgA-deficient patients are that platelet recipients with anti-IgA antibodies should receive platelets and FFP from other IgA deficient donors. These patients can receive washed RBCs, but frozen deglycerolized RBCs are preferred in this population.

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CMV Negative Components

CMV is carried in a small minority of donor monocytes. CMV negative components should be used for CMV seronegative patients who are immunoincompetent including infants <6 months old, those with congenital or acquired immunodeficiencies, and bone marrow or organ transplant candidates or recipients of CMV negative grafts.

Special Transfusion Considerations: Hereditary Disorders of Hemostasis

A thorough medical history is the foundation of assessing the risk of bleeding/clotting. A disorder may be identified from the family history. Hemophilia A (factor VIII deficiency) and hemophilia B (factor IX deficiency) are sex-linked disorders occurring in the male children of mothers carrying the mutation. Autosomal dominant inheritance occurs with von Willebrand's disease. It is important to ascertain whether bleeding is spontaneous, longstanding, or follows injury. Seemingly spontaneous bleeding occurs in more severe bleeding disorders, such as the hemophilias or von Willebrand's disease. Milder bleeding disorders, such as mild factor deficiency, factor XI or XII deficiency, may present after surgery or following trauma. Bleeding from factor XIII deficiency typically occurs 24 – 48 hours after surgery. The physical location of bleeding can help identify the cause. Mucosal bleeding, bruising or petechiae typically occur with hereditary platelet disorders. Hemophilias present with spontaneous bleeding into joints, particularly elbows and knees.

Procoagulant Disorders: Congenital Platelet Disorders

Platelets can be abnormal in number (quantitative) or function (qualitative). Disorders can be inherited or acquired. Inherited platelet abnormalities are rare. von Willebrand's disease (VWF) and afibrinogenemia, which do influence platelet function, are more common but are factor abnormalities (see below) rather than platelet abnormalities.

Acquired quantitative abnormalities in which platelet numbers are low but function is normal are more common and are due to either decreased bone marrow production, as that caused by viral infection (HIV, EB virus, CMV, hepatitis B), aplastic anemia, paroxysmal nocturnal hemoglobinemia, or leukemia. More commonly, it can be caused by peripheral destruction of platelets, often due to antibodies against the platelet membrane, as occurs in autoimmune thrombocytopenia, or following transfusion, platelet consumption due to endothelial damage, as occurs with Rocky Mountain spotted fever, meningococemia, HELLP syndrome in pregnant women, or in the renal microcirculation, as in hemolytic uremic syndrome. Platelets can also be sequestered in the spleen, which normally contains one third of circulating platelets.

Intrinsic platelet abnormalities include Bernard-Soulier syndrome, Glanzmann's thrombasthenia, and storage pool disorders. In Bernard-Soulier syndrome there is an absence of the platelet

surface receptor GP 1b to which von Willebrand’s factor binds, inhibiting normal subendothelial binding. These patients have mild thrombocytopenia, giant platelets, and a prolonged bleeding time. Platelets fail to agglutinate on exposure to ristocetin. Glanzmann’s thrombasthenia is an autosomal recessive disorder in which there is an absence of the platelet GP IIb-IIIa binding site for fibrinogen. Patients with thrombasthenia have severe mucous membrane and postoperative bleeding. Platelet transfusion may be required to stop bleeding. Bleeding time is very prolonged and platelets do not aggregate with exposure to physiologic stimuli (ADP, collagen, epinephrine, thrombin). Storage pool disorders are disorders of platelet granule secretion. Bleeding is mild and patients experience easy bruising, occasional excess mucous membrane bleeding, and postoperative bleeding. Platelets may be deficient in dense granules (decreased storage pool ADP), alpha granules (thrombospondin, factor V; “grey platelet syndrome”), absent thromboxane A2 synthetic material (cyclooxygenase or thromboxane synthetase), or thromboxane A2 functional deficiencies (defective surface membrane receptors for thromboxane A2). These patients have normal appearing platelets, moderately prolonged bleeding times and normal ristocetin-induced platelet agglutination but impaired or absent aggregation with exposure to collagen, epinephrine, and low concentration of ADP.

Table 4-4: Procoagulant Disorders: Congenital Factor Deficiency

von Willebrand’s Types	
I	Low levels of vWF (80% of cases)
II a	Abnormal vWF
	Have low amounts of high LW multimers
II b	Abnormal vWF
	Avidity to platelet glycoprotein Ib
	Low platelets
III	Low vWF and low factor VIII
*Platelet type	Increased avidity to platelets

Deficiency of von Willebrand’s factor (VWF), a multimeric molecule that stabilizes circulating factor VIII and binds platelets to one another and to endothelium, is the most common bleeding disorder. There are three main forms of deficiency: decreased normal multimer (80% of cases), abnormal multimers, and absent multimers. VWF is synthesized in the endothelium, as is factor VIII. Bleeding in mild to moderate cases can be treated with desmopressin (DDAVP), which releases VWF from the endothelium. Endothelial VWF is released within 30 – 60 minutes. Synthesis of new endothelial VWF requires six hours. The platelet types of VWF (IIb and platelet type) are not treated with DDAVP because increased abnormal VWF lowers the platelet count. Type III VWF is not treated with DDAVP, since there is a complete absence of VWF. Other

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treatments include intermediated purity factor VIII concentrates (Alphanate, Humate-P), fresh frozen plasma, and cryoprecipitate. Mucosal surfaces are rich in fibrinolytic agents. Consequently, antifibrinolytic agents, such as epsilon aminocaproic acid (EACA) and tranexamic acid (10 times more potent than EACA), are useful adjuncts to other treatments for mucosal bleeding. Antifibrinolytic agents are contraindicated during episodes of hematuria, due to concerns with urinary tract obstruction.

The two most common coagulation factor disorders are *hemophilia A* (factor VIII deficiency) and *hemophilia B* (Christmas disease or factor IX deficiency). Both are X-linked congenital disorders occurring in 1 out of 5,000 males (80 – 85% hemophilia A and 10% hemophilia B). Hemophilia is graded as severe (<1% of normal FVIII or FIX levels), moderate (1 - 5% of normal levels) or mild (>5% of normal levels). Individuals with severe hemophilia bleed with little or no apparent trauma, those with moderate disease bleed following mild to moderate injury, while mild hemophiliacs bleed after severe trauma or surgery. Normal individuals have 100% or 100 units of factor/ml of plasma.

Patients with severe hemophilia are increasingly treated with scheduled prophylactic factor concentrate infusion to prevent spontaneous bleeding and its chronic sequelae. Mild to moderate trauma or bleeding is treated with sufficient factor to raise plasma factor levels to 40% of normal. For severe bleeding or major surgery, factor levels are raised to 100% of normal and maintained at a trough level of 80 – 100% for some time after the event. Some patients with mild hemophilia A will experience mild to moderate bleeding benefit from treatment with DDAVP. Episodes of severe bleeding are not controlled with DDAVP. DDAVP is not useful in the treatment of severe hemophilia A or in the treatment of any form of hemophilia B. Replacement factor concentrates are either high purity plasma concentrates or recombinant concentrates. Each unit of factor VIII concentrate increases the plasma factor VIII level by 2% with a half-life of 10 – 12 hours. The desired rise in plasma factor VIII level is calculated by: $\text{desired rise (U/dl)} \times \text{kg body weight} \times 0.5$ with repeat dosing every 12 hours. For factor IX concentrate, each unit increases plasma factor IX levels by 1% due to increased extravascular distribution; its half-life of 18 – 24 hours. Recombinant factor IX in children is generally dosed 1.2 – 1.4 times higher than adults due to the higher volume of distribution in children. The desired rise in plasma factor IX level is calculated by: $\text{desired rise (U/dl)} \times \text{kg body weight} \times 1.2$ with repeat dosing every 24 hours. Ideally, factor levels should be monitored during treatment whenever possible due to individual and product variable responses. Mucosal hemostasis can be augmented with EACA or tranexamic acid.

Some hemophiliacs (20-30% hemophilia A, 1-4% hemophilia B) form inhibitors i.e. neutralizing alloantibodies to factor concentrates. Subsets of hemophilia B patients with inhibitors develop anaphylaxis. Increasing the dose of factor concentrate can successfully treat patients with low levels of inhibitors. Bleeding episodes in hemophilia A patients with high levels of inhibitors are

treated with continuous infusion of factor concentrate or bypassing agents, such as recombinant factor VIIa or activated prothrombin complex concentrates (Factor VIII inhibitor bypassing agent, FEIBA). Bleeding hemophilia B patients who have inhibitors can be treated with recombinant factor VIIa or prothrombin complex concentrates (contraindicated in patients with anaphylaxis). Immune tolerance has been achieved in some patients using high dose factor concentrate, immune globulin infusion, immunosuppression and extracorporeal immunoabsorption.

Deficiency of other coagulation factors is rare and may or may not be associated with bleeding. Congenital deficiency of fibrinogen, prothrombin, Factors V, V/VIII, VII, X, XI and XIII may be clinically significant and inherited as autosomal recessive traits. Each of these deficiencies may present as mucosal bleeding or as unexpected and sometimes life threatening bleeding during surgery. Quantitative specific factor assay may or may not predict the severity or frequency of clinical bleeding, particularly with factor VII and factor XI deficiency. Specific factor concentrates should be used, if available, during bleeding episodes or when the risk of bleeding is high (surgery) in order to minimize the risk of transfusion transmitted infection.

Deficiency in Factor XII, prekallikrein, or high molecular weight kininogen, has no effect on hemostasis but may protect from arterial thrombus formation.

Clotting Disorders: Congenital Hypercoagulable Disorders

Hereditary thrombophilias are a group of disorders in which individuals are more likely to develop either venous or arterial clotting. They comprise a group of mutations involving procoagulants (factor V Leiden, factor II, factor VII), natural anticoagulants (antithrombin, protein C, protein S), inhibitors of fibrinolysis (plasminogen-1 activator inhibitor) or other mutations (methylene tetrahydrofolate reductase deficiency- MTHFR). All of these deficiencies have in common a propensity to abnormal clotting.

Antithrombin, a serine protease inhibitor, preferentially binds and neutralizes the active site of thrombin, Xa, IXa, XIa inhibiting coagulation. Antithrombin activity is markedly enhanced (100x) by heparin binding. Heparin is inactive in the absence of antithrombin (heparin resistance). Local release of platelet factor four from lysis of activated platelets also inhibits antithrombin. Deficiency of antithrombin leads to uninhibited thrombin conversion of fibrinogen to fibrin and consequent thromboembolic events. Homozygous AT deficiency is incompatible with life. Heterozygous AT deficiency, occurring in 1:2,000 individuals, is usually asymptomatic, but spontaneous thromboemboli and habitual abortion may occur.

Protein C and protein S work together and require vitamin K for anticoagulant effect. Both protein C and protein S are manufactured in the liver in inactive forms. When activated, protein C neutralizes Va and VIIIa. Protein C is also thought to participate in activation of fibrinolysis. Protein S accelerates the process. Homozygous protein C or protein S deficiency is thought to be

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incompatible with life and heterozygous deficiency results in a propensity to thromboemboli. Factor V Leiden (8% of the white population) is resistant to activated protein C.

Perhaps the most common type of hereditary thrombophilia, particularly in individuals of Northern European ancestry, is resistance to activated protein C (APC resistance) caused by a single amino acid mutation on factor V that results in idiopathic recurrent thromboembolism, particularly when other factors are present, such as surgery, pregnancy, birth control pills, or prolonged inactivity.

Red Cell Disorders Requiring Repeated Red Cell Transfusion

In *Sickle Cell Disease (SCD)*, the two β globin molecules of hemoglobin (two α and two β globins) each have a single amino acid substitution (valine for glutamic acid) in SCD. This substitution of a hydrophobic residue for a hydrophilic residue results in a predilection to hemoglobin polymerization on deoxygenation. Red cell dehydration and deoxygenation occur as red cells traverse the microcirculation. Hemoglobin then polymerizes trapping leukocytes and platelets, damaging the endothelium and occluding small vessels. Patients who have persistent high levels of fetal hemoglobin experience less severe disease. These changes give rise to the adverse consequences of SCD: acute anemia, stroke, acute chest syndrome and rarely hyperhemolysis syndrome.

Chronic transfusions are often used to reduce the incidence of these complications. However, complications of chronic transfusion therapy can include transfusion reactions, transfusion of transmitted infections, red cell alloimmunization, delayed hemolysis, and iron overload. The high rate of red cell alloimmunization (25% vs. 0.5 – 1.5% in the general population) is attributed to racial differences between donors and recipients, leading to discordance between donor and recipient red cell antigens, inflammation, immune responsiveness, number of transfusions, and age at first transfusion. Several strategies are currently used to minimize antibody formation and delayed hemolysis. Some institutions phenotypically match the red cells to be transfused either before any transfusion or after the formation of the first red cell antibody. Limited antigen matching is done with C, E, and K. Extended red cell antigen matching usually entails the addition of Duffy, Kidd and MNS. Nevertheless, antibody may form to Rh variant alleles and/or uncommon red cell antigens. The distribution of red cell antigens is population dependent and influences alloantibody specificity. For example, in Asians the prevalence of K antigen is low and anti-K alloantibody uncommon. In comparison, the MNS antibodies anti-Mi^a/Mur are found in 31% of chronically transfused Chinese thalassemia patients with alloantibody.

Delayed transfusion reactions are estimated to occur in 5% of transfused SCD patients. These reactions generally occur two days to two weeks after transfusion and may or may not be accompanied by measurable antibody or a positive DAT. *Hyperhemolysis syndrome* is an uncommon but is a serious complication following transfusion; the post transfusion hematocrit

falls to a level lower than the pre-transfusion hematocrit. Although not well understood, hyperhemolysis syndrome is thought to be due to hemolysis of autologous red cells, suppression of erythropoiesis, or macrophage activation.

Individuals with sickle cell trait (one abnormal β globin) do not experience the complications of those with SCD. Individuals with HbSC and HbSb⁰ (β thalassemia with no normal beta chain) may experience complications similar to those with SCD. Patients with HbSb⁺, in which some normal beta chain is produced, do not experience the complications of SCD.

Chronic transfusion is often necessary for treatment of severe forms of β thalassemia, β thalassemia major and hemoglobin E- β thalassemia (HbE- β thal). HbE- β thal is relatively common in parts of India, Bangladesh, Myanmar and Southeast Asia.

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