Overview of Inflammation and Coagulation
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Inflammation and Coagulation: Review of Normal Responses

Inflammation is the body’s immediate response to all types of cell injury. It is usually short in duration (hours to days). Within seconds of any tissue injury (e.g., hypoxia, trauma, infection), proinflammatory cytokines are released, activating a host of responses. Inflammation in turn will activate coagulation. Inflammation and coagulation are so vital to our survival that both responses are triggered by a number of different mediators. These multiple triggers promote amplification of the inflammatory and coagulation responses when a large or persistent stimulus is present.

Normally, homeostasis is achieved by the simultaneous release of anti-inflammatory cytokines and anticoagulants, and by initiating coagulation. Knowledge of the inflammatory and coagulation responses is needed to understand problems common in critical care (e.g. sepsis, Adult Respiratory Distress Syndrome, Acute Coronary Syndrome, Acute Brain Injury) and provides the basis for understanding common pharmacological interventions. This discussion will provide an overview of normal inflammation, coagulation and homeostasis.

Normal State

Endothelial cells make up the lining of all blood vessels (called endothelium). Capillary walls are made up of a single endothelial cell layer. Normally, tight junctions located between adjacent endothelial cells permit only small substances to pass across the capillary endothelium (e.g., oxygen, carbon dioxide, glucose).

For every single white blood cell (leukocyte) in normal blood, there are approximately 700 red blood cells (erythrocytes). Only 1% of blood cells are white or platelets; RBCs make up 99%.

During blood flow, the heavier red cell mass travels down the centre of the vessel lumen, pushing the platelets and leukocytes toward the endothelium. In normal states, leukocytes and platelets continue to flow without sticking to the blood vessel endothelium because non-activated endothelium releases substances that keep the vessel wall relaxed and non-adherent (e.g., prostacyclin, nitric oxide).

INFLAMMATION

Inflammation is the non-specific response of the microscopic circulation to tissue injury. Tissue injury causes mast cells to degranulate, leading to the release of dozens of inflammatory mediators, including histamine. Histamine augments blood flow to the affected area and increases blood vessel permeability. Proinflammatory substances stimulate neutrophil production and activate endothelial cells at the site of injury.

Neutrophils are the “first responders” of the leukocytes. They begin to increase in number within seconds of an inflammatory response. Neutrophils are phagocytes that “gobble up” pathogens and dead cells. Once trapped within the neutrophil, toxic substances that kill and digest the pathogen are released. When phagocytes die, their contents (dead bacteria and toxic agents) are released into surrounding tissue and appear as pus. The same substance that is toxic to invading bacteria can be injurious to surrounding healthy tissues, thus, persistent exudate can become a trigger for more inflammation such as seen at the edges of draining wounds.

Endothelial cells also become activated at the onset of inflammation. Activated endothelial cells contract and increase the spacing between endothelial cells, increasing the blood vessel permeability. Activated endothelial cells also express proteins onto their surface that are adhesive or “sticky” for neutrophils and platelets.

Recall that neutrophils and platelets are naturally pushed toward the endothelium during blood flow. During inflammation, neutrophils and platelets are pushed toward activated endothelial cells and begin to adhere to the vessel surface. Neutrophil adherence (called margination) causes the neutrophils to roll along the vessel surface. This slows their rate of flow in the area where the blood vessels are also more permeable (leaky). Thus, during inflammation, the increased number of neutrophils are drawn to the site of inflammation where they quickly migrate toward the injured tissue (diapedesis). Water, protein and other cells also move across permeable endothelial membranes. This provides substrates for tissue repair, but also causes the accumulation of tissue edema.
Advancements in technology such as hand held microscopes have enabled scientists to examine the microscopic circulation during inflammation. Sluggish white cell circulation and increased permeability on the venule side of the capillary can be observed.

When inflammation continues for days, or becomes persistent, the short life span and limited phagocytic capacity of the neutrophil may become inadequate. Monocytes are the next leukocytes to be activated. When monocytes are activated, they begin to migrate into injured tissues where they mature to become tissue macrophages. Macrophages are larger phagocytes, with longer survival times and more powerful killing capabilities than neutrophils. When inflammation persists, toxic contents released from dying neutrophils and macrophages can cause harm to healthy tissues, leading to increased or chronic inflammation.

Activated neutrophils, monocytes and macrophages all release substances called cytokines. Proinflammatory cytokines help to eradicate infection, remove dead cells and promote tissue repair. Examples of proinflammatory cytokines are tumour necrosis factor-α (TNF-α), interleukins 1, 2, 6 and 8 (IL-1, IL-2, IL-6 and IL-8) and interferon-γ. To achieve homeostasis, the release of proinflammatory cytokines triggers the release of anti-inflammatory cytokines. Examples of anti-inflammatory cytokines are interleukins 4, 10, 11 and 13 (IL-4, IL-10, IL-11 and IL-13), soluble TNF-α and IL-1ra. When the inflammatory trigger subsides, proinflammatory cytokine release stops, allowing the anti-inflammatory cytokines to dominate and restore homeostasis.

Activated endothelial cells and various other proinflammatory mediators also trigger an increase in blood flow to the injured area. Initially, vasoconstriction of affected blood vessels and surrounding arteries occurs in order to decrease bleeding. This is followed by a vasodilatory response, most notably in the venules. The net effect is that blood flow increases in the direction of the permeable venules. This enhances the delivery of neutrophils, nutrients and water toward the injured tissue.

Prostaglandins and bradykinins also trigger pain receptors. Pain encourages the individual to attend to the injured tissue and immobilize the area of injury. Activated white blood cells release cytokines that increase the metabolic rate and trigger fever production. The higher rate of metabolism enhances blood flow delivery and facilitates oxygen extraction by the tissues. The accumulative effect of an increased blood flow, increased metabolic rate and pain receptor stimulation is a warm, throbbing, painful area of inflammation.

**COAGULATION**

Coagulation can be divided into 3 distinct phases. Phase one is initiated when platelets begin to adhere to the surface of activated endothelium. Phase two is the creation of a platelet plug produced when platelets begin to aggregate or stick to each other. The final phase occurs when the coagulation cascade is activated, leading to the production of fibrin that will stabilize the clot by holding it in place. Endothelial cells play an important role in mediating adherence, aggregation and activation of the clotting cascade.

Inflammation stimulates an increase in production of platelets, similar to the increase in neutrophils. In early systemic inflammation, an increase in the number of platelets can be seen. In severe and persistent systemic inflammation, the ongoing consumption of platelets can lead to a drop in the platelet concentration. Thus, in systemic inflammation, platelet counts may be elevated or reduced.

**Homeostasis**

Normal endothelium inhibits coagulation by producing substances such as nitric oxide and prostacyclin. Both of these substances promote vasodilation and platelet inhibition. Non-activated endothelium also expresses the protein thrombomodulin, which facilitates anticoagulant and fibrinolytic activity by converting Protein C to activated Protein C (discussed later).

Additionally, normal endothelium expresses other anticoagulants such as glycosaminoglycans and heparinoids. Once formed, clots are removed from the vessel wall by a process called fibrinolysis. Fibrinolysis is the breaking down of a fibrin clot, a process that is triggered when tPA converts plasminogen to plasmin, the substance responsible for clot lysis. In the area of tissue injury, activated endothelial cells promote clot formation and inhibit clot lysis. In the uninjured areas, non-activated endothelium opposes clotting.
Platelet Activation

Normal blood contains a sufficient number of circulating platelets to achieve hemostasis when required. Following a break in the endothelial lining, platelets are exposed to subendothelial connective tissue. Circulating von Willebrand factor causes platelets to attach to the exposed collagen at the site of injury. Platelet attachment to the endothelium is referred to as adherence. Once activated, platelets release granules, which contain: ADP, serotonin, fibrinogen, lysosomal enzymes and platelet factor 4. Activated platelets also stimulate the production of the prostaglandin thromboxane A2 (TXA2).

Platelet aggregation is the attachment of platelets to other platelets that are already bound to the endothelial wall. ADP and TXA2 both stimulate platelet adhesion and aggregation. In addition, platelet aggregation leads to the exposure of binding sites on the platelet surface known as Glycoprotein IIb and IIla receptor sites (GPIIb/IIla). These receptor sites facilitate platelet-to-platelet connections that enhance aggregation and increase the size of the platelet plug.

A number of drugs used in critical care influence platelet activity. For example, DDAVP (desmopressin) increases coagulation by enhancing the release of Factor VIII components, including von Willebrand factor. Aspirin blocks TXA2 to decrease platelet adhesion. Because prostaglandins also stimulate inflammation and trigger pain receptors, aspirin also has anti-inflammatory and analgesic properties. Although heparin is used more commonly for its anticoagulant properties (preventing stabilization of the clot), heparin also inhibits platelet aggregation by blocking the thrombin binding sites on platelets. Clopidogrel (Plavix) blocks ADP receptors to decrease platelet adhesion and aggregation, whereas, drugs like abciximab (ReoPro) and epifibatide (Integrilin) block the GP IIb/IIla receptors. Multi-drug therapy is often used in acute coronary syndrome to ensure adequate platelet inhibition by using various drugs that each block a different platelet binding receptor.

Activation of Clotting Cascade

Activated or injured endothelium loses its natural anticoagulant property at the site of the tissue injury. Activated endothelium stops producing nitric oxide and prostacyclin, and decreases the amount of thrombomodulin. In addition, activated endothelial cells and monocytes express large amounts of Tissue Factor (TF), an important trigger of the coagulation cascade. Activation of the clotting cascade leads to the generation of thrombin. Thrombin in turn stimulates additional ADP and TXA2 increases platelet aggregation and triggers the conversion of fibrinogen to fibrin. Fibrin strands create a mesh-like structure to hold the platelet plug securely in place. Thrombin actually promotes more thrombin production by activating co-factors VIII and V of the coagulation cascade to stimulate additional thrombin production.

To ensure adequate coagulation occurs, the presence of TF and/or thrombin actually delays the onset of fibrinolysis. TF triggers the production of Plasminogen Activator Inhibitor (PAI 1), an endogenous inhibitor of tPA, whereas, thrombin mediates the production of Thrombin Activatable Fibrinolysis Inhibitor (TAFI). If the inflammatory trigger is large or persistent, platelet activation, TF generation and thrombin production will also persist, leading to ongoing clot production and inhibition of fibrinolysis. A pro-coagulant state will remain until the inflammatory trigger is removed.

As previously mentioned, TF is an important activator of the coagulation cascade. The coagulation cascade consists of a series of enzymatic reactions that terminate in the development of a fibrin clot. Activation of each coagulation factor initiates the activation of the next factor in a sequential fashion. All of the coagulation factors except fibrinogen are either proenzymes or cofactors. Proenzymes are transformed into activated enzymes after hydrolysis of one or more of the peptide bonds. Cofactors include Factor III (Tissue Factor), V and VIII. Each acts as a catalyst to accelerate other enzymatic reactions. A Roman numeral is used to symbolize the various coagulation factors (listed in Table 1). When one of these coagulation factors becomes activated, a small letter “a” is added to the Roman numeral to denote the activated form of the coagulation factor (Figure 1).
Historically, the initiation of coagulation was believed to occur as a result of activation of either the intrinsic or extrinsic pathways. Both of these pathways converge to become one common final pathway when factor Xa and Va are combined (see Figure 1). The factor Xa:Va complex is responsible for the conversion of prothrombin to thrombin. Thrombin causes fibrinogen to become fibrin.

It is now believed that most coagulation is initiated when tissue factor expressed on injured endothelial cells and activated monocytes combines with Factor VIIa (refer to the extrinsic side of pathway). In addition to the activation of Factor Xa:Va, the complex of Tissue Factor:Factor VIIa simultaneously activates Factor IX (refer to the “intrinsic pathway” in Figure 1). Consequently, both sides of the coagulation cascade are activated under the influence of Tissue Factor, amplifying the amount of coagulation. This suggests that the common pathway likely begins at the point when Factor IX becomes activated. The role that the intrinsic pathway plays in the initiation of coagulation is not clearly known.

Clotting provides hemostasis, helps to wall off infection, traps leukocytes in the area of injury and initiates the repair of injured blood vessel walls.
Anti-inflammation

Anti-inflammatory cytokines are released from activated leukocytes, in response to the release of proinflammatory cytokines. Once the inflammatory trigger is removed and proinflammatory cytokine release subsides, anti-inflammatory cytokines can dominate. Corticosteroids also have anti-inflammatory properties. Activated Protein C is an important inhibitor of inflammation and will be examined below.

Anticoagulation:

Following activation of the clotting cascade, circulating antithrombin binds to thrombin, to weakly inactivate thrombin activity. Antithrombin also inactivates Factors Xa, IXa, XIa, and XIIa. Endogenous heparin binds with the antithrombin-thrombin complex to significantly enhance the antithrombin activity. Thrombin triggers the conversion of Protein C to activated Protein C, a potent anti-inflammatory, anticoagulant and pro-fibrinolytic agent.

Pharmacological agents classed as anticoagulants work by inhibiting or de-activating steps within the coagulation cascade to delay or decrease the production of fibrin. Because anticoagulants block a different phase of coagulation than anti-platelet agents, both anti-platelet and anticoagulant agents may be used together when it is very important to prevent further clot formation (e.g., Acute Coronary Syndrome).

The administration of unfractionated heparin is believed to work the same way as endogenous heparin. Unfractionated heparin equally inhibits thrombin and Factor Xa on a 1:1 ratio. Heparin binds to the antithrombin-thrombin complex, to potentiate the antithrombin effect. When antithrombin levels are low (e.g., in procoagulant states such as septic shock), heparin resistance may develop. The need for higher doses of heparin to maintain therapeutic anticoagulation may indicate heparin resistance. Thrombin inhibition prolongs the PTT, therefore, heparin infusions are titrated to a desired PTT.

By contrast, low molecular weight heparins (LMWH) produce more anti Xa activity than antithrombin activity. This is because LMWH chains are not long enough to simultaneously link to both antithrombin and thrombin. LMWHs have an antithrombin to anti Xa ratio of 1:2 – 1:4, with minimal effect on the aPTT.

One advantage of LMWH therapy is the ease of use; monitoring is not required because weight based therapy produces consistent levels of anticoagulation. This is different than unfractionated heparin where variable levels of anticoagulation may be seen despite consistent weight based dosing. A number of physiological changes may contribute to this response variability, making it difficult to maintain consistent anticoagulation. An advantage of unfractionated heparin is that it is easy to reverse in the event of bleeding.

Another concern regarding the use of any type of heparin is the risk of heparin induced thrombocytopenia (HIT). While the risk is lower when LMWHs are used, both unfractionated heparins and low molecular weight heparins are contraindicated if heparin induced thrombocytopenia (HIT) or heparin allergy develops.

Danaparoid is an anticoagulant that can be administered to patients with heparin allergy or HIT. Danaparoid has a 1:20 antithrombin to anti Xa activity, producing anticoagulation without prolonging the aPTT. It may be used to block dialysis catheters. Although rarely used because there are alternative agents with shorter half-lives, anti Xa levels are used to titrate danaparoid infusions. The long half-life of the drug (25-26 hours) is a concern; bleeding cannot be quickly reversed with danaparoid.

A newer alternative to danaparoid is argatroban. It is a synthetic direct thrombin inhibitor, with a shorter half-life than danaparoid (half-life is prolonged in hepatic failure). Because it is a thrombin inhibitor, it will prolong the aPTT. The aPTT is used to adjust the dose.

Fibrinolysis:

Tissue plasminogen activator (tPA) is released in response to fibrin generation. TPA converts plasminogen to plasmin. Plasmin causes fibrinolysis, or the dissolution of preformed clot. The dissolution of a clot causes an increase in the number of circulating fibrin degradation products. While anticoagulants prevent new clot from forming, fibrinolytics are responsible for the break
down of clot that has already formed. The tPA-plasminogen-plasmin cascade is depicted in Figure 2.

In addition to the fibrinolytic activity of plasmin, endogenous activated Protein C also has important fibrinolytic effects.

The Role of Activated Protein C

As previously described, activated Protein C influences homeostasis by anti-inflammatory, anticoagulant and profibrinolytic actions. Protein C is an endogenous protein that circulates in the blood. When thrombin is produced, it circulates to areas where the endothelium is not in an activated state and combines with a protein called thrombomodulin. Recall that thrombomodulin is only located on the surface of non-activated endothelial cells. The thrombin-thrombomodulin complex attaches itself to Protein C and Protein S on the surface of the non-activated endothelium, triggering the conversion of Protein C to activated Protein C. The activated Protein C circulates to the areas of inflammation, turning off inflammation and clotting, and allowing fibrinolysis to begin.

In diseases where there is widespread and systemic activation of inflammation (with widespread endothelial activation) such as in severe sepsis or septic shock, thrombomodulin availability is reduced. This can limit the ability to convert Protein C to activated protein C, causing persistent inflammation, clotting and anti-fibrinolysis.

Once activated, Protein C helps to restore homeostasis by causing the following 3 effects:

**Anti-inflammatory:** Activated Protein C inhibits further cytokine production (e.g. Tumour Necrosis Factor [TNF] and interleukin 1 and 6 [IL-1, IL-6]) to decrease inflammation. A decrease in cytokine production will also reduce further production of TF (a mediator of clotting). Activated Protein C also decreases leukocyte rolling and adhesion to endothelial surfaces.

**Anticoagulant:** Activated Protein C deactivates factors VIIIa and Va, two cofactors that precipitate thrombin formation. Thus, Activated Protein C is an important anticoagulant.

**Profibrinolytic:** Activated Protein C blocks the formation of 2 antifibrinolytic substances that are produced during thrombin generation and endothelial injury (TAFI and PAI-1). By opposing these two inhibitors of fibrinolysis, clot lysis is facilitated.

The administration of activated Protein C (Xigris) was shown to reduce mortality in septic shock with multi-organ failure. For several years, we used Xigris for patients with persistent organ dysfunction despite fluid and vasopressor therapy. Unfortunately, studies that attempted to replicate the benefits shown in the original study (Prowess) did not demonstrate the same benefit, therefore, drug production was stopped by the manufacturer and Xigris is no longer available.
TESTS OF THE INFLAMMATORY AND COAGULATION RESPONSES

INFLAMMATION

Leukocyte Count (White Blood Cell Count): The inflammatory response mounts quickly during an acute infection or tissue injury. If the inflammatory response is large and the neutrophils are no longer only localized to the site of injury, the leukocyte count will elevate. An elevated leukocyte count that consists primarily of elevated neutrophils is characteristic of an acute systemic inflammatory response. While infection is one cause for this finding, not all inflammation is caused by infection. For example, trauma, major surgery, Acute Coronary Syndrome and pancreatitis are examples of powerful inflammatory triggers.

Inflammation stimulates an increase in production in neutrophils and a rise in the leukocyte count in most individuals with systemic inflammation. If the systemic inflammation is extreme or persistent, or the patient is immunocompromised (e.g., elderly, diabetic, oncology patients), they may become unable to sustain the leukocytosis and may develop a lower than normal leukocyte count. A low leukocyte count in the setting of sepsis is a very critical finding. In immunocompromised patients, a normal or low leukocyte count may mask the severity of their disease.

When a significant and persistent inflammatory response is present, the demand for neutrophils may exceed the ability to produce them. This can lead to the release of immature neutrophils into the circulation. This is identified on a blood smear or differential as a "left shift", or, as >10% bands. In persistent acute inflammation, monocyte production is triggered. This can produce a rise in the number of monocytes and macrophages.

An elevated leukocyte count that is mainly characterized by increased lymphocytes is not indicative of acute infection or inflammation. Lymphocytes are cells of the immune response (e.g. antibody complexes or helper/killer T cells). Lymphocytes are elevated in chronic or viral infection, and certain malignancies.

Platelet Count: Inflammation stimulates an increase in the production of platelets, similar to the increase in neutrophils. During systemic inflammation, the patient is hypercoagulable and may have a higher than normal platelet count. If the systemic inflammation and coagulation persists, the patient may develop a low platelet count due to increased platelet consumption. This is very common among patients ill enough to be admitted to critical care (e.g., following major surgery, trauma, sepsis or acute MI).

ESR or C Reactive Protein: An increase in the ESR (eosinophil sedimentation rates) or C reactive protein level indicates inflammation is occurring. These are both nonspecific indicators of inflammation (and hypercoagulability), therefore, neither provide any direction regarding the cause of the inflammatory response. ESR has little clinical value, as it will remain elevated for prolonged periods of time after inflammation has decreased. C reactive protein differs from the ESR because it declines quickly when the inflammatory response subsides. This makes C reactive protein useful in a variety of inflammatory conditions (e.g. Acute Coronary Syndrome, joint infection) as a marker of a patient’s responsiveness to therapy.

Sometimes, particularly in research studies, proinflammatory cytokines are measured to identify the presence of ongoing or resolving inflammation. Examples include IL1 and 6 or TNF-α.

COAGULATION

Prothrombin Time (measured as INR): Measurements of both PT(INR) and PTT (aPTT) are done from a sample of whole blood that is collected in a tube that has a precise ratio of blood to citrate. Citrate prevents the blood from clotting by inactivating calcium ions. Calcium ions are important catalysts that precipitate clotting at several steps in the clotting cascade.

A prothrombin time is measured by calculating the time (seconds) it takes for a fibrin clot to form after the initiation of the extrinsic pathway. This is accomplished by adding calcium (to reverse the citrate) and thromboplastin (Tissue Factor) to the blood sample. Reporting of the PT has been standardized by conversion of the results to an International Normalized Ratio (INR). Each lab converts a normal PT to an INR of 1.0, by dividing the patient PT into the lab control PT.
This simplifies the adjustment of anticoagulant therapy and gives universal meaning to the INR value, which can be invaluable for patients on Coumadin who wish to travel.

The PT measures the activity of clotting factors within the extrinsic (including Factor VII) and traditional common pathway (from Factor X to fibrin). It will be prolonged in a deficiency of Vitamin K or Vitamin K dependent clotting factors (e.g. VII, X, IX, Protein C and S). Coumadin or warfarin inhibit the synthesis of Vitamin K dependent clotting factors to prolong the PT. Because coumadin works by impairing production, its onset of action is much longer than heparin. Usually, it takes 2 days for an oral dose of Coumadin to influence the INR. When converting a patient from heparin or LMWH to coumadin, it is important to maintain therapeutic heparin or LMWH dosing until the INR is in a therapeutic range. Conversely, the initiation of anticoagulation with Coumadin should include simultaneous initiation of therapeutic dosages of unfractionated heparin or LMWH. This should continue until the INR has reached a therapeutic range.

Prolonged INR levels can be shortened by the administration of Vitamin K if the patient has received coumadin or has a low Vitamin K level. An example of a situation where INR may be high due to vitamin K deficiency is biliary obstruction. Bile is needed to absorb vitamin K from the GI tract, therefore, biliary obstruction can cause elevation in the INR that will respond to vitamin K administration.

Liver disease with hepatocyte destruction can prolong the INR by decreasing the uptake of Vitamin K from the GI tract and/or by impairing the ability to manufacture clotting factors. If the INR is prolonged due to Vitamin K deficiency, the INR will respond to one or two doses. Failure to respond indicates that hepatocyte damage is impairing clotting factor production; repeated doses of Vitamin K could lead to toxicity. Biliary obstruction can prolong the INR because bile salts are needed to absorb Vitamin K from the GI tract. Prolonged INR due to bile salt reduction should be responsive to Vitamin K.

Fresh Frozen Plasma can be given to replace deficient clotting factors and may be part of the treatment to correct the INR. If a patient has a prolonged INR because of Coumadin administration, and rapid reversal is needed because of bleeding or the need for an intervention (e.g., surgery, insertion of a central line), the INR can be rapidly reversed with plasminogen complex concentrate (PCC). Examples of PCCs are Beriplex and Octaplex. These blood products contain a concentrated supply of vitamin K dependent clotting factors (Factors VII, IX, II and X) and protein C and S. Both products also contain heparin, therefore, should not be used with suspected HIT.

**Activated Partial Prothrombin Time (aPTT):** Activated partial prothrombin time (aPTT) measures the time (seconds) that it takes for a fibrin clot to form after initiation of the intrinsic pathway. It measures the intrinsic and traditional common pathways (from Factor X to fibrin) and may become prolonged if there is a deficiency of any clotting factor EXCEPT Factor VII.

If the aPTT is prolonged and the INR is normal, Factor VII deficiency is excluded. The aPTT is most sensitive to antithrombin activity, therefore, heparin and argatroban therapy prolongs the aPTT.

Fresh frozen plasma will replace clotting factor deficits and correct a prolonged aPTT. If the prolongation is caused by intravenous heparin therapy, protamine sulphate can be administered to reverse the heparin (e.g. post bypass surgery). Protamine sulphate should not be administered if the aPTT prolongation is not due to exogenous heparin as its administration can precipitate more clotting.

If both the aPTT and the INR are prolonged, the problem is most likely occurring in the final common pathway or is due to significant reduction in multiple clotting factors. This can include deficiencies of Factors V, X or II (prothrombin), DIC or overanticoagulation with coumadin.

Clotting factor deficiency identified by prolonged INR and aPTT, along with a reduction in the platelet count is seen in any disorder that causes severe systemic activation of inflammation and clotting due to accelerated consumption of clotting factors and platelets. Disseminated Intravascular Coagulation (DIC) is a prolonged INR/aPTT and low platelet count due to excessive clotting factor consumption.

**Thrombin Time (TT) and Fibrinogen Levels:** The thrombin time is the time required for blood to clot following administration of thrombin. It tests the lower half of the common pathway
and will be prolonged if the fibrinogen level is low. Because heparin potentiates antithrombin activity, heparin therapy also prolongs the thrombin time.

Measurement of the fibrinogen level is a more specific indicator of fibrinogen deficiency than thrombin time, therefore, it is more common to measure the fibrinogen level than to measure TT. Fibrinogen levels can be low in DIC as a result of excessive consumption of coagulation factors, including fibrinogen. Fibrinogen should be measured for any patient with massive bleeding, and it is almost always low in a major obstetrical bleed. Fibrinogen can be replaced with plasma, and the administration of a 1:1 red cell:plasma ratio during massive transfusion can prevent severe fibrinogen loss. Cryoprecipitate (Cryo) concentrate contains factor VIII, von Willebrand factor and fibrinogen at a higher concentration than plasma alone. It is currently the only IV preparation available to correct fibrinogen deficiencies, however, other forms of fibrinogen concentrates may be available in the future.

**Factor VII:** Factor VII has begun to gain interest as an important treatment modality in severe trauma. Because most clotting is initiated when Tissue Factor combines with Factor VII, increased Tissue Factor can lead to depletion of Factor VII. Factor VII levels can be measured and Factor VII concentrate can be administered. It is being given at the roadside following massive trauma in war-torn regions of the world.

**Fibrinogen Degradation Products (FDP) and D-Dimer:** When a fibrin clot is broken down, fibrin degradation products increase in the bloodstream (measured as FDP or D-dimer). An elevated FDP or D-dimer is a non-specific indicator that increased clot lysis (and therefore clotting) has occurred. FDP only measures the byproducts of degradation, therefore, it only demonstrates the effects of plasmin. D-dimer is a much more specific indicator of DIC, measuring both thrombin and plasmin activity by detecting both newly formed and newly degraded fibrin.

Although elevated FDP and D-dimer levels indicate increased coagulation activities, levels are generally elevated in all critically ill patients for a variety of reasons (e.g. sepsis, intravascular lines, surgery). Consequently, these tests are of limited value during critical illness. It is generally more useful to measure the INR/PTT, platelets and specific clotting factors such as fibrinogen and/or Factor VII levels.

D-dimer can be useful as a means of excluding increased clotting in patients with no other reason for increased coagulation activity. For example, in the emergency room setting, a normal D-dimer can be useful as a means of ruling out DVT or pulmonary embolus, whereas, a positive test necessitates further investigation. A red D-dimer (or simply red) is a rapid method for measuring D-dimer using whole blood. It is available in some emergency room settings. D-dimer may also be useful when differentiating Thrombotic Thrombocytopenia Purpura (TTP) from DIC (D-dimer will be normal in TTP).

**Anti Xa Levels**

Although coagulation monitoring of LMWH is rarely indicated, anti Xa level monitoring would be the appropriate test. Danaparoid infusions should be adjusted to maintain a therapeutic anti Xa level. Normal anti Xa levels are 0, with a therapeutic goal of .5-.8 units/ml during systemic anticoagulation. Anti Xa levels are prolonged by unfractionated heparin, LMWH and danaparoid.

**Correction of INR prolongation due to warfarin:**

Coumadin: Blocks Vitamin K dependent clotting factors. Prolongs INR.

- Strategies to correct INR if warfarin induced (goal INR <1.5):
  - STOP Warfarin therapy (~3 days for effect)
  - Vitamin K orally (12 -24 hours for effect)
  - Vitamin K intravenously (6 -12 hours for effect)

  Urgent correction:
  - Plasma (effect immediately after infusion)
M PCCs (effect immediately after infusion)

* The preferred correction for warfarin induced INR correction if patient is bleeding or requires urgent procedure such as line placement or surgery is Prothrombin Complex Concentrates. Two products are available through Canadian Blood Services:
  * Octaplex® by Octapharma
  * Beriplex® by CSL Behring

M PCC’s are manufactured from large pools of human plasma (tested and virally inactivated)

M PCCs contains Factors II, VII, IX, X (Vitamin K dependent factors), Proteins C and S as well as heparin

M Differences between use of PCCs and platelets are shown to the right.

<table>
<thead>
<tr>
<th>Plasma</th>
<th>PCC’s</th>
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<tr>
<td>Group specific</td>
<td>No group required</td>
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<tr>
<td>Time required to thaw (25 minutes)</td>
<td>Time required to reconstitute (15 minutes)</td>
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<tr>
<td>~ 1 – 1.5 liters</td>
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<td>Varying content of coagulation factors</td>
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<td>May decrease INR to 1.5 or less</td>
<td>INR dependably 1.5 or less immediately after infusion</td>
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<tr>
<td>Risk of acute reaction (allergic, TACO, TRALI)</td>
<td>Very low risk of reaction</td>
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<tr>
<td>Risk of viral transmission</td>
<td>Virally inactivated: extremely low risk</td>
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Hematological Disorders

Disseminated Intravascular Coagulation (DIC):

DIC at varying degrees of severity is the most common hematological disorder. It is also called “consumption coagulopathy” and “defibrination syndrome”. It is a process of accelerated thrombosis, consumption of clotting factors, and hemorrhage due to clotting factor deficiency.

Characteristics:
- Procoagulant state (e.g., any event that can trigger systemic inflammation and clotting)
- Formation of fibrin due to clotting activation
- Fibrinolysis
- Depletion of clotting factors
- End-organ damage due to clotting, hemorrhage, shock and/or precipitating event

Treatment:
- Correct precipitating event (e.g., sepsis, retained placenta, hemorrhage)
- Replace clotting factors (e.g., with plasma administration)
- Administer platelets if indicated
- Heparin may be beneficial to stop clotting
- Blood products as required if bleeding occurs

The most severe form of DIC occurs in the setting of profound and acute illness such as post partum hemorrhage, septic shock or hemorrhage shock. The outcome and severity is usually dependent on the underlying cause and ability to correct before multi-organ failure ensues.
Heparin Induced Thrombocytopenia (HIT)

Thrombocytopenia is a common complication of heparin therapy, producing a mild reduction in platelet count in many patients after 2 or 3 days of heparin therapy. This has little clinical significance and is known as Type I Heparin Induced Thrombocytopenia.

A much more serious form of thrombocytopenia due to heparin (Type II HIT) is due to an immune-mediated response that is characterized by the formation of antibodies to the heparin-platelet factor 4 complex. We will refer to this as HIT syndrome.

HIT syndrome, also called HITT (Heparin Induced Thrombocytopenia and Thrombosis) produces a drop in platelets that usually develops ~10 days after an exposure to heparin. The longer the exposure the greater the risk and this is true with either unfractionated or LMWH. Females receiving unfractionated heparin who have a surgical versus medical admission are at the greatest risk. Patients who develop HIT syndrome after exposure to LMWH are more likely to have been previously exposed to unfractionated heparin than those who develop HIT syndrome with LMWH and were never exposed. HIT syndrome can develop with exposures as low as 250 units of heparin flush or exposure to a catheter that is heparin coated.

Antibodies to heparin develop more frequently among cardiac surgery patients than among those undergoing orthopedic surgery, but orthopedic surgery patients who do develop antibodies are more likely to develop HIT syndrome than cardiovascular surgery patients with antibodies.

Antibody Formation:

IgG, IgM and IgA type antibodies can be triggered by a complex of heparin and platelet factor 4. Platelet factor 4 is a protein that is released from activated platelets. These are called anti-PF4/heparin antibodies. About 3-8% of the population may have these naturally.

Platelets that have anti-PF4/heparin antibodies attached will aggregate, due to a number of proposed mechanisms. This causes both an increase in clot formation and reduction in the plasma platelet concentration. In its worst form, patients experience thrombosis that may be arterial in nature, with thrombocytopenia.

Clinical Findings:

- Drop in platelet count by > 50 percent 5-10 days after initiation of unfractionated or LMW heparin
- Delayed onset HIT may occur; identified by drop in platelet and thrombosis after heparin removal
- Thrombosis, if present is the most serious finding and can be both venous and arterial. A new thrombosis in a patient being treated with heparin for thromboembolic disease is a red flag.
- Skin necrosis at the site of heparin injections (erythema followed by purpura and hemorrhage that leads to necrosis)
- Adrenal hemorrhage, adrenal vein thrombosis and transient global amnesia are rare complications
- Anaphylactoid reaction (fever/chills, cardiopulmonary arrest) after IV heparin bolus

Diagnosis:

- HIT assay (Serotonin release assay)
- Must be approved by hematology: lab will not process until a hematologist has approved the test and contacts the hematology lab (LHSC)
Treatment:
- Anticoagulation with a non-heparin agent (e.g., fondaparinux, argatroban)
- Stop ALL heparin and LMWH
- Monitor for limb ischemia, DVT, PE or other thrombotic events

Thrombotic Thrombocytopenic Purpura (TTP) and Thrombotic Thrombocytopenic Purpura-Hemolytic Uremic Syndrome in Adults:

- Thrombotic Thrombocytopenic Purpura (TTP) and Thrombotic Thrombocytopenic Purpura-Hemolytic Uremic Syndrome (HUS) are both acute syndromes that have multiple organ abnormalities.
- Both syndromes have microangiopathic hemolytic anemia and thrombocytopenia.
- Both syndromes are essentially the same findings, with HUS including uremia
- Thrombosis can cause stroke, seizures, renal failure
- Thrombocytopenia may cause purpura
- Most accurately, patients with both neurological and renal findings have TTP-HUS. HUS alone is usually a disease of children. TTP-HUS or HUS in children has been seen most frequently as a result of the Shiga toxin-producing Ecoli. Several years ago, contamination of the water supply in Walkerton, Ontario produced many severe and several fatal cases of this condition.
- Other causes or precipitating events: drugs (quinine, chemotherapy, immunosuppressive agents, antiplatelets, valacyclovir), DIC, pregnancy, antiphospholipid antibodies, CV surgery, kidney transplant, allogeneic bone marrow transplant
- Can also be idiopathic
- One variant of TTP is due to ADAMTS13 deficiency. This form of TTP usually has minimal or no neurological or renal involvement. ADAMTS13 (A Disintegrin And Metalloprotease with a ThromboSpondin type 1 motif, member 13) is an enzyme that normally degrades von Willebrand factor (which is an important activator of clotting). In the absence of ADAMTS13, clotting is not turned off.

Diagnosis of TTP (Classic Pentad):

- Thrombocytopenia
- Microangiopathic hemolytic anemia
- Neurologic findings
- Renal abnormalities
- Fever

Classic pentad may not always be found, but is more common among those sick enough to arrive in critical care. Urgent and prompt treatment is curative, therefore, patients are treated without meeting all criteria. Only thrombocytopenia and microangiopathic hemolytic anemia is required to make diagnosis.

Findings:
- Thrombocytopenia
- Pathological hyaline thrombi (90% of patients died before therapeutic plasma exchange)
- Hemolytic anemia (diagnosed by presence of fragmented red cells called shistocytes) on blood smear
- Non-immune mediated hemolysis (negative direct Coombs’ or direct antiglobulin test)
- Thrombi composed primarily of platelets can compromise flow to affected organs. Endothelial injury (inflammatory response) is evident.
• Microangiopathic hemolytic anemia can produce a low hemoglobin and MCV, elevated bilirubin (dying red cells release bilirubin), reduction in haptoglobin (a protein that binds the free hemoglobin being released from damaged cells, thus the haptoglobin gets “used up”) and elevated LDH (released from dying red cells)
• Neurological symptoms may include confusion, headache, TIA, stroke and seizure
• CT may show PRES (Pattern of Reversible Leukoencephalopathy Syndrome)
• Full neurological recovery is possible
• Fever is present in most severe cases or when secondary to infectious cause
• Abdominal symptoms of pain, nausea, vomiting and diarrhea common in ADAMTS13 deficiency. Abdominal symptoms and an elevated neuroph is common in children with HUS. Blood diarrhea is a hallmark of Shiga toxin-producing bacteria
• Cardiac involvement with thrombi and hemorrhage can lead to myocardial necrosis (MI) with the associated complications

**Treatment:**
• Untreated, typically progresses to irreversible renal failure, neurological deterioration, cardiac ischemia and death
• Early TPE (Therapeutic Plasma Exchange) is the mainstay of therapy (removal of patients plasma and replacement with donor plasma) and is indicated for non-diarrheal TTP (may be given if neurological symptoms present)
• TPE may either remove something bad from the plasma or replace something that is missing.
• In the absence of TPE, plasma infusion may buy time until transfer to an appropriate centre. It may also be sufficient in ADMATS13 deficiency.
• Delay in treatment or failure to treat patients who only have some of the diagnostic criteria is associated with worse outcomes
• Treatment for sepsis if present; adequate fluid resuscitation is essential in HUS as dehydration worsen renal failure and fluid may help to clear toxins spontaneously.
• Platelet transfusion is restricted to those at risk for bleeding (<10,000) as there is anecdotal evidence that transfusion may increase thrombosis and neurological complications
• Plasma exchange

**Immune Thrombocytopenia Purpura (ITP):**
• Previously called Idiopathic Thrombocytopenia Purpura
• Less clinical severe than TTP
• Diagnosis is made by excluding any other reason for thrombocytopenia
• Petechial hemorrhage may be evident
• Identified by normal CBC, normal differential, normal smear, but low platelet count
• Exclude drug or herbal causes (including herbal or quinine containing drinks)
• Likely caused by destruction or platelets and suppression of new platelet production as a result of antibodies (that may or may not have been identified and are not routinely tested)

**Primary ITP:** no identified cause
**Secondary ITP:** immune mediated but in conjunction with another disorder
**Chronic ITP:** lasts longer than 12 months
**Refractor:** fails to respond to therapy including splenectomy, and is associated with severe complications of bleeding

**Treatment:**
All adults with severe thrombocytopenia are usually treated (platelets < 30,000); bleeding is rare an usually doesn’t occur until < 10,000. Children often go into spontaneous remission and may not be treated; this is rare in adults.
Treatment of asymptomatic adults with >30,000 not usually recommended. Only about 15% with platelets 30,000 – 50,000 develop more severe disease.

**Steroids:** steroids are the first line treatment and may be all that is required

**Immune globulin:** Immune globulin (IV IgG 1 gm/kg/day X 1-2 days) with anti-D (50 to 75 mcg/kg per day, given once) may be used (only works in RH positive patients who have not had splenectomy).

Immune globulin does not produce long term remission but is useful in an acute setting when a patient has life-threatening thrombocytopenia. Anti-D only works in RH positive patients because it binds to the erythrocyte D antigen (not available in RH negative patients).

There is a small potential risk of hemolysis with anti-D, therefore, the risk should be weighed against the potential benefit.

**Second Line Therapy:** Splenectomy, rituximab, and the thrombopoiesis-stimulating agents (eg, romiplostim and eltrombopag) are second line agents for patients who fail steroid therapy and have severe thrombocytopenia. Splenectomy and rituximab can induce remission, but thrombopoiesis-stimulating agents improve platelets only during continued therapy.

Splenectomy is the preferred method. It is believed to work by:

a. Splenectomy removes the major site where antibody-coated platelets are trapped and destroyed

b. The spleen contains ~ 25 percent of the total lymphoid mass, splenectomy may decrease the number of B-lymphocytes responsible for anti-platelet antibody production
References

Information on blood product replacement can be found at the following two websites:

**Bloody Easy: Sunnybrook Health Sciences Centre**

**Blood Transfusion Manual: London Health Sciences Centre**


[http://www.wadsworth.org/chemheme/heme/microscope/celllist.htm](http://www.wadsworth.org/chemheme/heme/microscope/celllist.htm)


