SEED Coagulation

Sysmex Educational Enhancement and Development
April 2012

Principles of haemostasis

The primary principle of haemostasis is to minimise blood loss at sites of vessel injury by forming a thrombus (clot) and at the same time maintaining blood flow (Fig. 1). In order to achieve this, there is a highly regulated, fine-tuned interaction of multiple processes, involving the blood vessel wall, principally the endothelium, platelets and non-cellular blood constituents.

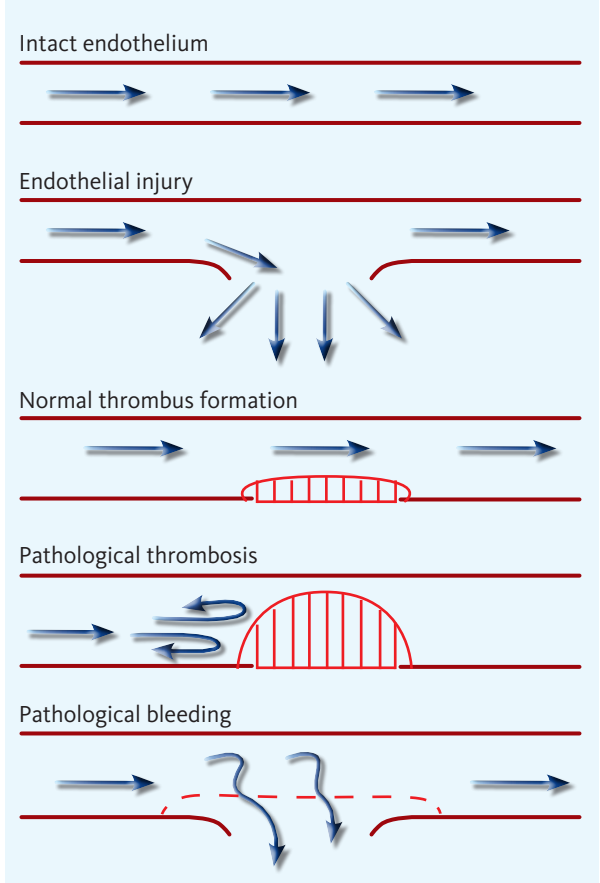

Fig. 1 *Schematic representation of the principles of haemostasis*

The core elements of haemostasis include blood vessel constriction, platelet activation, coagulation and fibrinolysis. All of these processes are initiated at the same time in response to blood vessel injury. Damage to the endothelial cell lining of the blood vessel results in exposure of collagen which under normal circumstances is not in contact with the blood. When circulating platelets come into contact with collagen they are activated and start to stick to the damaged surface. At the same time a substance called tissue factor is also exposed which activates the coagulation cascade resulting in the formation of a fibrin clot. Platelet adhesion and activation is conventionally known as primary haemostasis and the process of coagulation and fibrin formation as secondary haemostasis. In simplistic terms, the platelet plug can be considered to be the 'bricks' and the fibrin meshwork the 'cement', both being required for the formation of a stable thrombus.

Normal haemostasis depends on the interaction of the following:

a) Blood vessel
b) Platelets
c) Coagulation system
d) Fibrinolytic system

A defect in one or more of these systems will result in either a bleeding disorder or a tendency to clot.

a) The blood vessel

The blood vessel wall which is lined with endothelial cells is the first line of defence for normal haemostasis. The endothelium is a highly regulated 'organ' in that it is responsible for ensuring that blood remains fluid at all times so that flow continues without hindrance. The surface is therefore regulated to be highly 'anticoagulant' most of the time.

However, at the first sign of injury where blood loss could be incurred, the endothelium switches to be highly 'procoagulant'.

b) Platelets

Platelets are produced in the bone marrow. They circulate in the blood and have a life span of about 10 days. Platelets have a very complex structure which facilitates the critical role that platelets play in the normal physiology of haemostasis. The major components of the platelet include the surface membrane composed mainly of phospholipids, intracellular granules, surface receptors and the canalicular system.

Ordinarily, platelets circulate as 'inert' particles, without interacting with the blood vessel wall or any blood constituent proteins. However, at sites of blood vessel damage, platelets stick to a variety of structures via a multitude of platelet surface receptors.

Von Willebrand factor (VWF) is a plasma protein the function of which is to act as an anchor for platelets at sites of injury. VWF and platelets ordinarily do not interact, but once VWF has bound to subendothelial tissues, it changes its own structure which the platelet then recognizes and binds tightly. The bond takes place via one of the major surface receptors, which in turn triggers multiple reactions inside the platelet which lead to 'activation' of the platelet.

Platelet activation includes several key end points:

- Shape change

  Platelets undergo a shape change from disc like to flattened spread out structures with multiple finger-like extensions. This physically assists in plugging the hole in the vessel wall. The platelet is able to do this because it stores an abundance of 'excess membrane' in the form of the canalicular system. This canalicular system is connected to the outside of the platelet and when activated, this excess membrane is pushed to the outside thereby greatly increasing the surface area.

- Granule release

  Platelets contain granules that are secreted when platelets are activated. These granules contain a number of key elements, such as factor V, VWF and calcium which further amplify the haemostatic process.

- Membrane flip-flop

  Platelet activation results in an irreversible flip-flop of its bilayer membrane resulting in the exposure of phospholipids (especially phospatidylserine) to the plasma which are normally housed primarily within the inner leaflet of the platelet membrane and hence separated from plasma proteins. This is a critical step in the haemostatic process as phospholipid is an essential element in the support of the coagulation cascade.

- Fibrinogen receptor

  Another vital consequence of platelet activation is the induction of a shape change in the main surface receptor for fibrinogen. As the fibrinogen molecule has two identical arms, one fibrinogen molecule is able to bind to fibrinogen receptors on adjacent platelets thereby linking them together.

The overall effect of this is that there is now a physical seal comprising of stretched out platelets which are tethered to collagen in the vessel wall at the site of injury. The primary platelet plug by itself is however only a temporary seal, and the formation of a proper clot is needed in order to seal the vessel wall securely whilst the damaged vessel repairs itself. The exposed phosphatidylserine on the activated platelets surface is ready to support the coagulation cascade which has its substrate, fibrinogen right there, holding the platelets together.

c) Coagulation

Coagulation is the process of converting soluble fibrinogen into insoluble fibrin in order to form a stable blood clot around the platelets that were initially trapped at the site of injury. The plasma contains a large number of proteins called coagulation factors that participate in a series of enzymatic reactions that end in the formation of insoluble fibrin. A very small trigger results in the progressive amplifi-

cation of each subsequent step which concludes in the production of relatively large amounts of fibrin. This sequential reaction is called the coagulation cascade. The clotting factors involved are factors XI, X, IX, VIII, VII, V and II. Factor II, also called prothrombin, becomes thrombin when activated.

For normal haemostasis to occur, a sufficient amount of thrombin needs to be rapidly generated in order for fibrinogen to be converted to fibrin. Fibrin is relatively 'watertight' and therefore further blood loss is prevented. Fibrin is also relatively resistant to fibrinolytic breakdown (see later). When the surface of the endothelium is damaged, coagulation is immediately activated by the exposure of tissue factor (TF), which is present below the endothelial cells in the vessel wall, to the coagulation factors present in plasma.

The coagulation cascade

The coagulation cascade as we understand it to exist today is shown in Fig 2. The process of coagulation starts with the presence of TF, which binds and activates FVII to FVIIa. The activated clotting factors are enzymes that sequentially cleave each other in a highly regulated fashion. This process happens wherever TF has become exposed. Once the TF:FVIIa complex is formed, it has a potent positive feedback loop on itself thereby increasing the generation of more TF:FVIIa several thousand fold. TF:FVIIa in turn forms a complex with FX and converts it to FXa. It is also able to bind and activate FIX to FIXa although the intensity of this reaction is far less potent. FXa, together with the co-factor FVa, converts prothrombin to thrombin (FIIa). This initial generation of thrombin is very short-lived as TF:FVIIa is very rapidly inactivated by tissue factor pathway inhibitor (TFPI).

Furthermore thrombin activates FXIII to FXIIIa which in turn is responsible for irreversibly cross-linking fibrin creating insoluble polymers and a stable clot.

It can be said that the primary role of the initial small quantity of thrombin generated is to activate platelets within and close to the growing thrombus via thrombin platelet receptors and the thrombin produced from the secondary positive feedback activation loop of FXI by thrombin is responsible for the sustained burst of thrombin that generates the stable fibrin clot.

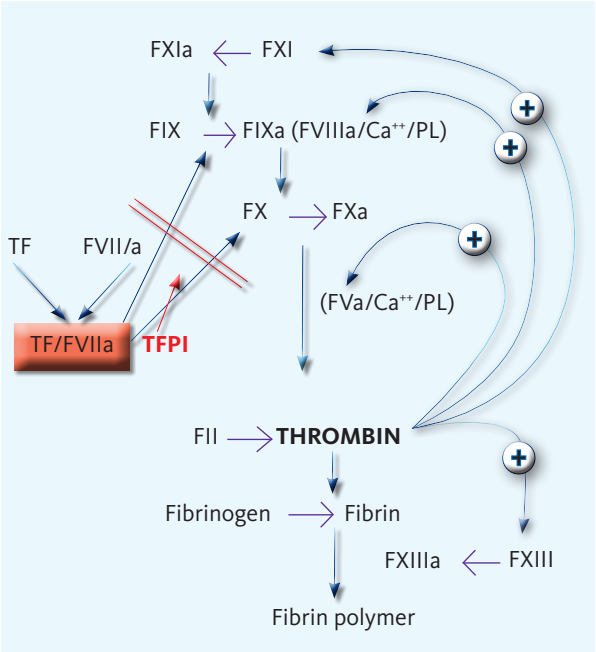

Fig. 2 *The revised coagulation cascade. PL refers to phospholipid which is the phosphatidylserine provided by the activated platelet surface.*

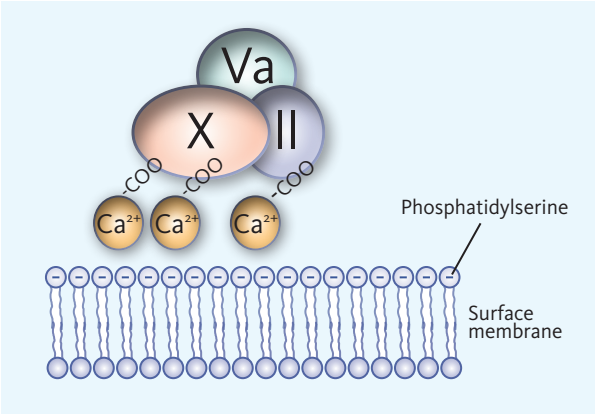

Fig. 3 *The role of calcium in coagulation*

The importance of vitamin K and calcium

An essential element in the success of coagulation cascade is the assembly of the various proteases in an orderly fashion on the surface of the platelet. The key to this process is the presence of GLA (γ-carboxyglutamic acid) domains of the vitamin K dependent clotting factors which are factors II, VII, IX and X. These GLA domains of these clotting factors are the key to the assembly of these complexes on platelet surfaces as they provide the critical link. Calcium ions which are positively charged bind to various GLA domains and to the phospholipid of the platelet, both of which are negatively charged, thereby localizing the clotting factors to the platelet sur-

face. The calcium ions essentially form a bridge between the clotting factor and the phospholipid on the activated platelet surface. This is how the clotting process is kept localized at the original site of endothelial injury. An absence of functional vitamin K will result in reduced ability for the coagulation factors to assemble on the platelet surface and compromise clot formation.
NB: Calcium is a critical element in the laboratory tests of coagulation!

d) Fibrinolysis

In the absence of adequate quantities of thrombin, a stable clot cannot be formed resulting in bleeding whereas unregulated production of thrombin will result in pathological thrombosis. A variety of mechanisms that collectively inhibit thrombin production have been well described. These include:

- Removal of activated clotting factors by blood flow past the clot
- Inactivation of clotting factors by circulating natural anticoagulants (protein C, protein S and antithrombin)
- Fibrinolysis

Fibrinolysis is the process of controlled clot breakdown. As soon as clot formation is triggered, so is the fibrinolytic pathway activated. The primary enzyme involved here is plasmin which degrades fibrin into fibrin breakdown products (FDPs) which includes D-Dimers.

The haemostatic balance

Despite the complexities of the control of haemostasis, ultimately the outcome is quite simply a matter of balance between clot formation and clot breakdown, both of which in turn are influenced by blood flow (or stasis), the vessel wall and the constituents of the blood (Fig. 4).

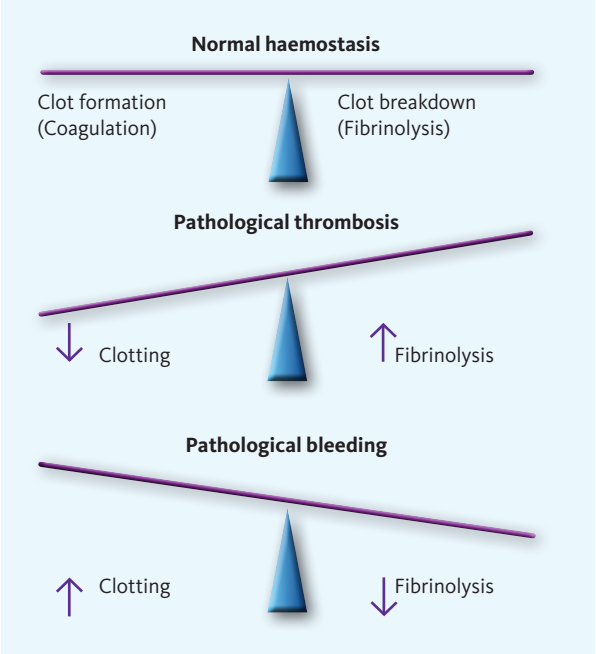

Fig. 4 *A schematic representation of the concept of haemostatic balance. Normal haemostasis requires fine balance between clot formation and clot breakdown. Pathological thrombosis occurs when there is excess clotting and/or diminished fibrinolysis and pathological bleeding when clotting is reduced and/or fibrinolysis is enhanced. In this context 'pathological thrombosis' refers to thrombus formation in excess of what is required to seal any damaged blood vessel and 'pathological bleeding' refers to any episode of bleeding which is more severe than one would ordinarily expect based on the nature of the injury.*