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Haemostasis: An Overview

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Abstract

Haemostasis is the process of blood clot formation at the site of vessel injury. When a blood vessel wall is disrupted, the haemostatic response must be quick, localized, and carefully regulated. Haemostasis is maintained in the body via three mechanisms: Vascular spasm (Damaged blood vessels constrict), Platelet plug formation (Platelets adhere to damaged endothelium to form platelet plug i.e, primary Haemostasis) and then degranulate and Blood coagulation (Clots form upon the conversion of fibrinogen to fibrin, and its addition to the platelet plug i.e, secondary Haemostasis). Various steps involved are: The first step is immediate constriction of damaged vessels caused by vasoconstrictive paracrine released by the endothelium. Vasoconstriction temporarily decreases blood flow and pressure within the vessel. When you put pressure on a bleeding wound, you also decrease flow within the damaged vessel. Vasoconstriction is rapidly followed by the second step, mechanical blockage of the hole by a platelet plug. The plug forms as platelets stick to the exposed collagen (platelet adhesion) and become activated, releasing cytokines into the area around the injury. Platelet factors reinforce local vasoconstriction and activate more platelets which stick to one another (platelet aggregation) to form a loose platelet plug. Simultaneously, exposed collagen and tissue factor (a protein-phospholipid mixture) initiate the third step, a series of reactions known as the coagulation cascade. The cascade is a series of enzymatic reactions that ends in the formation of a fibrin protein fiber mesh that stabilizes the platelet plug. The reinforced platelet plug is called a clot. Some chemical factors involved in the coagulation cascade also promote platelet adhesion and aggregation in the damaged region.

Keywords: Haemostasis, Platelet, Coagulation, Vasoconstriction, Fibrinogen.

Introduction

Haemostasis may be defined as the process that maintains flowing blood in a fluid state and prevents loss of blood from sites of vascular disruption. This definition implies two major

components: First, a potent procoagulant mechanism that is capable of forming haemostatic plugs at sites of vascular disruption, and second, regulatory systems that confine normal haemostatic plug formation to sites of vascular disruption [1]. Active components in this system include circulating platelets, procoagulant and regulatory plasma protein, and endothelial cells lining the vascular wall. Normal Haemostasis [2-5] is maintained by a delicate balance between the procoagulant and regulatory sides of Haemostasis, disruption of this balance can lead to either excessive bleeding or clot formation. The procoagulant mechanism is normally initiated vascular damage or disruption. This leads to deposition of platelets at the damaged site; the platelet

Plug [6-14] is then reinforced by a meshwork of fibrin strands. This process is normally halted as it extends out into areas of the vessel lined by intact endothelial cells. The formation of the platelet plug is often referred to as primary Haemostasis, while the formation of the fibrin meshwork is referred to as secondary Haemostasis. The mechanisms involved in turning off the process are called the regulatory systems. As the limitation of clot formation [15-21] to sites of vascular disruption would suggest, the process of regulation is very dependent on intact endothelial cells.

Methodology

The procoagulant mechanism is normally initiated vascular damage or disruption. This leads to deposition of platelets at the damaged site; the platelet plug is then reinforced by a meshwork of fibrin strands. This process is normally halted as it extends out into areas of the vessel lined by intact endothelial cells. The formation of the platelet plug is often referred to as primary Haemostasis, while the formation of the fibrin meshwork is referred to as secondary Haemostasis. The mechanisms involved in turning off the process are called the regulatory systems. As the limitation of clot formation to sites of vascular disruption would suggest, the process of regulation is very dependent on intact endothelial cells.

Primary Haemostasis

Platelet Morphology and Production

Platelets are central components of Haemostasis and anucleate cytoplasmic fragments derived from bone marrow megakaryocytes that are themselves derived from the pluripotent hematopoietic stem cell. At least two stages of megakaryocyte progenitors have been demonstrated by in vitro culture techniques, the burst-forming unit-megakaryocyte and the colony forming unit megakaryocyte. Each of these stages appears to be sensitive to a different set of cytokines. Maturation of the colony forming unit megakaryocyte leads to the formation of recognizable megakaryocytes. Megakaryocytes are large cells with multilobated nuclei derived from a process of endomitosis. Normal megakaryocytes are usually 8N-32N, but the ploidy number can vary depending on the rate of platelet production and consumption. Platelets are formed and released from megakaryocytes by a process of cytoplasm fragmentation along lines of demarcation formed by enfolding of the cytoplasmic membrane. Platelet size thus depends on the size of the zone of demarcation. When platelet production is increased, the zones of demarcation are typically larger. This results in the increased mean platelet volume (MPV) characteristic of thrombocytopenia secondary to increased consumption. The peripheral platelet count appears to be regulated by the total platelet mass rather than the platelet count. This is

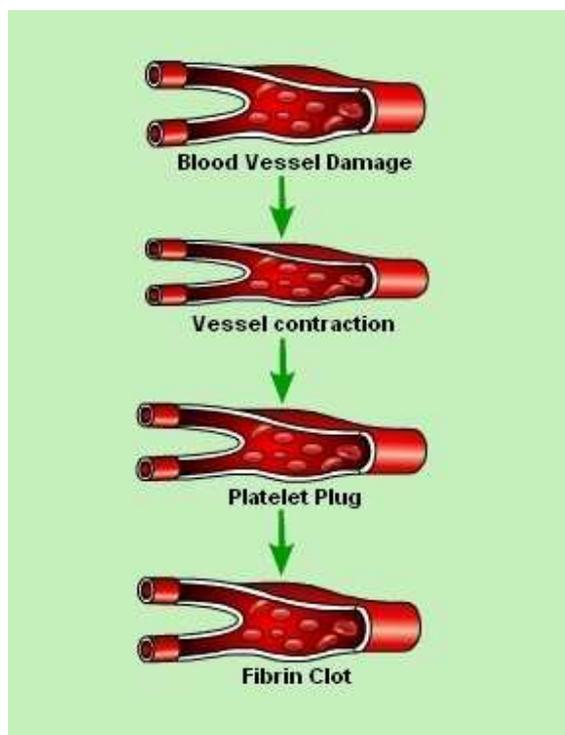
reflected in disorders such as Bernard-Soulier syndrome and May-Hegglin anomaly, which are characterized by thrombocytopenia and an increase in MPV.

The resting platelet normally has a discoid shape that is maintained by a circumferential band of microtubules. The Platelet membrane is physically and biochemically complex. There are numerous in folding of the plasma membrane to form the surface-connected open canalicular system. The membrane contains a variety of receptors, some of which are linked to membrane associated enzyme systems such as phospholipase A₂, phospholipase C, and adenylate cyclase. Beneath the cytoplasmic membrane there is a sol-gel zone that is rich in actin and myosin. These proteins are involved in the shape change and contraction characteristic of platelet activation. Juxtaposed to the surface-connected open canalicular system is a series of membrane bound tubules referred to as the dense tubular system. The dense tubular system corresponds to the endoplasmic reticulum of smooth muscle cells and functions as a storage site for calcium.

Within the cytoplasm of the platelet are several organelles, including mitochondria, peroxisomes, α granules, and dense granules; storage pools of glycogen are also frequently present in resting platelets. α Granules contain a variety of proteins, many of which are homologues of plasma proteins. A long standing questions has been whether or not such proteins are derived from passive adsorption by the platelets or active synthesis by the megakaryocyte. Evidence to date suggests that both mechanisms are important. Platelet factor 4, β -thromboglobulin, and von Willebrand factor, are synthesized by the megakaryocyte. Some α -granule proteins are specific for the platelet; thus platelet factor 4 and β -thromboglobulin can serve as markers of platelet release. Thrombospondin, a major constituent of α granules, has a limited tissue distribution and has been used as a marker of platelet activation. The platelets dense granules contain a variety of small organic compounds, including ADP, ATP calcium, and serotonin. Perhaps the most important component of the dense granules is ADP an essential platelet agonist. As circulating platelets are anucleate, their ability to synthesize new proteins is relatively limited. Thus, once a platelet has undergone release of its granule contents or inactivation of one or more of its enzymes (e.g. aspirin effect), it remains functionally impaired until it is removed from the circulation.

Platelet Response to Vascular Injury (fig-I)

The typical platelet response to vascular injury can be divided into three major steps. These are platelet adhesion, the interaction of platelets with non platelet surfaces; platelets activation, during which platelets undergo shape change and secrete ADP and other granule constituents; and platelet aggregation, the interaction of platelets with each other. The adhesion of platelets to exposed sub endothelial structures is mediated primarily by von Willebrand factor (vWF), a large adhesive protein. Von Willebrand factor is synthesized by endothelial cells and megakaryocytes; plasma vWF is primarily a product of endothelial cell synthesis and release, whereas platelet α graule vWF is primarily a product of megakaryocyte synthesis. In addition, endothelial cells secrete vWF into the sub endothelial cell space so that vWF is present in the sub endothelial cell matrix itself. All three sources of vWF are probably necessary for normal platelet adhesion.

Fig-I (Platelet Response to Vascular Injury)

Current evidence suggests that when vWF interacts with sub endothelial components such as collagen, it undergoes a conformational change, exposing binding sites for its platelet receptors. The primary platelet receptor for vWF is glycoprotein IB (GP IB). Under conditions of high shear stress, vWF can also bind to the glycoprotein IIb /IIIa (GP IIB / IIIa) complex. Von Willebrand factor is normally synthesized as a polymer consisting of a variable number of dimeric subunits. The longer polymers (high – molecular weight multimers) are capable of mediating platelet adhesion to sub endothelial structure, perhaps because the larger forms maximize surface contact between the platelet and vessel wall. Activation of the platelet membrane by adhesion or other platelet agonists leads to dynamic metabolic and morphologic changes in the responding platelet. A number of metabolic pathways are involved in this platelet response, among these; the phospholipase C- phosphatidylinositol pathway appears to be critical in initiating the platelet response. Activation of phospholipase C by any one of a number of membrane signals leads to cleavage of phosphatidylinositol-bisphosphate (PIP₂), resulting in the release of two critical intracytoplasmic mediators, diacylglycerol (DG) and Inositol trisphosphate (IP₃). Inositol trisphosphate causes rapid release of calcium from its storage sites into the platelet cytoplasm. The increase in cytoplasm calcium activates calmodulin dependent phosphokinases and leads directly to phosphorylation of myosin light chain. This pathway is essential for platelet shape change and contraction. Diacylglycerol binds to and activates protein kinase C. Protein kinase C then phosphorylates a number of proteins, including a 45 – kd protein essential for platelet secretion. In addition, DG can lead to activation of the phospholipase A₂ pathway.

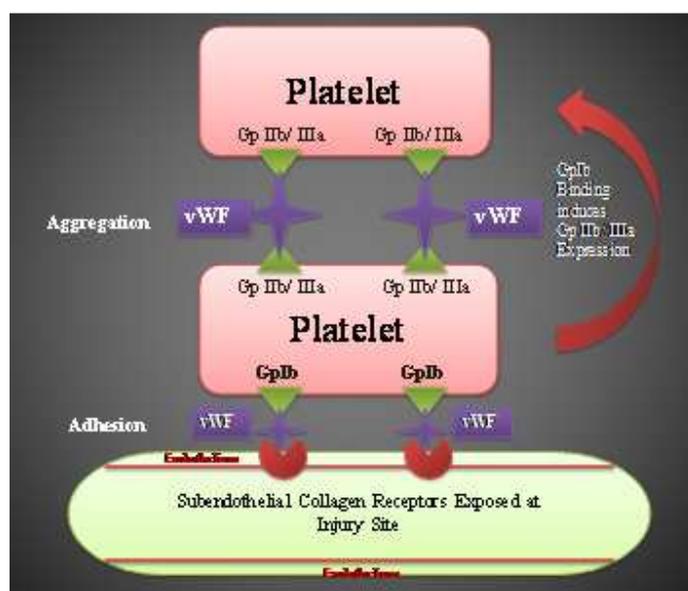
Activation of phospholipase A₂ leads to the release of arachidonic acid from membrane phospholipids, particularly phosphatidylcholine. Arachidonic acid can be converted into cyclic

endoperoxides by cyclo-oxygenase or metabolized by 12 – lipoxygenase. The cyclo-oxygenase pathway leads to the formation of thromboxane A₂ which itself is a potent stimulator of phospholipase C. Aspirin and other nonsteroidal anti-inflammatory agents have an antiplatelet effect because of their ability to inhibit cyclo-oxygenase and block formation of thromboxane A₂. The response of the platelet leads to extension of pseudopodia, secretion of important mediators and proteins, and ultimately contraction of the haemostatic plug. One of the mediators released from the dense granules is ADP, a platelet agonist that can further stimulate surrounding platelets. In addition, it is capable of activating the GP IIB/ IIIa complex to mediate the third and final stage of the platelet response, platelet aggregation. Platelet aggregation is another example of “molecular gluing”. GP IIB/IIa can be cross- linked by a variety of adhesive proteins; the major protein involved in cross-linking platelets *in vivo* appears to be fibrinogen. Intact fibrinogen is a dimeric molecule that allows reciprocal interaction of its terminal ends (D domains) with GP IIB / IIIa receptors on adjacent platelets. Under resting conditions, the GP IIB/ IIa receptor cannot interact with or bind fibrinogen. However, once a platelet has been activated or exposed to ADP, fibrinogen can bind to and cross- link responding platelets. Thrombospondin, a major component of platelet α granules, appears to stabilize and augment the platelet – platelet interaction mediated by fibrinogen.

Secondary Haemostasis (Fig-II)

Secondary Haemostasis is marked by the conversion of a soluble protein, fibrinogen, into an insoluble form. Formation of the fibrin meshwork results in reinforcement and stabilization of the platelet plug at the site of vascular disruption. The process of fibrin clot formation can be divided into two phases; the first phase consists of formation of the potent procoagulant enzymes thrombin; the second phase consists of the conversion of fibrinogen into fibrin with subsequent polymerization and stabilization.

Fig-II (Secondary Haemostasis)



Thrombin is formed as a result of multiple enzymatic steps, which are known collectively as the coagulation cascade. Most of the enzymatic steps involve formation of an “enzymes activation complex” composed of an active enzyme, a proenzyme substrate protein, a protein cofactor, and a phospholipid surface that serves to localize the process and concentrate the reactants. The coagulation cascade can be viewed as the formation of a series of these activation complexes. As each enzyme activates many substrate proenzymes, each activation complex represents an amplification step. The enzymes of the coagulation cascade are serine proteases; that is they are enzymes that split specific peptide bonds through the formation of unstable bonds with the reactive serine at the enzyme’s active site. Coagulation serine proteases can be divided into two families, the vitamin K- dependent proteins and the contact system proteins. The contact system serine proteases share several characteristics:

- (a) The molecular weight of each monomeric unit is 80 to 85 KD.
- (b) They do not require vitamin K for complete synthesis.
- (c) They are not adsorbed by BaSO₄ or Al (OH)₃; and
- (d) They are not completely consumed during in vitro coagulation of blood. Factor XI is unique among these serine proteases in that it circulates as a dimer linked by disulfide bonds, and it is the only protein of this family that is associated with a bleeding tendency when the factor is absent.

The vitamin K – dependent factors are all characterized by the presence of between nine and 12 γ carboxyglutamic acid residues near the amino terminal end of the protein. Vitamin K is necessary for the posttranscriptional carboxylation of these glutamic acid residues, these proteins do not bind calcium and do not interact with phospholipid surfaces. These descarboxy forms, which are found in vitamin K deficiency, oral anticoagulant therapy, and liver disease, are thus ineffective at mediating secondary Haemostasis. Vitamin K - dependent proteins are heat stable and, with the exception of prothrombin, are not entirely consumed during coagulation in vitro. Some of the proteins (Protein C, protein S, factor IX, and factor x) are characterized by an additional posttranscriptional modification, the hydroxylation of an aspartic acid to form β hydroxyaspartic acid. The functional significance of this modification is still uncertain, but may involve calcium binding by these proteins. Protein S is unique among the vitamin K-dependent proteins in that it has non enzymatic function; rather, it functions as a cofactor for activated protein C.

Thrombin Formation (fig- III)

There are at least two mechanisms for initiating thrombin formation; these have been designated the intrinsic and extrinsic pathways. Their names derive from the fact that all components of the intrinsic system are found in circulating blood, while the extrinsic pathway requires the presence of tissue factor, a component not normally present in circulating blood. Recent studies suggest that the extrinsic pathway is the major physiological mechanism for initiating the coagulation cascade; the intrinsic system may be involved in pathological conditions and may also play a role in fibrinolysis.

Activation of the intrinsic system occurs when factor XII binds to negatively charged surfaces, resulting in a conformational change in the molecule and expression of enzymatic activity. Factor XII can activate both prekallikrein and factor XI; HMW kininogen serves as a

cofactor for both of these activation or prekallikrein thus leads to formation of a positive feedback loop, permitting generation of large quantities of factor XIIa in a very short period of time. Calcium is not required during these initial steps involving vitamin K –independent proteins.

Factor XIa activates factor IX in the presence of calcium and phospholipid surfaces, particularly activated platelet membranes. This is the only step for which there is no known protein cofactor. Factor IXa activates factor X in the presence of VIIa, calcium, and phospholipid. Factor VII, as it circulates in plasma, has minimal procoagulant activity; however, limited proteolytic cleavage, primarily by thrombin, results in the expression of potent procoagulant cofactor (not enzymes) activity. Factor Xa then converts prothrombin to thrombin in the presence of factor V a, calcium and phospholipid. Analogous to factor VII, factor V must be activated by thrombin for full expression of its procoagulant cofactor activity.

The extrinsic pathway begins with exposure of blood to tissue factor. In the presence of tissue factor and calcium, VIIa activates both factor IX and factor X. Factor Xa then converts prothrombin to thrombin, as described previously. Because this step is common to both pathways, it is often designated the “final common pathway. The activation of factor IX by VII-a tissue factor provides a connection between the intrinsic and extrinsic pathways of coagulation. However, this connection is not evident with the routine tests of Haemostasis namely, the prothrombin time (PT) and activated partial thromboplastin time (APTT). The lack of dependence of the PT on factor VII and IX concentration is more related to concentration of tissue factor and phospholipid used in the assay than the biological importance of this pathway.

Fibrin Formation (fig- III)

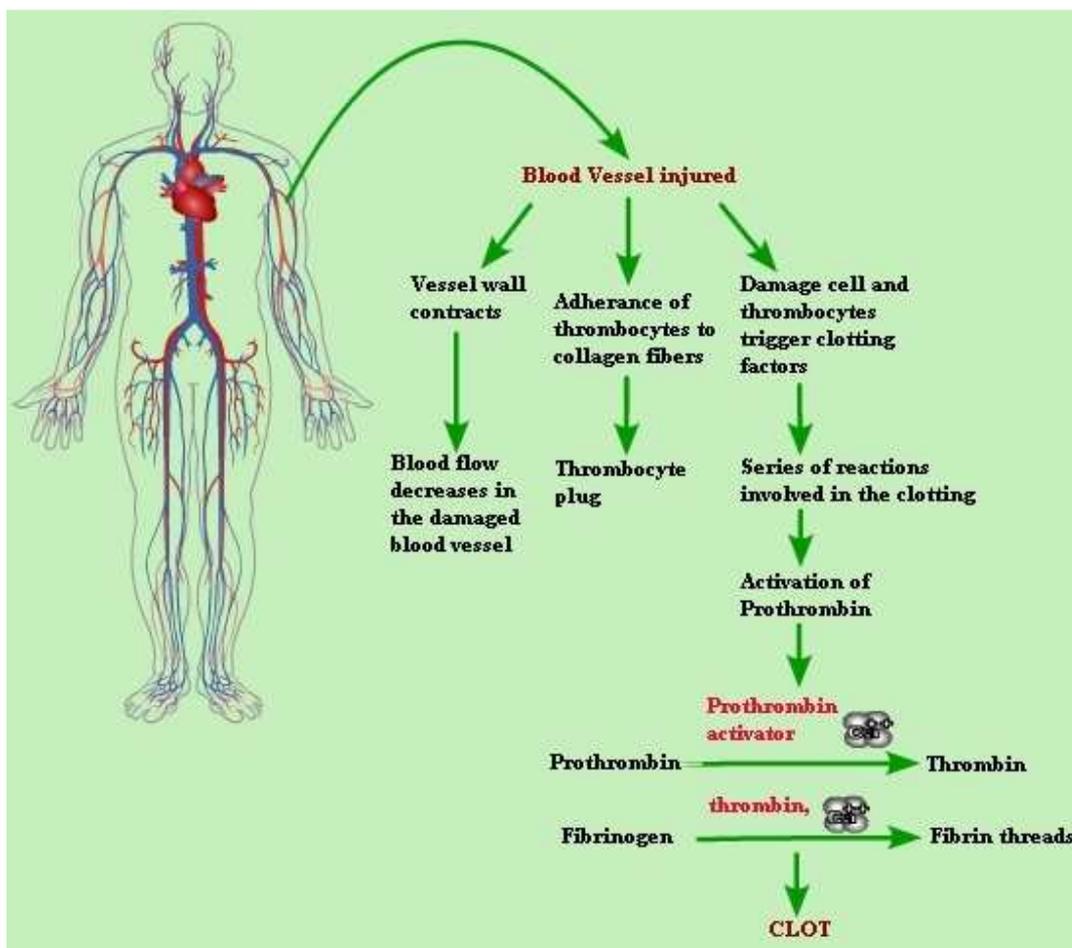
Thrombin is a potent enzyme that serves several key roles in Haemostasis. These include conversion of fibrinogen to fibrin, activation of factors V and III, activation of factor XIII, activation of protein C, and activation of platelets. In addition, thrombin can interact and stimulate a variety of other cells, including endothelial cells. For example, interaction of thrombin with endothelial cells can lead to fundamental changes in the surface of the endothelial cell. Many of these changes increase the procoagulant activity of the endothelial cell and promote thrombosis. Critical to these functions of thrombin is the fact that conversion of prothrombin is accompanied by separation of the amino terminal, phospholipid – binding portion of the protein from its catalytic (enzymatic) portion. This large fragment is referred to as prothrombin fragment 1.2 (F_{1,2}) Thus thrombin becomes a soluble enzyme in contrast to the other vitamin K-dependent enzymes, which require a phospholipid surface for effective function.

Fibrinogen is a large dimeric protein having a molecular weight of 340 kd. It is composed of six peptide chains: two A α , B β and two γ chains. Each half of the fibrinogen molecule is composed of one A α , one B β and one γ chain linked together by extensive disulfide bonds that are concentrated in the amino and carboxy terminal ends. The extensive cross – linking gives these regions of the molecule a globular structure, while the intervening sequences are arranged in a long helical structure. The two halves of the fibrinogen molecule are linked by disulfide bonds between the amino terminal ends of the γ chains.

Thrombin cleaves a 16-amino acid peptide from the amino terminal end of each A α chain (fibrinopeptide A). And a 14-amino acid peptide from each amino terminal end of the B β chains (fibrin peptide B). Loss of these peptides from the central domain of the fibrinogen molecule alters the charge distribution and exposes binding sites for the carboxy terminal ends of fibrin, known as the D domains. The fibrin monomers then polymerize in a staggered overlap, with D domains interacting with the central E domain of the adjacent fibrin monomer. Lateral association then leads to gelation of the fibrin.

The fibrin gel is stabilized by factor XIIIa, which covalently cross-links fibrin monomers. Factor XIII is activated by thrombin, but full enzyme activity is expressed only after the active enzymatic subunit (the 'a' subunit) separates from its carrier molecule (the 'b' subunit), a process that occurs within the fibrin matrix. Factor XIIIa covalently links glutamine to lysine amino acids. The principal sites of cross linkage are between D domains of adjacent fibrin monomers and between the carboxy terminal ends of the α chains of distant fibrin monomers. Covalent cross linkage greatly stabilizes the fibrin clot and makes it resistant to fibrinolysis.

Fig-III (Clotting system)



Regulation of Haemostasis

The procoagulant mechanisms described in the previous sections lead to rapid formation of haemostatic plug at sites of vascular damage. Under normal circumstances, coagulation is limited to sites of vascular disruption and is not propagated throughout the vessel. There are multiple systems involved in limiting clot formation to sites of vascular disruption. These are known collectively as the regulatory systems. Knowledge about the various systems involved in the regulation of Haemostasis has grown tremendously in recent years.

Regulation of the Platelet Response in Haemostasis

A number of factors that contribute to the regulation of the platelet response have been identified; most of these components are derived from intact endothelial cells. A major inhibitor of the platelet response is prostacyclin, which is formed by metabolism of arachidonic acid through the prostaglandin pathway in endothelial and vascular wall cells. Prostacyclin is a short-lived, but potent inhibitor of platelet aggregation. It stimulates platelet adenylate cyclase, which leads to an increase in cytoplasmic cyclic AMP. Cyclic AMP inhibits the phospholipase C pathway and the release of calcium from storage sites. More recently a second short lived metabolite has been found to have profound effects on the platelet response. Endothelial derived relaxing factor (EDRF) is a potent vasodilator that causes an increase in platelet cyclic GMP levels. The increase in cyclic GMP is associated with inhibition of both platelet adhesion and aggregation. Furthermore, the effect of EDRF and prostacyclin is synergistic; thus, the low basal levels of these two factors may be sufficient to minimize platelet activation under resting conditions.

Endothelial cells also release the enzymes ADPase, which converts ADP into inactive metabolites. ADP is essential for exposure of the GP IIb/ IIIa binding sites, which mediate platelet aggregation. Another barrier to platelet activation is the glycocalyx on the surface of intact endothelial cells. The glycocalyx has a negative charge and tends to repulse platelets under resting conditions. In addition, intact endothelial cells serve as a barrier between circulating platelets and any sub endothelial vWF. Thus, platelet adhesion is prevented in areas where the endothelium remains intact. Under normal circumstances, these components work in concert to limit platelet activation to sites of endothelial disruption.

Regulation of Fibrin Clot Formation

At least four systems have been identified as having a role in the regulation of fibrin clot formation. Each of these component mechanisms appears to be critical for effective regulation of Haemostasis, and isolated deficiencies of these component systems have been associated with clinical manifestations. Each of the component systems has a unique effect on the formation of fibrin clot, as discussed in the following sections.

Tissue Factor Pathway Inhibitor

Tissue factor pathway inhibitor (TFPI) is the most recently characterized protein involved in the regulation of Haemostasis. TFPI is a 40 kd- protein that has also been called lipid associated coagulation inhibitor (LACI) and extrinsic pathway inhibitor (EPI). A portion of circulating TEPI is present in the lipoprotein fraction, while the remainder is free in plasma. TEPI is a Kunitz- type inhibitor and rapidly combines with free Xa, inactivating the enzymatic activity for factor Xa. The TEPI – Xa complex then binds to the membrane associated tissue factor – factor

VIIa complex, inhibiting VIIa enzymatic activity. Thus, TEPI is responsible for inhibition of the major physiological initiator of Haemostasis, the factor VIIa- tissue factor complex.

Serine Protease Inhibitors

The serine protease inhibitors (SERPINs) are proteins that act as pseudo substrates for specific enzymes and form covalently linked complexes between the active site serine of the target enzyme and the susceptible bond in the inhibitor. The SEPRIN family includes a large number of proteins. Although most of these proteins are capable of inhibiting multiple enzymes, each is primarily directed toward a few select enzymes.

The major inhibitors involved in the regulation of fibrin clot formation are antithrombin III (AT III) and heparin cofactor II. AT III requires heparin – like molecules for effective neutralization of factor Xa and thrombin. Heparin binds to AT III, including a conformational change that results in exposure of the enzyme neutralizing site. In addition, heparin simultaneously binds to thrombin and AT III, resulting in approximation of the inhibitor to its target enzymes. Relatively long chains of heparin are necessary to mediate this function. In contrast, only the conformational change is necessary for the inhibition of factor Xa. Accordingly, this step can be accelerated by both short (low molecular weight) and long heparin polymers. Endothelial cells are probably the source of heparin for this reaction *in vivo*.

Dermatan sulfate appears to be the major cofactor for heparin cofactor II, heparin can accelerate the activity of this inhibitor, but high concentrations (greater than 1.0 U/ml) are necessary. In contrast to AT III, heparin cofactor II inhibits only thrombin among the coagulation enzymes. Levels of heparin cofactor II are decreased in patients with acute consumptive coagulopathy, suggesting that the inhibitor does play a role in the normal regulation of Haemostasis.

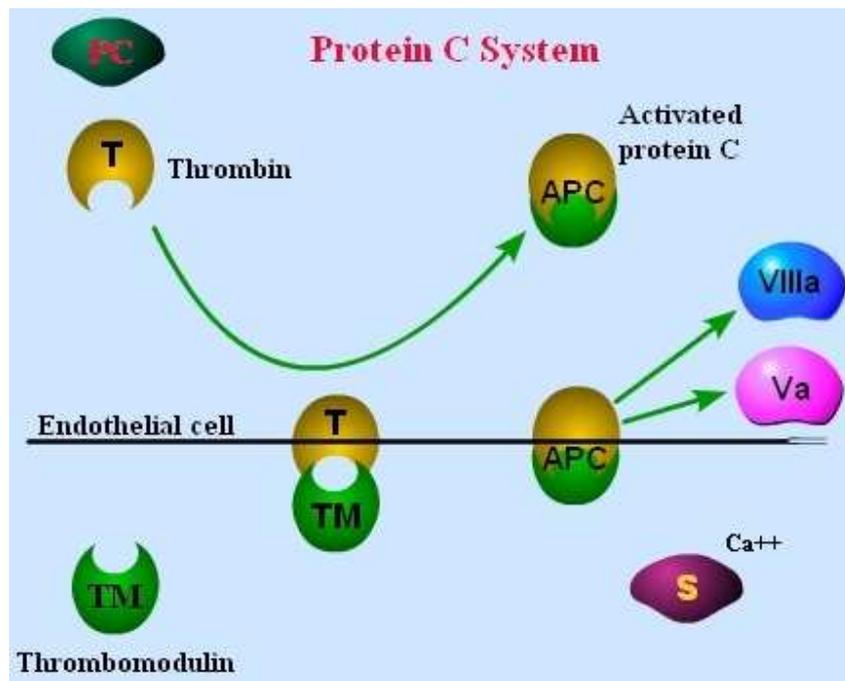
Protein C System (Fig-IV)

The protein C system is necessary for regulating the major cofactors of the coagulation cascade, factors Va and VIIa. The protein C system involves multiple protein components and can be divided into three phases; activation, activity, and regulation. Activation of protein C occurs on the surface of intact endothelial cells, which express thrombomodulin, a membrane associated protein that functions as a cofactor for the activation of protein C by thrombin. Thrombin binds to thrombomodulin, loses its procoagulant activity and no longer converts fibrinogen to fibrin, activates Va or VIIa, activates XIII, or activates platelets. Thus, binding of thrombin by endothelial cell surface thrombomodulin is itself an anticoagulant step. The altered thrombin, however, readily activates protein C, a vitamin K- dependent protein and can still be inhibited by AT III.

Activated protein C (APC) is a proteolytic enzyme which degrades factors Va and VIIIa. This step requires a phospholipid surface, usually supplied by the platelet, and a protein cofactor, protein S. Protein S is another vitamin K – dependent factor however, it is unique among the vitamin K- dependent coagulation factors in that it is not a serine protease. Protein S is produced by a variety of cells, including hepatocytes, endothelial cells, and megakaryocytes. Thus, protein S is present within platelet α granules and is released in the environment of a forming haemostatic plug, where it is most needed.

Protein C is inhibited by several SERPINs. The two major inhibitors involved in the regulation of APC appear to be protein C inhibitor, which is identical to plasminogen activator inhibitor-3, and plasminogen activator inhibitor -1. Experimental evidence suggests that APC has profibrinolytic activity. This effect appears to be related to competition between APC and tissue plasminogen activator for these two SERPINs. In the presence of increases amounts of APC, the inhibitors are utilized to neutralize APC, leaving tissue plasminogen activator free to activate plasminogen to plasmin.

Fig-IV (Protein C System)



Fibrinolytic System

The fibrinolytic system is also a complex multicomponent system. The major effect or enzyme of this system is plasmin, which is derived from plasminogen by limited proteolytic cleavage. Although a number of serine proteases can convert plasminogen to plasmin, the major physiological activator appears to be tissue plasminogen activator (tPA). Urokinase is another important activator of plasminogen, particularly in pathological conditions and within the genitourinary tract. Tissue plasminogen activator requires fibrin for effective activation of plasminogen to plasmin; fibrin appears to function as a cofactor for the activation of plasminogen by tPA. In contrast, urokinase is a direct activator of plasminogen and does not require fibrin as a cofactor.

Plasmin is a potent proteolytic enzyme that degrades fibrin clots. Under usual circumstances, plasmin remains bound to the fibrin clot through interaction of lysine binding sites on plasmin with the fibrin matrix. However, if plasmin breaks free of the fibrin clot, it is capable of digesting fibrinogen, factors V and VII, and platelet membrane glycoproteins. Cleavage of fibrin by plasmin results in the release of adjacent D domains. If these regions have been covalently cross-

linked by factor XIIIa, these end products of plasmin digestion are known as D dimmers. Antibodies that are specific for D dimmers have been developed and can be used to assess fibrinolysis.

Regulation of the fibrinolytic system occurs on at least two levels. Activation of plasminogen to plasmin is regulated by the SERPINS plasminogen activator inhibitor-1 (PAI-1) and protein C inhibitor. As discussed in the section on protein C, these inhibitors also inhibit APC, and their availability for regulation of tPA is dependent on the amount of APC in the environment. The second level of regulation involves the SERPIN α_2 – antiplasmin (α_2 -AP). α_2 -Antiplasmin rapidly inhibits free plasmin. However, it competes with fibrin for the same lysine binding sites on plasmin. Thus, as long as plasmin is bound to the fibrin clot matrix, it is not inhibited by α_2 -AP. Pathological fibrinolysis can occur because there is normally twice as much plasminogen as α_2 -AP on a molar basis. With marked activation of the fibrinolytic system, the α_2 -AP regulatory mechanism is easily overwhelmed. This is one of the key pathological changes underlying disseminated intravascular coagulation.

Summary of the Regulatory System

Numerous components participate in the down regulation of the haemostatic response. This coordinated effort is normally successful in limiting haemostatic plug formation to sites of vascular disruption. A number of hereditary and acquired defects of the regulatory system have now been described. Alterations that result in a decrease in the effectiveness of any one of these regulatory components can lead to increased clot formation *in vivo*, giving rise to a thrombotic tendency.

An Approach to Haemostatic Disorders [22-25]

There are a number of reasons why the haemostatic system may be evaluated as part of the clinical management of patient. Common reasons include therapeutic drug monitoring, presurgical evaluation of haemostasis, evaluation of a possible bleeding tendency, evaluation of a possible thrombotic tendency, evaluation for the possibility of a circulating lupus anticoagulant, evaluation for the possibility of disseminated intravascular coagulation, and evaluation for other specific disorders. The laboratory plays a key role in monitoring oral anticoagulant and heparin therapy. In contrast, the laboratory has only a minor role in monitoring fibrinolytic therapy (e.g. tPA, therapy) or antiplatelet therapy. In general, laboratory parameters measured during these therapeutic regimens provide little useful information, at least currently.

Routine presurgical evaluation of Haemostasis remains a controversial topic. Several recent reports have indicated that routine preoperative prothrombin times (PTs) and activated partial thromboplastin times (APTTs) do not predict the risk of bleeding during surgery. Others have suggested that routine evaluation of the haemostatic system may be of use in determining whether or not the patient has an underlying coagulopathy. This controversy highlights the need to distinguish between a test's ability to provide a measure of the risk of bleeding and its ability to detect an abnormality that may or may not place a patient in a somewhat higher risk group. The following sections outline an approach to the evaluation of patients with apparent bleeding or thrombotic disorders.

Haemostasis is a balanced system with the potent procoagulant mechanisms capable of forming a haemostatic plug at sites of vascular damage and equally potent regulatory systems capable of limiting clot formation to such sites. Bleeding or thrombotic disorders can arise whenever this balance is disturbed. Two mechanisms may lead to an increase in bleeding: a decrease or abnormality on the procoagulant side or an increase in regulatory activity. Von Willebrand's disease and hemophilia A provide examples of the first mechanism, whereas heparin therapy provides an example of the second mechanism. Similarly, thrombosis may occur because of defects in the regulatory system (e.g. antithrombin III deficiency) or increased activity on the procoagulant side, as may occur during the course of metastatic carcinoma. As with most problems in medicine, the clinical evaluation of these disorders begins with the clinical history and then moves to the laboratory for identification of specific disorders.

Evaluation of a Potential bleeding Disorder

The clinical history of a patient with a potential bleeding disorder is used to determine whether the patient truly does have a bleeding problem, whether the problem is likely to be congenital or acquired, and whether the defect is in primary Haemostasis, secondary Haemostasis, or fibrinolysis. As perceptions of the seriousness of bleeding problems vary significantly from patient to patient, the clinician must establish the frequency of bleeding, sites of bleeding, and severity of bleeding to determine the actual presence of a significant bleeding diathesis. Age of onset and family history are very helpful in distinguishing between congenital and acquired disorders, an early age of onset is suggestive of a congenital bleeding disorder, and where as the sudden appearance of bleeding in a previously healthy adult suggests an acquired problem. Family history can help establish a pattern of transmission. Some disorders are characterized by sex-linked recessive inheritance; others are characterized by either autosomal dominant or autosomal recessive inheritance.

The pattern of bleeding is very helpful in constructing a differential diagnosis. Mucocutaneous bleeding, characterized by epistaxis, ecchymosis, and genitourinary bleeding, is suggestive of a platelet disorder. Soft tissue bleeding, including hemarthrosis, hematomas, and retroperitoneal bleeding, is more suggestive of a coagulation disorder such as hemophilia A. Delayed bleeding is a classic manifestation of fibrinolytic type bleeding. The clinical history should include a thorough medical history to document any other medical problems that may predispose to a bleeding diathesis. In addition, a careful medication history, including over the counter medications, should be obtained. It is helpful to remember that the most common cause of abnormal platelet function is during therapy, particularly aspirin therapy.

Following the clinical history, routine screening tests are commonly performed. These include the platelet count and bleeding time to assess platelet function and the PT and APTT to assess fibrin clot formation. Note that these screening tests do not assess fibrin stabilization or fibrinolysis. The bleeding time is a commonly performed test to assess primary Haemostasis. A number of techniques for determining the bleeding time have been described, but the most commonly performed method is the template bleeding time using a disposable device. Although this test appears to be simple and straightforward, a number of technical variables can confound results. The bleeding time test is usually performed on the volar surface of the forearm, and incision is made either perpendicular or parallel to the elbow crease. Those advocating a parallel incision point to the increased sensitivity to platelet defect associated with this type of incision,

while those advocating a perpendicular incision point of the diminished scarring with this tube of incision. It is important that any one laboratory use only one direction for the incision to obtain consistency in their results. A blood pressure cuff should be applied to the arm, and a constant pressure of 40 mm Hg maintained throughout the procedure. Fluctuation of the venous blood pressure because of a leaky cuff will cause erratic results in the bleeding time. Uniform and consistent pressure must be applied to the bleeding time device to ensure a uniform incision. Excessive pressure can result in a deeper cut and artificial prolongation of the bleeding time, whereas insufficient pressure may lead to a superficial cut and an artificially short time. Blotting is normally performed every 30 seconds and should be done in a manner to prevent disruption of the forming platelet plug. Following the completion of the bleeding time, the wound should be closed with a butterfly-type bandage and covered with a bandage.

The PT and APTT are tests of fibrin clot formation. The PT evaluates the extrinsic system of coagulation, beginning with activation of coagulation by tissue factor – factor VII and is sensitive to defects in fibrinogen, prothrombin, factor V, factor X, and factor VII. The APTT evaluates the intrinsic systems of coagulation and is sensitive to defects in fibrinogen, prothrombin, factor V, factor X, factor VIII, Factor IX, factor XI, factor XII, prekallikrein, and HMW kinogen. A number of technical variables can affect performance of the PT and APTT. Among these specimen acquisition, sample processing, and choice of reagent are key components. Care should be taken to obtain a clean venipuncture sample and to anticoagulate it promptly with an appropriate amount of citrate. The sample should be processed as soon as possible to yield platelet-poor plasma. PT and APTT reagents vary significantly in their sensitivity and responsiveness to various haemostatic disorders. Therefore, the laboratory should be aware of the performance characteristics of their reagents and should carefully construct their own normal range to distinguish normal from abnormal adequately. Now-a-days Carrier erythrocytes have been evaluated in thousands of drug administration in humans Proving safety and efficacy of the treatments [26]. Based on the results of the clinical history and screening laboratory tests, a limited number of patterns emerge. Platelet disorders are usually characterized by mucocutaneous bleeding and a long bleeding time and / or a low platelet count. In contrast, coagulation disorders are usually characterized by a history of soft tissue bleeding and an abnormality of the APTT or PT > Fibrinolytic disorders are classically characterized by a history of delayed bleeding, often following trauma, and normal screening tests of Haemostasis. Based on these initial results, further evaluation for a specific disorder can be undertaken. It is helpful to keep in mind the relative frequency of congenital disorders associated with bleeding. The three most common are von Willebrand's disease, hemophilia A and hemophilia B.

Evaluation of Potential Thrombotic Tendency

As with the evaluation of bleeding disorders the evaluation of a potential thrombotic tendency begins with a thorough medical history. One of the primary goals of the history is to determine the likelihood of a congenital defect. In this regard, it is important to note the age of onset of symptoms and any family history of thrombosis. Most congenital defect is associated with onset of symptoms prior to the age of 45, and the family history for most of these disorders is also positive. Congenital thrombophilia is usually associated with recurrent deep venous thrombosis but may, on association, be associated with arterial thrombosis. A completely medical history must be obtained to determine if there is an underlying medical illness that may predispose to thrombosis (e.g. metastatic tumor, autoimmune disorders etc.). A thorough

medication history is also important particularly before prescribing oral contraceptives in young women.

There are also screening assays that assess the regulatory system of coagulation or the degree of activation of the procoagulant mechanisms. Therefore, the laboratory approach to potential thrombotic disorders involves measuring the individual components of the regulatory systems. In general, assays that reflect the biological activity of the protein in question should be selected for use. In practice, such functional assays have been difficult to develop for some components of the system, notably protein C and protein S. In addition, assays for lupus anticoagulant activity and anti-phospholipid antibodies may be of use. More recently, a variety of markers of the level of activation of the haemostatic system have been described these include such peptides as prothrombin F_{1, 2}. Thrombin - anti thrombin complexes, protein C activation peptide, and plasminogen-anti plasmin complexes. The usefulness of these assays in determining the risk of thrombosis, however, remains undetermined.

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