

Studies on Components of Blood & Their Functions

Chapter 6

Coagulation Factors: An Overview

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1. Introduction

Hemostasis is a critical physiological function defined as the process that leads to the cessation of bleeding from a blood vessel [1]. Broadly, the process of hemostasis involves the following: local vasoconstriction, platelet plug formation, and the coagulation cascade. The initial brief reflexive vasoconstriction with platelet adhesion, aggregation, and activation constitutes primary hemostasis, while fibrin clot formation and stabilization are referred to as secondary hemostasis [2]. Blood coagulation is among the most critical steps involved in mediating hemostasis. It has been defined as a process in which flowing liquid blood plasma is converted to a soft, viscous gel entrapping the cellular components of red cells and platelets, thereby preventing blood's extravasation [3]. It is executed through two distinct pathways: extrinsic and intrinsic, eventually leading to a common pathway. The extrinsic pathway is known as the tissue factor pathway. Tissue factor released by the endothelial cells is responsible for the induction of the coagulation process. The intrinsic pathway is the contact activation pathway. Exposure of specific coagulation factors to subendothelial collagen is the inciting event for the initiation of this pathway [4] **Figure 1** depicts both extrinsic and intrinsic coagulation pathways. In this narrative review, we discuss the important coagulation factors, their applications in the laboratory, clinical medicine, and pharmacology.

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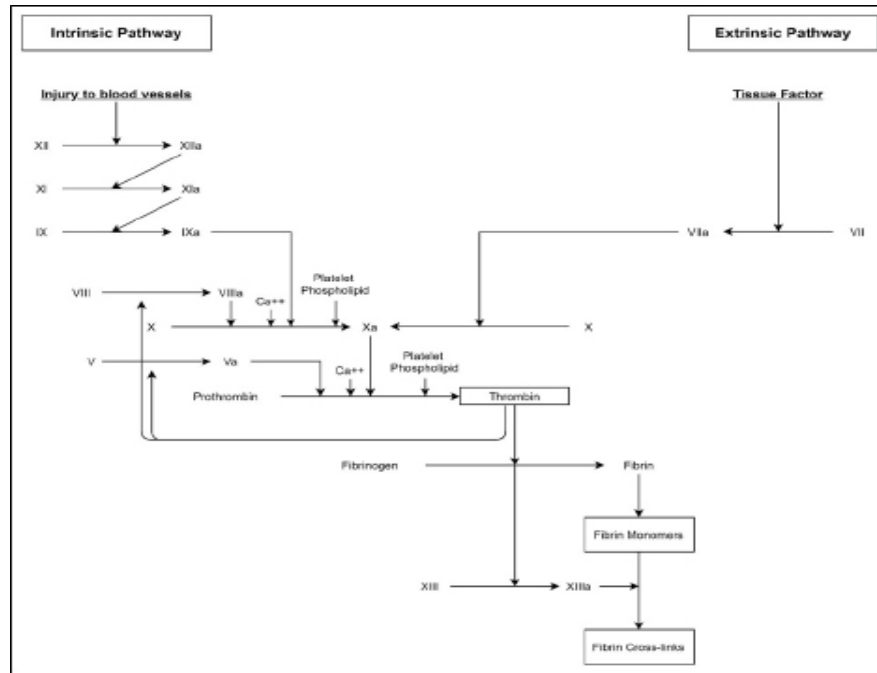


Figure 1: Intrinsic, Extrinsic and Common coagulation pathways.

2. The Coagulation Factors - Biochemistry, Physiology, and Functions

2.1. Factor I

Fibrinogen, also known as Factor I of the coagulation cascade, is a protein synthesized by the liver. Approximately 2-5 g of fibrinogen is produced in the body each day, resulting in plasma levels of 1.5 to 4 g/L [3,5]. Structurally it is a 340 kDa protein and consists of three pairs of polypeptide chains $A\alpha$, $B\beta$, and γ weighing 66.5, 52, and 46.5 kDa, respectively [5]. These are encoded by a gene cluster located on Chromosome 4 and are held together by disulfide bonds [3,6]. Fibrinogen is a soluble macromolecule, but upon activation of the coagulation cascade, ultimately gets converted into insoluble fibrin. The enzymatic protein Thrombin is responsible for this change, which brings about the transformation by cleaving the N terminal peptide off the $A\alpha$ and $B\beta$ chains. This allows for strong non-covalent interaction between the fibrinopeptide cleaved molecules, resulting in insoluble fibrin strands [3].

2.2. Factor II and III

Prothrombin is the coagulation factor II. It is synthesized by hepatocytes as a protein containing 622 amino acids and bears a molecular weight of 72 kDa [3]. Activation of prothrombin to the active enzyme thrombin can be initiated with proteolytic cleavage at either the 271st or the 320th amino acid residue [7,8]. Cleavage at residue 271 yields a substrate by the name of prethrombin, whereas cleavage at residue 320 gives rise to meizothrombin. Further cleavage of prethrombin at residue 320 or Meizothrombin at residue 271 produces active thrombin. Thrombin acts on multiple substrates of the coagulation cascade, including fibrinogen and factors V, VIII, XI, XIII [3]. Thromboplastin or coagulation factor III is a combination of tissue factor and phospholipids, which serves as an enzymatic complex to aid prothrombin

conversion to thrombin. Thromboplastin has been used to evaluate the prothrombin time (PT) due to its action of activating the extrinsic pathway [9]. Partial thromboplastin, which consists of only phospholipids, can also be used to assess the intrinsic coagulation pathway's function due to its role in the activated partial thromboplastin time (aPTT) test [4].

2.3. Factor IV and V

Calcium plays a part at multiple junctures in the coagulation cascade and is also called Factor IV. Owing to its positive charge, it interacts with negatively charged phospholipids and mediates binding of Factors II, VII, IX, X via the carboxylated glutamic residues to the platelet surface. It is also involved in the activation of Factors X and V [4]. Besides, it has been shown that calcium functions along with factor XIII to stabilize the fibrin clot protein C production is also upregulated by Calcium [10]. Factor V or proaccelerin is a coagulation factor protein synthesized by hepatocytes, which serves as a cofactor and possesses no enzymatic activity. It is encoded by a gene locus on chromosome 1 and has a molecular weight of 330 kDa [3]. It is a part of the common coagulation pathway functioning as a cofactor in the prothrombinase complex. It forms in conjunction with Factor X. Prothrombinase serves to convert prothrombin into active thrombin. It should be noted that the generated thrombin itself is responsible for further conversion of Factor V into Factor Va. Factor V is usually degraded by Protein C upon stimulation by thrombin and thrombomodulin [4]. A variant known as Factor V Leiden is characterized by resistance to protein C degradation and leads to a thrombophilic state resulting in complications such as Deep Vein Thrombosis and Pulmonary embolization [11].

2.4. Factor VII and VIII

Factor VII also known as proconvertin, is synthesized initially as a precursor protein that undergoes modification to yield a 406 amino acid polypeptide [3]. It is a molecule belonging to the serine protease class of enzymes. It cannot express its proteolytic action unless associated with tissue factor, which acts as an allosteric activator [12,13]. Once bound to the tissue factor, it can be converted to its active state by several moieties, including factor Xa and thrombin. When activated, it catalyzes the conversion of both factors IX and X to their respective active states [14].

Factor VIII or Antihemophilic globulin is a protein encoded by a gene locus on the long arm of Chromosome X. Contrary to most coagulation factors that are produced within hepatocytes, factor VIII is synthesized by liver sinusoidal cells, high endothelial venules as well as lymphatic capillary endothelium [15,16]. Factor VIII circulates in the plasma in close association with the von Willebrand factor (vWF), which shields it from proteolysis and prolongs its half-life. Along with Factor V, it is the only other coagulation factor to not possess enzymatic activity [17]. Upon activation by thrombin, Factor VIII dissociates from vWF and exerts its function as a cofactor to factor IXa. Together they bring about activation

of factor X. Due to loss of protective effect of vWF after dissociation, factor VIII ultimately gets inactivated by protein C, amongst others.(4) Deficiency of this clotting factor results in the most common severe inherited coagulopathy in humans, namely, hemophilia A [18].

2.5. Factor IX, X, XI and XII

Factor IX or Christmas Factor is a clotting factor that, like several others, has serine protease activity. Synthesized within hepatocytes, the protein's mature form is 415 amino acid residues long [3]. Factor XIa can bring about this factor's activation due to the intrinsic pathway's progression or less commonly by 'factor VIIa - tissue factor – calcium' complex [19]. The prime function of Factor IXa occurs in conjunction with Factor VIII as together they form 'tenase', i.e. a protein complex acting on Factor X, resulting in its activation. Deficient levels of this coagulation factor lead to Hemophilia B [18].

Factor X Is also known as the Stuart Prower factor. Its a molecule produced by the liver and structurally consists of 448 amino acids [3]. The involvement of Factor X in the coagulation cascade signifies the beginning of the so-called common pathway, i.e., where the intrinsic and extrinsic pathways culminate. Factors VIIIa-IXa and factor VIIa-tissue factor can cleave the polypeptide chain at specific sites causing activation. Thereafter, factor Xa alongside Factor Va constitutes the chief prothrombinase enzyme complex responsible for converting prothrombin into active thrombin [4].

Factor XI or Plasma thromboplastin antecedent is a coagulation factor structurally a homodimer with a molecular weight of 160 kDa [20,21]. Factor XI is a part of the intrinsic coagulation pathway and may be activated by either thrombin, factor XIIa, or factor XIa itself. Its substrate is factor IX, which is converted to Factor IXa [4].

Factor XII, also known as Hageman factor, is an 80kDa protein consisting of multiple domains. It serves as the starting point for coagulation's intrinsic or contact pathway as it gets activated upon contact with negatively charged biologic surfaces [22]. Besides coagulation, activated Factor XII also modulates the inflammatory response owing to its function of generating bradykinin, thereby initiating the Kallikrein- Kinin system [4].

2.6. Factor XIII

Factor XIII or Fibrin Stabilizing Factor is an enzymatic protein with transglutaminase activity [23]. It consists of two 'A' subunits which possess enzymatic activity and two 'B' subunits, which act as carrier proteins [3]. It is converted by thrombin to its active form. It covalently cross-links fibrin monomers under its transglutaminase action, thus providing stability to the formed clot. Deficiency, especially of the A subunit, causes a rare but potentially severe hemorrhagic disorder. On the contrary, the paucity of the B subunits causes a relatively

mild coagulopathy [24].

2.7. Additional coagulation factors:

High molecular weight kininogen (HMWK) is a 620 kDa protein comprising a 622 amino acid chain [25]. It has been subdivided into domains numbered 1-6, each possessing a distinct function. Amongst the most critical roles of HMWK is its cleavage by plasma kallikrein at domain 4 to yield bradykinin. Domain 1 acts to inhibit atrial natriuretic peptide, whereas data suggests that domains 2 and 3 are responsible for inhibiting calpain and papain. Domains 5 and 6 have a role to play in the contact activation pathway [3]. Prekallikrein is a hepatic synthesized precursor of Plasma kallikrein. It bears close structural resemblance with factor XI, although they are products of separate genes [3]. It circulates in the blood bound to HMWK. Coagulation Factor XII is responsible for the conversion of prekallikrein into active plasma kallikrein [26].

Kallikreins are a group of serine proteases with diverse physiological functions. They are grouped into plasma Kallekrein, of which there is only one subtype KLKB1 and Tissue Kallekreins, of which there are 15 subtypes, ranging from KLK1 to KLK15. The tissue kallikreins are products of the largest contiguous cluster of protease genes in the human genome located on chromosome 19 [27]. Plasma Kallikrein, derived from prekallikrein, is responsible for generating vasoactive molecules like bradykinin and kallidin under its action on kininogens [28]. Tissue kallikreins have a broad spectrum of activity across various organ systems. KLK1, KLK2, and KLK3 also participate in bradykinin production, similar to plasma kallikrein [29]. Kallikreins also have been found to regulate neural plasticity, skin desquamation, and semen liquefaction [30-32]. Platelet phospholipids are a critical component of Coagulation Factor III. Owing to raised intracellular calcium, phospholipids are expressed widely across the platelet cell membrane and provide attachment sites to clotting factors, especially activated Factor Xa [3,33]. This action allows it to exert its prothrombinase activity.

3. Clinical Importance and Application of Coagulation Factors

3.1. Investigations Used in Clinical Practice

Prothrombin Time (PT) / International Normalized Ratio (INR) - is an in vitro assay used to evaluate the function of the extrinsic and common coagulation pathways. Precisely, it measures the time required to form a clot after thromboplastin addition to a given blood sample, which under physiological conditions ranges between 10 to 13 seconds [34]. The patient's blood is initially collected in a vial containing citrate, which chelates calcium and prevents coagulation cascade progression [35]. Once ready to be tested, calcium is added in excess alongside supraphysiological amounts of tissue factor, thereby initiating the extrinsic coagulation pathway. However, owing to wide variations in the constitution of tissue factors

provided by different manufacturers, standardization was deemed necessary. The INR was thought of as a solution. Each manufacturer assigns a particular numerical figure known as the international sensitivity index (ISI) based on comparisons drawn with an international standard tissue factor substrate. The INR thus is the ratio of the PT of the patient's sample to the PT of a control sample raised to the power denoted by the ISI [4]. Prolonged PT can occur due to Vitamin K deficiency, liver disease affecting synthetic function, disseminated intravascular coagulation (DIC) as well as Warfarin therapy [36]. PT can be used for laboratory evaluation for warfarin therapeutic efficacy and diagnosis of toxicity. For most conditions treated with warfarin, an INR of 2-3 is usually targeted [37].

Activated partial thromboplastin time (aPTT) is a test performed in vitro to assess the intrinsic and common coagulation pathways' dynamics. Since the intrinsic pathway relies on contact activation, this requirement is sufficed in vitro by adding kaolin or silica or other similar negatively charged molecules and the phospholipid cephalin. It is one of the initial tests used in the evaluation of Hemophilia. The normal range is 25 to 35 seconds, although it may vary across several laboratories [38]. A prolonged aPTT could be denotive of conditions such as Hemophilia, von Willebrand disease, and DIC [38].

Thrombin time is a laboratory test used to assess fibrin's generation from fibrinogen by thrombin's action. Excess of thrombin is added to a centrifuged blood sample, and the time needed for clot formation is measured. The normal thrombin is usually less than 20 seconds and typically ranges between 14 -16 seconds. Prolonged thrombin time could be suggestive of hypofibrinogenemia, dysfibrinogenemia, or the presence of thrombin inhibitors [39].

3.2. The Hemophilias

Hemophilia refers to an inherited bleeding disorder caused by deficiency or rarely dysfunction of a particular coagulation factor. It is the most common severe hereditary hemorrhagic disorder in humans.(40) Hemophilia almost always results from a defect or mutation in the gene encoding the clotting factor [41]. The hemophilia spectrum includes the following three conditions, namely hemophilia A, hemophilia B, and hemophilia C.

Hemophilia A, also known as classic hemophilia, is the most commonly encountered subtype and results from deficiency of the coagulation factor VIII. Factor VIII is an integral component of coagulation's intrinsic pathway and enhances thrombin production and fibrin clot formation; its deficiency hampers these critical processes. It is inherited via the X linked recessive mechanism. As a result, men are predominantly affected, with this condition presenting in 1 in 5000 males with close to 400,000 cases worldwide [42]. Females are usually carriers with symptoms rarely manifesting; except in conditions where one of the X chromosomes is inactivated or absent. The disease characteristically presents as bleeding out of proportion to the causative trauma, i.e., major bleeding in response to relatively minor trauma. Clinical

features may be apparent as early as birth in the form of subgaleal/ intraparenchymal bleeds or unexplained excessive bleeding after circumcision [41]. A classical manifestation is bleeding within joints known as hemarthrosis, which presents as tense, tender, swollen joints and can lead to long term joint destruction. This typically occurs in infants learning to walk and suffering falls and often presents in conjunction with muscular or cutaneous bleeds [41]. Recurrent bruising, epistaxis, hematoma formation, and excessive oral mucosal bleeding after minor surgical procedures like dental extraction also fall within the spectrum of the disease. Occult intraparenchymal bleeds in internal organs may also occur in severe cases. The severity of bleed often correlates with the coagulation factor's plasma levels, with the greatest severity seen at levels < 0.01 IU/ml. Laboratory evaluation reveals prolonged activated partial thromboplastin time with a normal platelet count [43]. Levels of Factor VIII may be measured for confirmation. Treatment is usually using the administration of Factor VIII [18].

Hemophilia B, also known as the Christmas disease, is a X-linked recessive disease caused by deficiency of clotting factor IX. This subtype frequency is much lower than Hemophilia A and is estimated at 1 in 40,000 males [44]. Like the previous variant, a deficiency in factor IX also disrupts the intrinsic coagulation pathway resulting in impaired hemostasis. The clinical features are similar, although much less severe than Hemophilia A [45]. Laboratory evaluation also may show a similar picture with reduced factor IX levels confirmatory for the diagnosis. Treatment is with replenishment of Factor IX [18].

Hemophilia C, which is a result of Factor XI deficiency, is the least common subtype with an occurrence of close to 1 in 100,000 males [46]. Hemophilia C does not usually present with spontaneous bleeding, hemarthrosis, or intramuscular bleed as opposed to the other two variants. Bleeding most often occurs secondary to surgery or trauma. Like the rest, it may also cause aPTT prolongation on laboratory evaluation. Treatment of hemophilia C is by Factor XI administration [46].

3.3. Anticoagulants

Heparin or Unfractionated Heparin (UFH) is a naturally occurring glycosaminoglycan comprising 10 to over 100 saccharide units that function as an anticoagulant. It mediates its action by potentiating the function of the intrinsic enzyme antithrombin III (ATIII) through induction of a conformational change in its structure, and thus, the activity of this enzyme rises several-fold [47]. The activated ATIII scavenges Factor II (Thrombin), Factor Xa, and other proteases, thereby halting the coagulation cascade. It is worth noting that a 16-18 saccharide sequence is necessary to inhibit thrombin's function, while a mere pentasaccharide sequence is deemed sufficient to stop factor Xa activity [48]. This knowledge led to the development of fractionated or low molecular weight heparins (LMWH) – e.g., enoxaparin, dalteparin containing between 10 - 23 saccharide units, explicitly targeting the pentasaccharide sequence

and inhibit coagulation by means of suppressing only factor Xa activity with no effect on thrombin [47,48]. UFH administered subcutaneously/intravenously, and LMWH given subcutaneously has found widespread utility in managing various thromboembolic disorders such as deep vein thrombosis, pulmonary embolism, Acute Coronary Syndrome as well as in Atrial Fibrillation and mechanical heart valve thrombosis prophylaxis.(49) Although aPTT monitoring is needed to evaluate for efficacy/toxicity of UFH, LMWH does not require any such evaluation. Protamine Sulphate is used as an antidote to the toxicity of UFH and LMWH, although it cannot neutralize the anti-factor Xa activity of LMWH effectively [47].

Warfarin is a coumarin derivative and a widely used oral anticoagulant which exerts its action via Vitamin K activity antagonism. It derives its nomenclature from the Wisconsin alumnus research foundation (warf) + arin (coumarin). Vitamin K in a reduced state (hydroquinone) is essential for enzyme-mediated gamma-carboxylation of glutamic acid residues on clotting factors II, VII, IX, X [50]. During this process, Vitamin K itself is converted to an epoxide form and requires VKOR activity to retransform it into the hydroquinone state. Warfarin inhibits the (VKORC1) activity and indirectly prevents activation of the clotting as mentioned above factors, affecting the extrinsic pathway and the overall coagulation process [51]. Since Protein C and Protein S are natural anticoagulants and rely on the method mentioned above, there is a transient procoagulant action of Warfarin in the first 1-2 days. Hence concomitant heparin administration is needed usually for the initial 4-5 days. It finds use mainly as long term prophylaxis in conditions such as Deep Vein Thrombosis, Pulmonary Embolism, Atrial Fibrillation [50]. Laboratory monitoring is usually carried out with Prothrombin Time (PT) and International Normalized Ratio (INR), with most clinical scenarios demanding an INR between 2-3 for optimal therapeutic effect. Overdose warrants immediate treatment with fresh frozen plasma (FFP) and definitive management with vitamin K [52].

Novel Oral Anticoagulants (NOACs) or Direct Oral Anticoagulants(DOACs) are a relatively newer anticoagulant class. They include the drugs Dabigatran, Rivaroxaban, Apixaban, and Edoxaban, among others. Dabigatran acts via direct thrombin antagonism, while the rest are inhibitors of factor Xa. These drugs' advantages over heparin or warfarin include: All-cause mortality primarily due to intracranial hemorrhage is much lower, Less frequent laboratory monitoring for effect, and Observational studies indicating lower fracture risk [53]. However, the following circumstances necessitate treatment with heparin or warfarin over NOACs – Prosthetic heart valves, Severe liver disease, Chronic Kidney disease, and Pregnancy [54].

4. Conclusion

Thus, understanding the coagulation pathways and clotting factors is crucial to understand the underlying pathophysiology of several life-threatening diseases and their

potential therapies. Research is continuing on several fronts involving novel therapies to treat coagulation factor deficiencies and dysfunctions. Emerging treatment options for hemophilia A, like gene therapy and Efficizumab, are proving to be more efficacious than the conventional factor VIII replacement. Of course, longitudinal studies and randomized controlled trials are required to draw definitive conclusions on their efficacy and safety. A thorough understanding of the coagulation factors, their clinical applications, and the basics of managing the associated pathology is essential in optimizing patient care.

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