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Understanding the Clotting Cascade, Regulators, and Clinical Modulators of Coagulation

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Abstract

The circulatory system plays a vital role in the survival of an organism by supplying it with essential nutrients, signaling molecules and eliminating the waste or toxic products from the body. This flow is tightly regulated by various factors, procoagulants support the formation of hemostatic plugs to prevent the leakage or blood loss and anticoagulants prevent the formation of unwanted clots. Disruption or dysregulation of procoagulants and anticoagulants lead to clinical complexities. In this chapter defects in the coagulation system, hereditary, acquired coagulation disorders, their diagnosis and recent clinical modulators of the coagulation system are discussed.

Keywords: coagulation, hemophilia, thrombosis, pro-coagulants and anticoagulants

1. Introduction

Blood carries a set of zymogen serine proteases called procoagulants these serine proteases are activated upon injury and promote the formation of a clot [1]. The clot formation initiates by two mechanisms [1–4]. One of the mechanisms is termed as tissue factor pathway or extrinsic pathway, and the other pathway is called as contact pathway or intrinsic pathway [2]. Extrinsic pathway or tissue factor pathway is initiated by the tissue factor (TF) released form the damaged cell [1–4]. TF proteolytically cleaves a zymogen factor VII (FVII) and activates it [1–4]. Activated factor VII (FVIIa) forms a complex with TF, forming a potent protease complex which activates the downstream cascade by limited proteolysis. TF-FVIIa complex converts the inactive factor IX (FIX) and factor X to activated factor IX (FIXa) and activated factor X (FXa). The activated FIXa binds to activated Factor VIIIa(FVIIIa) to form X-ase complex on the

phosphatidylserine-rich membrane surface, this complex converts FX to FXa. Intrinsic/ contact pathway is initiated by artificial surfaces in the plasma. Artificial surfaces induce conformational change in factor XII (FXII) and results in activation of small amount of FXII. Activated factor XII (FXIIa) activates high molecular weight kininogen (HK) and plasma prekallikrein (PK) and this acts as a positive feedback loop for FXII activation [1–4]. Further, FXIIa activates Factor XI (FXI) to FXIa, which intern activates FIX. Both extrinsic and intrinsic pathways collaborate with a common pathway that involves activated FXa. FXa binds to activated factor V (FVa), forming prothrombinase complex and Prothrombinase complex cleaves prothrombin to generate activated thrombin. Thrombin cleaves fibrinogen to fibrin, these fibrin monomers polymerizes to form insoluble fibrin polymer (**Figure 1A**).

Along with the clotting factors, platelets play a vital role in regulating the hemostasis by forming a cellular plug at the site of injury. The circulating platelets get immobilized at the sub endothelial surface of the site of injury by binding to the von Willebrand factor (VWF) [3]. Platelet receptor GPIb-IXV is essential for this process. Similarly, receptor GPVI helps to anchor the platelets at the site of injury with the help of collagen. Further these platelets get activated and expose phosphatidylserine, which provides a lipid surface for the clotting factors [3]. Among the clotting factors, fibrin helps in activating the platelets by cleaving the protease activated receptors (PARs) that include PAR1 and PAR4 (**Figure 1B**).

Hemostasis is tightly monitored by feedback mechanisms, where anticoagulants inhibit the protease function of coagulation favors by directly inhibiting them or their cofactors [1–4]. The natural anticoagulants include tissue factor pathway inhibitor (TFPI), Activated protein C (APC), Protein S (PS) and Protein Z (PZ). These anticoagulants help in regulating blood clot
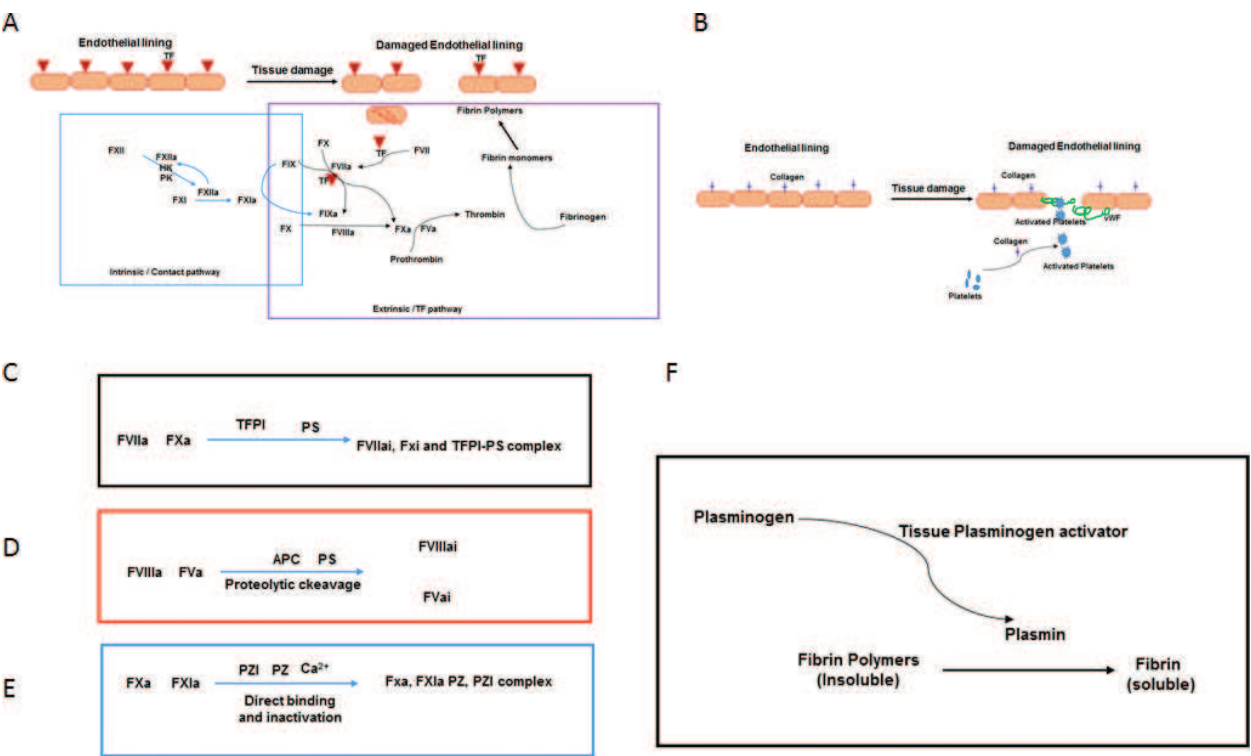

Figure 1. (A) Schematic representation of coagulation cascade. (B) Schematic representation of platelet plug formation. (C) TFPI pathway. (D) APC function. (E) PZI pathway. (F) Clot lysis.

formation [5–7]. TFPI directly binds to FVIIa and Xa complex and inhibits their function and the TFPI function is accelerated in presence of PS. APC proteolytically cleaves FVIIIa and FVa [7]. PS was discovered as a cofactor for TFPI, APC and recent reports demonstrated that PS can directly bind and inhibit the functions of FVa, FIXa and FXa [5–7]. Protein Z-dependent protease inhibitor inhibits FXa and FXIa, in the presence of PZ and calcium [8] (**Figure 1C**-**E**).

Blood clots from the healthy system are removed by fibrinolytic system [9]. In the fibrinolysis process tissue specific plasminogen activators (tPA) or urokinase plasminogen activator activates plasminogen by proteolytically cleaving it into activated plasmin. Plasmin cleaves the insoluble fibrin polymers into soluble peptides [9] (**Figure 1F**).

2. Defects in hemostasis

Quantitative or qualitative defects in the coagulation factors lead to hemostatic defects such as hemophilia or thrombosis [10, 11]. Hemophilia is characterized by defects in clotting factors and it is characterized by spontaneous or periodic bleedings [11]. Whereas, thrombosis is caused by the high amount of procoagulants in the plasma, hyper activation of procoagulants or defects in anticoagulants [10]. Thrombosis is characterized by systemic clots which impair the normal hemostasis. Bleeding disorders are also classified hereditary and acquired disorders. Hereditary disorders are associated with gene mutations and inherited to the offspring [11]. The major hereditary disorders are hemophilia, rare bleeding disorders and thrombosis. Acquired disorders are caused by several factors such as infections, habits and environmental effects [12].

3. Hemophilia

Hemophilia is an inherited bleeding disorder, caused by the deficiency of procoagulants. Deficiency of FVIII is known as hemophilia A, deficiency of FIX is known as hemophilia B and deficiency of FXI is known as hemophilia C [13–17]. The hemophilia A and B are X chromosome linked disorders and they are mainly observed in the male population [14, 15, 17]. Hemophilia A cases are observed in 1 in 5000 males whereas, hemophilia B cases are observed in 1 in 20,000 males (https://www.hemophilia.org/About-Us/Fast-Facts). Hemophilia is classified based on the functional antigen levels. Patients with <1% activity with spontaneous bleeding are termed as severe hemophilia, patients with 1–5% activity are called moderate hemophilia and individuals with >5%, <40% are termed as mild hemophilia [17].

3.1. Hemophilia a and factor VIII

Hemophilia A is majorly caused by deficiency in FVIII antigen levels or mutations in FVIII gene that effect FVIII functions [18, 19]. FVIII is encoded by the gene that localized on the long arm of X chromosome and the gene consists of 26 exons [18, 19]. A total of 2537 mutations are identified on FVIII gene [20]. FVIII is highly expressed in the liver [21, 22]. The mature FVIII protein consists of 2332 amino acids with 6 domains. These domains include A1, A2, B, A3, C1 and C2 [23, 24]. In the blood FVIII is activated by thrombin or FXa [25, 26]. Thrombin cleaves FVIII at R372, R740 and R1689 and removes B domain [26]. Similarly, FXa cleaves FVIII at

K36, R336, R562, R740, R1689 and R1721 [27]. FXa mediated cleavage of FVIII at K36 and R336 leads to inactivation of FVIIIa [27]. APC inactivates FVIII by proteolytically cleaving FVIIIa at R336 and R562 which leads to destabilization of A1 and A2 domain interaction [28, 29] (**Figure 2**). In 1960s, major treatment for hemophilia A is whole blood or plasma transfusion [30, 31]. This treatment has a drawback of viral transfusion along with the coagulation factors. Treatments of mild hemophilia A include vasopressin analogs to enhance the synthesis of FVIII, 1-Desamino-8d-arginine vasopressin (DDAVP) is a vasopressin analog clinically used to enhance the plasma levels of FVIII [32, 33]. Recombinant FVIIa and FVIII are also used to prevent bleeding events in the hemophilia patients [34].These clotting factors are also supplemented with FVIIa or factor FVII inhibitor bypassing agent (FEIBA) to enhance the function of FVIIa and FVIII, whereas FEIBA enhanced the risk of thrombosis [35, 36]. Recent studies elucidated that stabilized recombinant FVIII can be used as a therapeutic for hemophilia A, this includes more stable isoforms of FVIII such as B domain deleted FVIII (BDD FVIII) [37] (**Table 1**). The ongoing research is focusing on using BDD FVIII as a gene therapy by incorporating it into the viral vectors and delivering it into the patient [38].

3.2. Hemophilia B and its treatment

Hemophilia B is another bleeding disorder considered to be indistinguishable from hemophilia A whereas, recent evidences elucidated that hemophilia B patients have less severe bleeding phenotype lower bleeding frequency and better long term outcomes compared to hemophilia A [42]. Hemophilia B is caused due to FIX deficiency [43]. FIX is encoded by a gene present on X chromosome, FIX is a major component of intrinsic pathway of coagulation cascade and it is activated into FIXa by FXIa or FVIIa [44–46]. Activated FIX forms a X-ase complex with FVIIIa and phosphatidylserine [47]. Tenase complex converts X to Xa [47]. The bleeding tendency depends on FIX activity in the plasma [48]. FIX deficiencies are classified based on the plasma FIX activity and they are severe (<1% FIX activity), moderate (1–5%) and
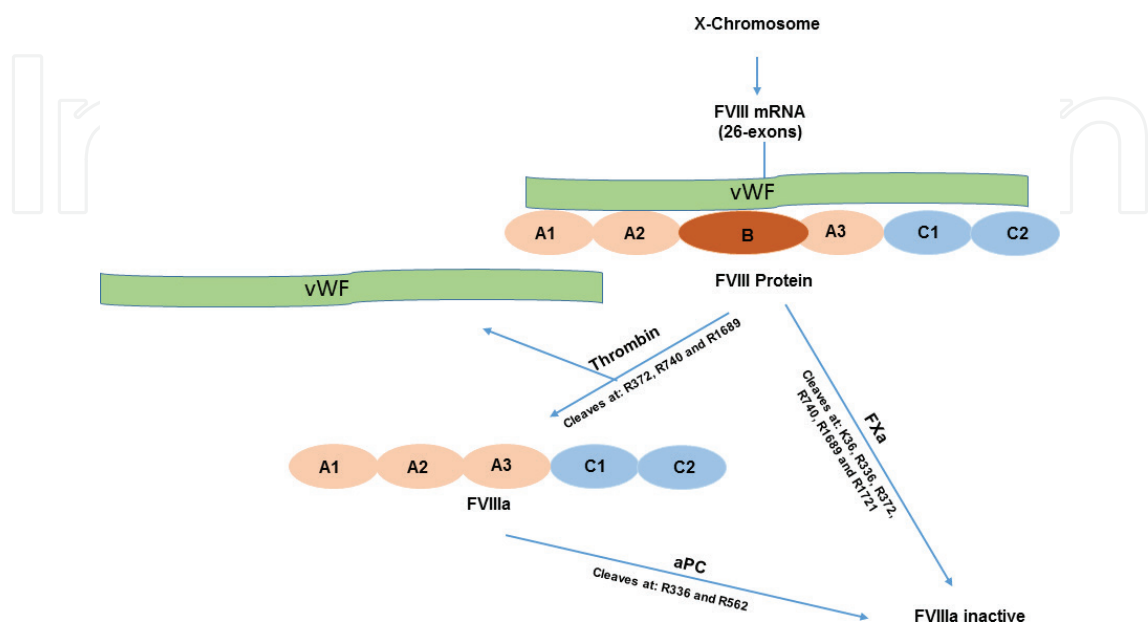

Figure 2. Schematic representation for FVIII biosynthesis and inactivation.

FVIII-product	Half-life in hours
Full length Plasma derived [31]	14.8–17.5
Plasma derived FVIII-vWF complex [39]	12.2–17.9
Recombinant Full length FVII [34]	14.6 ± 4.9
B-Domain Deleted FVIII [34]	14.5 ± 5.3
BDD-PEGylated [40]	14.69 ± 3.79
BDD-rFVIII-Fc [41]	19.7 ± 2.3
BDD-rFVIII EHL single chain [30]	14.2

Table 1. Development of FVIII therapy for hemophilia.

mild (5–40%) [48]. Current therapies for hemophilia B include plasma derived FIX, recombinant FIX, recombinant FIX fused with polyethylene glycol (PEG), recombinant FIX fused with Fc portion of immunoglobulin G, FIX fused with albumin mutant FIX [48]. The disadvantages of plasma derived FIX is that it has a very short half-life in the patient plasma and plasma derived FIX has chances of viral contamination. Recombinant FIX is produced in Chinese hamster ovary cells [48]. rFIX has an increased half-life compared to plasma derived FIX, it over came the problem of viral contamination, however rFIX showed 30% less activity compared to plasma derived FIX, due to variations in the glycosylation. Conjugation of FIX with PEG is known as PEGylation. PEG serves as a shield for PEGylated FIX and protects it from proteolytic cleavage. PEGylated FIX's half-life is five times in mice compared to the half-life of rFIX [48]. FC fused FIX has a half-life of 48 h. The other FIX fusion protein in clinical trial is FIX fused with albumin. Single amino acid mutation in the catalytic domain of FIX (R338L) increased its Tenase activity by 2 fold and thrombin generation activity by 6 fold, therefore by FIX R338L usage in gene therapy is under investigation [49].

3.3. Hemophilia C

Hemophilia C is caused by FXI deficiency where factor XI activity of 15–20 U/dL or lower. Surprisingly FXI deficiency does not show a severe bleeding phenotype [16].

4. Platelets in hemophilia

Platelets are key components of primary coagulation system [50]. TF released from the damaged endothelial cells activates the platelets [51].Activated platelets get adhere to the site of damage with help of vWF [52]. Upon activation platelets expose phosphatidylserine which gives the lipid surface to the coagulation cascade [51]. Recent studies elucidate that platelets can play a major role in hemophilia, as hemophilia patients with same FIX or FVIII antigen levels has different clotting time due to variations in the platelet mediated coagulation activity [53–55]. Platelets store FVIII in the alpha granules therefore, platelets are being used as therapeutic components for hemophilia treatment, majorly in gene therapy. In a recent study, hemophilic dogs were transfused with genetically modified platelets (which can over express FVIII gene). Bleeding events were stopped in the hemophilic dogs after transfusing them with genetically altered platelets [53].

5. Rare bleeding disorders

Rare inherited bleeding disorders (RBDs) include deficiencies of coagulation factors such as fibrinogen, factor II (FII), FV, combined deficiency of FV and FVII, FVIII, FX, FXI, FXIII and vitamin K dependent factors. RBDs are mostly autosomal recessive disorders varying from 1 in 500,000 to 1 in 2–3 million [56]. These disorders are diagnosed by clotting assays such as thrombin time, prothrombin time and activated partial thromboplastin time followed by molecular diagnosis [56].

5.1. Fibrinogen deficiency

Fibrinogen is a 340 kDa hexamer assembled by the combination of 3 homologous polypeptide chains (Aα, Bβ and γ) [57]. Fibrinogen plays an important role in clot formation where thrombin converts fibrinogen into soluble fibrin which further forms an insoluble polymer mesh, fibrin also plays an active role in platelet aggregation by binding to glycoprotein IIb/IIIa on the activated platelets [57]. The genes encoding for Bβ (FGB), Aα (FGA) and γ (FGG) are located on chromosome 4 from centromere to telomere [58]. Fibrinogen is primarily synthesized in liver [59]. Fibrin deficiency is identified as two phenotypes termed as afibrinogenemia/hypofibrinogenemia and it is characterized by low plasma and platelet fibrinogen antigens whereas, dys/hypodysfibrinogenemia is characterized by the deficiency of functional fibrinogen levels [60, 61]. Afibrinogenemia is detected by prolonged prothrombin time, thrombin time, activated partial thromboplastin time, impaired platelet adhesion and impaired platelet aggregation induced by ADP [60, 62]. Clinical manifestations of fibrinogen include umbilical stump bleeding, possible gastrointestinal bleeding, recurrent episodes of intracranial hemorrhage [60, 63]. Treatment for fibrinogen deficiency include replacement therapy by cryoprecipitate [63].

5.2. Prothrombin deficiency

Prothrombin is a vitamin K dependent glycoprotein synthesized in the liver [64]. Prothrombin is encoded by 21 kb gene present on chromosome 11 [65, 66]. Prothrombin deficiency is observed in 1 in 2 million [65]. Prothrombin deficiency is classified into two types, hypoprothrombinemia caused by low prothrombin production and dysprothrombinemia is caused by deficiency of functional prothrombin [65]. Hypoprothrombinemia with less than 5% prothrombin antigen is characterized by severe bleeding whereas dysprothrombinemia causes variable bleeding tendencies [65]. Treatments for prothrombin deficiency include prothrombin complex concentrate and fresh frozen plasma [67].

5.3. Factor V deficiency

FV is a single polypeptide encoded by chromosome 1 and primarily synthesized in the liver and some evidences show that FV is also produced by megakaryocytes [68–70]. The activated FV acts as a cofactor for FXa, to form a prothrombinase complex and it also serves as a target for APC-PS complex in inhibiting the coagulation cascade [71]. Patients with FV deficiency

surprisingly do not show bleeding phenotype. Recent evidences elucidated that platelets endocytose FV from plasma, modify them intracellularly and release it at the site of injury. This platelet released FV is resistant for inhibition. If symptomatic patients usually have umbilical stump bleeding, skin and mucosal tract hemorrhage [72].

5.4. FVII deficiency

FVII is a 50 Kda single chain polypeptide encoded by F7 gene located on chromosome 13 and FVII levels are influenced by age, sex and health condition such as blood cholesterol and triglyceride levels [73, 74]. FVII deficiency is observed in 1 in 500,000, with variable phenotypes [74]. Some patients do not show bleeding phenotype despite very low FVII levels, whereas others with similar FVII antigen levels show severe bleeding phenotype [74]. The bleeding phenotypes of FVII deficiency include central nervous system hemorrhage, epistatic and menorrhagia [74]. Frozen fresh plasma, prothrombin complex concentrates, plasma derived FVII concentrate, recombinant FVIIa are typically used to treat FVII deficiency [75, 76].

5.5. FX deficiency

Factor X is a single chain polypeptide with a molecular weight of 58,900 kDa and circulates in plasma with a concentration of 10 μg/ml [77]. FX is encoded by FX gene present on chromosome 13 [78]. FX deficiency is characterized by central nervous system and gastro intestinal bleeding [79, 80]. FX deficiency is one of the very rare disorders observed in 1 in 500,000–1000,000 [79, 80]. Treatments of FX deficiency include highly purified plasma FX, recombinant FX, fresh frozen plasma and prothrombin complex concentrates [79, 80].

5.6. FXI deficiency

FXI is a 80 kDa protein with a plasma concentration of 30 nM, encoded by a 23 kb gene present on chromosome 4 [81–83]. Mutations in the coding region are the major causes for FXI deficiency and the prevalence of FXI deficiency is 1 in 1000,000 [83, 84]. The common symptoms of FXI deficiency are oral and post-operative bleeding. FXI deficient women are prone to menorrhagia. Fresh frozen plasma, FXI concentrate and antifibrinolytic agents are used to treat FXI deficiency [84].

5.7. FXIII deficiency

The functional FXIII consist 2 catalytic A subunits (FXIII-A) and 2 carrier subunits (FXIIIB) [85]. FXIII-B is encoded by chromosome 6 and synthesized by the cells derived from bone marrow, whereas FXIIIA is encoded by chromosome 1 and secreted from liver [85, 86]. FXIII crosslinks α and γ subunits of fibrin thereby increases the strength of fibrin clot and increases fibrinolytic resistance [86]. Prevalence of FXIII deficiency is 1 in 2 million, patients with FXIII-A have high tendency of bleeding [87]. 2–5% plasma FXIII is sufficient to prevent bleeding, FXIII concentrates are usually used to treat FXIII deficiency and frozen fresh plasma and cryoprecipitate are also recommended [87].

5.8. Vitamin K dependent coagulation factors deficiency (VKCFD)

Procoagulants such as FII, FVII, FIX and FX, as well as anticoagulants Protein C, S and Z contain a Glutamic acid rich domain [88, 89]. The Glutamate residues require γ-carboxylation to enable these proteins to bind to the phospholipid membrane in the presence of calcium and carry out their functions [90]. Hepatic γ-glutamyl carboxylase (GGCX) and its cofactor, reduced vitamin K (KH2) aids the carboxylation process and in this process vitamin K is converted into vitamin K epoxide [91, 92]. The vitamin K epoxide is recycled to reduced vitamin K by the vitamin K epoxide reductase (VKOR) enzyme complex [91, 92]. GGCX is encoded by the gene located on chromosome 2 and VKORC1 is encoded by the gene present on chromosome 16 [93, 94]. Mutations in these gene cause loss of GGCX or VKOR complex function and lead to vitamin K dependent coagulation factor deficiency [95]. The clinical manifestations of VKCFD include intracranial hemorrhage or umbilical stump bleeding [95]. Viral inactivated frozen fresh plasma is the agent of choice for VKCFD patients, who require surgical procedures or have acute bleeding [95].

6. Thrombosis

Blood clotting occurs at the site of injury to prevent the leakage of the blood However in thrombosis, blood clots are formed in the blood vessel without any damage response and occlude the blood vessel [96]. Thrombosis is classified based on the location of the clot formation, it includes atrial thrombosis, venous thromboembolism (VTE) and pulmonary embolism (PE) [97, 98]. Thrombosis causes high mortality in United States where, annually 900,000 patients develop VTE and 300,000 people die due to PE [99–101]. Atrial emboli is found predominantly in surgical and intensive care patients due to preexisting conditions such as age, hypercoagulability, cardiac abnormalities and atherosclerosis [102]. Most often the clots are found in the veins due to low shear rates in veins (20–200/s) compared to arteries (300–800/s) [103]. Thrombosis found in veins is termed as venous thrombosis. The thrombus formation in the deep veins is termed as deep vein thrombosis. The risk factors for thrombosis are classified by Virchow and they referred as Virchow's Triad [104]. The triad includes endothelial injury, stasis or turbulence of blood flow, and blood hypercoagulability. Endothelial injuries generally happen during surgery, the turbulence of blood flow occurs due to cardiovascular disorders or hypertension [104]. Hypercoagulability is caused by the environment, unhealthy habits and age. The environmental risk factors include exposure to high altitudes and hypoxic environment [103]. The external risk factors for the thrombosis include smoking, chronic alcoholism and consumption of oral contraceptive pills [103]. Similarly, health conditions like cancer, obesity and aging promote the risk of thrombosis [103]. The molecular mechanisms under these risk factors are yet to be understood. Thrombosis is also caused by inherited factors such as mutations in the genes that encode for coagulation factors or anticoagulants.

6.1. Procoagulants - thrombosis

Serine proteases of coagulation cascade play a vital role in the progression of clot formation [3]. Mutations in the proteases convert them into hyper active forms and some of the mutations

prevent their degradation and enhance thrombin generation. High levels of FVIII, FIX, FVII and TF are known to cause the thrombosis [105].

6.1.1. FVIII and thrombosis

FVIII is secreted from the hepatocytes, the mature FVIII zymogen circulates in the blood stream at a concentration of 0.1–0.2 µg/ml (<100 IU/dl) [105]. In blood FVIII is bound to vWF produced by the endothelial cells, with a dissociation constant of 0.2–0.4 nM [106]. The complex of vWF-FVIII stabilizes FVIII by preventing the cleavage of inactive FVIII by FXa and APC and it also blocks the procoagulant of FVIII by allowing the selective activation of FVIII by thrombin (**Figure 2**). vWF anchors and multimerizes at the site of tissue damage and helps in the formation of platelet plugs [106]. These vWF multimers are cleaved by ADAMTS13 (ADAMTS13 is a Disintegrin like and Metalloprotease with ThromboSpodin repeats family metalloprotease) [107]. Mutations in vWF or ADAMTS13 increases plasma FVIII levels. Increase in the plasma FVIII above 150 IU/dl increases the risk of thrombosis by 4.8 fold [105, 108]. Further each increase in FVIII level with 10 IU/dl is associated with a 10% increase in the risk of a first event of thrombosis.

6.1.2. FIX and thrombosis

FIX is a key component of intrinsic/contact pathway. Levels of FIX are important to regulate the hemostasis [3]. Lower levels of FIX antigen leads to hemophilia and recently two studies showed that higher levels of FIX lead to thrombosis [109]. Saenko et al. demonstrated that risk of thrombosis increases by 2.3–2.8 fold in the subjects with plasma FIX activity >150 IU/dl and van HylckamaVlieg et al. demonstrated that risk of thrombosis increases by 2.8 fold with plasma FIX levels >129 U/dl [110, 111]. Age, increase in blood lipids and use of oral contraceptive pills are some of the reasons for elevated plasma FIX levels [110, 111]. Some of the mutations in FIX gene lead to increase in FIX activity thereby, increase in the risk of thrombosis. FIX Padua variant is a one among the FIX mutants to show enhanced risk of thrombosis. FIX Padua is a single amino acid substitution variant where arginine 388 is mutated to leucine [112].

6.1.3. Tissue factor and thrombosis

Tissue factor is also known as Factor III (FIII), it is a 47 kDa glycoprotein highly expressed in the pericytes and adventitial fibroblasts, low levels of TF expression are observed in CD14-positive monocytes [113]. TF is expressed in the parenchyma of highly vascularized organs such as placenta, brain, heart, kidneys, and lungs [114]. Circulatory TF is found in macrovesicles produced by apoptotic bodies, smooth muscle cells, monocytes and cancer cells. TF expression is enhanced by pathological conditions such as bacterial infections and cancer [115, 116].

6.1.4. Other procoagulants and thrombosis

FXI levels more than 110 IU/dl increases the chances of thrombosis by 2 fold and inhibition of FXI in thrombosis models rescues the DVT. Prothrombin levels more than 115 IU/dl increases the risk

of thrombosis by 2.1 fold. Plasma thrombin levels are reported to increase due to polymorphic variations. FV leiden is one the well-known FV variant that causes high risk of thrombosis. Koster et al. reported increase in fibrinogen levels increases the risk of thrombosis by 2.8 fold [105].

6.2. Anticoagulants - thrombosis

Activated Protein C, Protein S, Protein Z and Tissue factor pathway inhibitor are natural anti-coagulants that help in preventing the accidental or pathological thrombi formation. Defects in these clotting factors lead to thrombosis [10].

6.2.1. Protein C (PC)

PC is a vitamin K dependent serine protease majorly synthesized by liver and its expression has also been identified in epididymis, kidney, lung, brain and male reproductive organ. PC is a single polypeptide of 461 amino acids, consist one Gla domain, a helical aromatic segment, two epidermal growth factor (EGF)-like domains, an activation peptide and a trypsin-like ser-ine protease domain [117]. In the presence of Calcium, PC binds to the endothelial membrane through its Gla domain and interacts with its receptor (endothelial PC receptor: EPCR) [117]. The complex of PC-EPCR facilitates the activation of PC by thrombin, thrombomodulin complex where, thrombin cleaves PC at Arg169-Leu170. This cleavage removes activation peptide from PC. Activated Protein C (APC) cleaves FV and FVIII, and inactivates them. Zymogen PC circu-lates in the blood at a concentration of 63 nM with a half-life of 2–3 hours whereas, plasma APC concentration is 40 pM with a half-life of 20 min. APC function is increased in presence of Protein S (PS) as PS acts as a cofactor for APC. Reduction of plasma APC antigen levels or loss of APC function is one of the causes for thrombosis. Causes for PC deficiency include congenital/ heredi-tary deficiencies due to mutations in PC gene. Till date 380 mutations are reported in PC gene (http://www.hgmd.cf.ac.uk/ac/gene.php?gene=PROC). Hereditary PC deficiency is treated by a protein C zymogen concentrate derived from human plasma known as Protexel® (Raosevich et al. 2003). Low plasma PC antigen levels (<10 IU/dl) are also caused by acquired PC deficiency. Acquired PC deficiency is caused by consumption of vitamin K antagonist or severe hepatic dysfunction. A recombinant analogue to the physiologic human activated PC (Drotrecogin alpha activated/ Xigris®) is used to treat the acquired PC deficiency. Thrombosis is also observed due to loss of APC function (APC resistance). APC resistance is observed due to mutations in FV (FV leiden) or APC resistance is acquired by smoking, chronic alcoholism and obesity [118].

6.2.2. TFPI

TFPI is a single chain polypeptide with specialized domains called Kuntz domains. It is pri-marily synthesized in endothelial cells, liver and macrophages [7]. TFPI is mainly bound to the endothelial cell surface through glycosaminoglycans. TFPI circulates in the plasma at a concentration of 1.0–2.5 nM with a half-life of 60–120 min. Major portion of plasma TFPI is bound to LDL and levels of TFPI are regulated by thyroid hormones. TFPI is cleared from the system by liver and kidney. TFPI directly binds to FVIIa, FXa complex and inhibits their func-tion. Inhibitory function of TFPI is enhanced in presence of Protein S [7]. Low levels of TFPI increases the risk of thrombosis by 2 fold [119].

6.2.3. Protein S

PS is a vitamin K dependent single chain polypeptide consist of one Gla domain, four EGF like domains and two Laminin G domains. PS is primarily synthesized in liver and it circulates in the plasma at a concentration of 450 nM. 60% of circulatory PS is bound to compliment component binding protein 4b (C4BP) and only 40% of the circulatory PS is free [6, 120]. PS acts as a cofactor for APC and TFPI in inhibiting FVIIIa, FVa, and TF-FVIIa-FXa complex [121, 122]. PS was reported to directly interact with procoagulants such as FV, FIXa and FX and inhibit their function [121, 122]. PS plays a key role in regulating inflammation and clearing the apoptotic bodies from the system. PS deficiency enhances the risk of thrombosis and PS deficiency is classified as hereditary PS deficiency and acquired PS deficiency. Hereditary PS is caused by mutations in the PS gene and till date ~200 mutations are reported in PS gene. Acquired PS deficiency is caused by several factors such as, usage of oral contraceptive pills, pregnancy, consumption of vitamin K antagonists and pathogen infections.

6.2.4. Protein Z and protein Z dependent protease inhibitor

Protein Z is a 62 kDa vitamin K dependent plasma protein that acts as a cofactor for 72 kDa serpin family protease inhibitor – Protein Z Dependent Protease Inhibitor (ZPI). ZPI-PZ complex rapidly inhibits FXa and FXIa [8].

6.2.5. Anticoagulants in the treatment for thrombosis

Thrombosis is treated by selectively inhibiting the major procoagulant proteins. Major drug targets for the treatment of thrombosis include vitamin K agonists, FXa, FIXa, Thrombin and platelet inhibitors. Current oral anticoagulants approved by FDA are rivaroxaban, Apixaban, dabigatran and endoxaban. Revaroxaban and Apixaban inhibit FXa, whereas dabigatran and endoxaban inhibit thrombin. Several other procoagulant inhibitors such as RNA aptamers are under investigation [123].

7. Acquired coagulation disorders

An individual can acquire coagulation disorders due to several reasons. Infections such as streptococci cause thrombosis by inactivating Protein S [124]. Chronic smoking and chronic alcoholism effect coagulation system by altering the liver functions. Environmental factors like hypoxia, drugs like aspirin, oral contraceptive pills, dietary problems like vitamin K deficiency affect the blood coagulation [12, 56–61].

7.1. Disseminated intravascular coagulation (DIC)

DIC is characterized by activation of clotting system within the vasculature which blocks the micro vessels and can cause further organ dysfunction [125]. In contrast, it can also accelerate fibrinolysis and cause severe bleeding. The international Society of Hematology (ISTH) has

defined DIC as *"an acquired syndrome characterized by the intravascular activation of coagulation with loss of localization arising from different causes. It can originate from and cause damage to microvasculature, which if severe, can produce organ dysfunction"* [125–127]. DIC occurs in all ages, races and all genders. DIC is classified as acute DIC, developed due to sudden exposure of pro-coagulants [125–127]. In acute DIC compensatory hemostatic mechanisms are quickly overwhelmed and leads to hemorrhage development. Chronic DIC is develops due to constant or intermittent exposure of small amounts of tissue factor (TF) [125–127]. DIC is acquired due to several reasons which include external agents such as infections, snake bite, trauma, severe transfusion reactions and environmental changes that cause hemocytopenia [125–127]. Disease conditions leading to DIC include malignancy, organ disfunctions such as hepatic failure and pancreatitis, vascular abnormalities. The phenotypes of DIC include non-symptomatic, bleeding, massive bleeding and organ failure type. If there is no observed phenotype in the patients, whereas the abnormalities were observed in clinical laboratory only, the diagnosis is known as Non-symptomatic DIC [125–127]. In the bleeding type is more predominantly observed phenotype in DIC, the primary symptom is bleeding due to hyperfibrinolysis [125–127]. This phenotype is observed in patients with leukemia, aortic aneurysm and obstetric diseases. Organ failure phenotype is observed in the patients with hypercoagulation, this phenotype is observed in patients with infections. Massive bleeding is observed when the fibrinolysis and hypercoagulation are remarkable. Massive bleeding often leads to death [125–127].

DIC is diagnosed by global tests such as platelet count, prothrombin time (PT), aPTT and the amount of fibrinogen, fibrin and fibrin degradation products. Other diagnostic markers include antithrombin, Protein C, Thrombin-Antithrombin (TAT) complex, VWF propeptide and plasminogen activator inhibitor-1 (PAI-1) (**Table 2**). Treatment of DIC depends on the type of phenotype (**Table 2**) [128]. Heparin treatment is recommended for the treatment of non-symptomatic type whereas, antifibrinolytic treatment is not recommended. The recommended treatment for the organ failure type DIC is natural protease inhibitor whereas, antifibrinolytic treatment is not recommended [128]. Recommended treatments for the bleeding phenotype DIC include blood transfusion, synthetic protease inhibitors and a fibrinolytic treatment, the non-recommended treatments include heparin and anti-Xa [128] (**Figure 3**).

7.2. Vitamin K deficiency and warfarin therapy

Vitamin K is an essential cofactor needed for carboxylation of glutamate residues of Gla domain containing proteins [95]. Dietary deficiency of vitamin K leads to acquired bleeding disorders. Vitamin K oral supplementation is recommended to treat the vitamin K deficiency [95]. Vitamin K deficiency is diagnosed by prolonged prothrombin time, detection of non-carboxylated proteins and measuring the plasma vitamin K by high performance liquid chromatography [129].

Warfarin is a coumarin-based anticoagulant and it is used as an oral anticoagulant. It inhibits vitamin K epoxide reductase (VKOR) thereby prevents vitamin K recycling which in turn limits the availability of vitamin K. Limitation in Vitamin K prevents carboxylation of glutamate residues of Gla domain containing proteins. Preventing Gla domain carboxylation of

Type of DIC	Phenotype	Diagnosis	Causes	Recommended treatments	Non-recommended treatments
Non symptomatic type DIC	No clinical symptoms whereas, abnormalities observed in the clinical examination	Elevation in the fibrin degradation product levels, decreased platelet count, elevation in levels of soluble fibrin and thrombin-anti thrombin complex	Venous thrombosis, operation and bone marrow disorders	Heparin	Afibrinolytic treatment
Organ failure type DIC	Hyper coagulation	Prolonged prothrombin time, elevated fibrinogen and fibrin degradation products, soluble fibrin, thrombin-antithrombin complex, VWF propeptide and plasminogen activator inhibitor-I. reduced platelet count, reduced antithrombin and Protein C levels, ADAMTMS 13	Liver dysfunction, Vitamin K deficiency, venous thromboembolism, operation, bone marrow disorder, capillary leak syndrome, renal disfunction, thrombotic microangiopathy and infection	Natural protease inhibitor	Afibrinolytic treatment
Bleeding type DIC	Hyperfibrinolysis	Prolonged PT, elevated fibrin degradation products, soluble fibrin, thrombin and antithrombin complex and plasmin-plasmin inhibitor complex. Reduced fibrinogen, platelet count.	Liver dysfunction, vitamin K deficiency, venous thromboembolism, operation, liver dysfunction and bone marrow disorder.	Blood transfusion, synthetic protease inhibitor and afibrinolytic treatment	Anti-Xa and heparin
Massive bleeding phenotype	Both hyper coagulation and hyper fibrinolysis	Prolonged PT, reduced fibrinogen and platelet count. Increased soluble fibrin, thrombin-anti thrombin complex and plasmin-plasmin inhibitor complex.	Liver dysfunction, vitamin K deficiency, venous thromboembolism, operation, liver dysfunction and bone marrow disorder.	Blood transfusion, synthetic protease inhibitor and antifibrinolytic treatment	Heparin, Anti-Xa and natural inhibitor

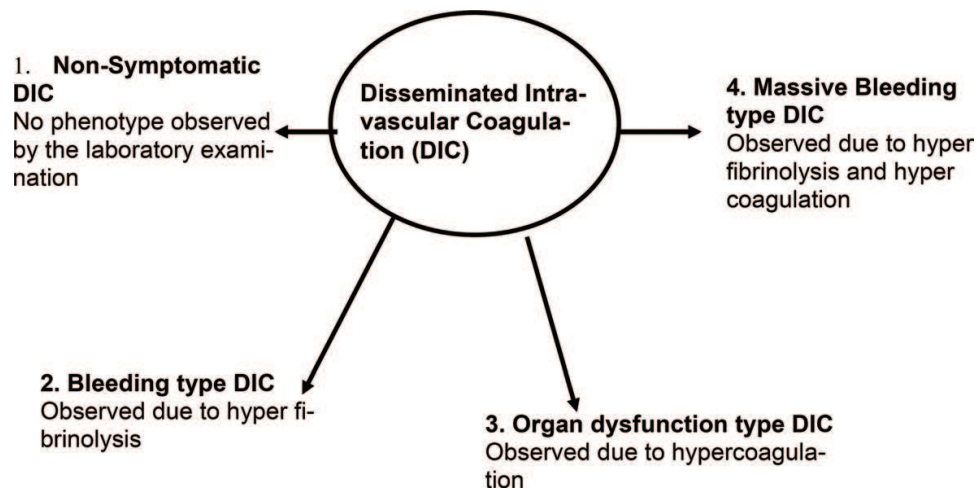**Table 2.** DIC phenotypes, diagnosis and treatment.

Figure 3. Schematic representation of DIC and its phenotypes.

clotting factors II, VII, IX and X prevents the risk of thrombosis, thereby Warfarin is used as an efficient oral anticoagulant/ blood thinner. Over dosage of Warfarin is lethal as it can cause severe bleeding. 2% of the warfarin consumers are prone to the risk of major hemorrhage [130]. Vitamin K is administered as an antidote for warfarin.

7.3. Acquired disorders of platelet function

Platelet activation and aggregation is essential for clot formation and fibrinolysis [131]. Decrease in platelet number or inhibition of platelet activation impairs blood clotting. Infections such as dengue virus, chickenpox, rubella and bacteria effect the circulating platelet number in the blood [132]. Antiplatelet drugs like aspirin impairs platelet aggregation therefore over dosage of aspirin can cause hemorrhage [133]. Acquired platelet disorders are analyzed by platelet count and their aggregation properties.

7.4. Liver disorders

Liver is the major source of coagulation factors. Chronic alcoholism, smoking and high fat consumption affect the function of liver and there by impact the synthesis of coagulation factors [134]. Blood transfusion is recommended for treating the coagulation defects caused by liver disorders.

8. Diagnosis of coagulation disorders

Coagulation defects are measured by the general assays such as aPTT and PT assays, direct measurement of antigen levels and specific coagulation factor activity assays. Some of the commonly used assays were discussed here.

8.1. Prothrombin time assay (PT assay)

PT assay is used to measure the functional integrity of extrinsic pathway. Clotting is initiated by supplementing tissue factor and calcium chloride to the platelet poor plasma. Elongation of PT indicates the increase in bleeding disorders, similarly shortening of PT indicates the enhanced chances of thrombosis [135].

8.2. Activated prothrombin time assay (aPTT assay)

aPTT assay is used to measure the integrity of intrinsic pathway. In this method the clotting is initiated by supplementing Kontact reagent and calcium chloride to the platelet poor plasma. Similar to PT assay, prolongation of aPTT indicates the risk of bleeding disorders and shortening of the aPTT indicates risk of thrombosis [136].

8.3. Clot waveform analysis (CWA)

CWA is a modified form of aPTT assay, where the light absorbance of the clot measured from the clot initiation to the lysis of the clot and the absorbance is plotted with respect to time using first and second derivates. This assay is more sensitive to measure the changes in FXII, X, IX, VII, V and II levels in the plasma [137, 138].

8.4. Coagulation markers

The coagulation activation and fibrinolysis markers are measured to determine the defects in the coagulation system. One of the diagnostic method to estimate the risk of thrombosis is measuring the D-Dimer antigen levels in the plasma. D-Dimers are the degradation products of cross linked fibrinogen generated during fibrinolysis, increase in the plasma D-Dimer antigen levels directly corresponds to an increase in the risk of thrombosis [139]. Prothrombin fragment 1 + 2 (F1 + 2) are the cleavage products generated from prothrombin and F1 + 2 levels are measured to diagnose the risk of thrombosis, sepsis and DIC [140]. Free thrombin that moves away from the site of clot formation forms a complex with antithrombin III and the

complex is known as TAT complex. TAT complex is used to measure the risk of thrombosis in patients with multiple trauma, liver dysfunction and septicemia [141, 142]. Coagulation factors like FXIII, Protein S and Antiphospholipid antibodies are also quantified by immune assays to measure the alterations in coagulation system [141].

9. Conclusions

Coagulation is a complicated biological phenomenon which maintains the hemostasis. Abnormalities in the genes that regulate the coagulation factors cause hereditary coagulation defects such as hemophilia and mutations in genes that encode anticoagulants such as Protein S, Protein Z cause thrombosis. Disruption in the anticoagulant and coagulation factors in the healthy individual causes acquired bleeding disorders. Acquired bleeding disorders include a bleeding disorder or a thrombotic disorder. These disorders can be diagnosed by current methods and can be treated with known methods. There is a high demand for efficient diagnostic and treatment methods for the abnormalities in the coagulation disorders.

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