Factor XIII

Congenital Deficiency Factor XIII, Acquired Deficiency, Factor XIII A-Subunit, and Factor XIII B-Subunit

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• Factor XIII (FXIII) is a transglutaminase consisting of 2 catalytic A subunits and 2 noncatalytic B subunits in plasma. The noncatalytic B subunits protect the catalytic A subunits from clearance. Congenital FXIII deficiency may manifest as a lifelong bleeding tendency, abnormal wound healing, and recurrent miscarriage. Acquired FXIII deficiency, with significant reductions in FXIII levels, has been reported in several medical conditions. The routine screening tests for coagulopathies—prothrombin time, activated partial thromboplastin time, and thrombin time—do not show abnormalities in cases of FXIII deficiency. A quantitative, functional, FXIII activity assay that detects all forms of FXIII deficiency should be used as a first-line screening test. Treatment consists of recombinant FXIII or FXIII concentrate. If these are unavailable, then fresh-frozen plasma and cryoprecipitates may be used. Factor XIII has a long half-life; therefore, the patients can lead near-normal lives with regular replacements. Patients with acquired FXIII deficiency with inhibitors need immunosuppressive therapy in addition to factor replacements.

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Factor XIII (FXIII) is one of the main contributors to clot formation in the final stages of coagulation. It is also important in wound healing and maintenance of pregnancy. Coagulation FXIII, also known as *fibrin-stabilizing factor*, circulates in plasma as a tetramer, requiring calcium and thrombin for activation.[1] Factor XIII consists of 2 potentially active, catalytic A subunits (FXIII-A) and 2 carrier/inhibitory B subunits (FXIII-B). In the plasma, FXIII-B is in excess, mainly in free, noncomplexed form.

The FXIII-A, a protransglutaminase enzyme, belongs to the family of transglutaminases. Its molecular mass is 83 kDa.[2] It consists of 4 main structural domains, β sandwich (amino acids 38–184), catalytic core (amino acids 185–515), β barrel 1 (amino acids 516–628), and β barrel 2 (amino acids 629–731) domains, plus an NH_2-terminal activation peptide, which is cleaved by thrombin during activation.[3] The Ca^{2+} binding sites on the surface of FXIII-A2 dimer are essential for its activation. The gene coding for human FXIII-A (*F13A1*) spans more than 160 kilobases (kb) and has been localized to band 6p24–25. It is transcribed into a 3.9-kb messenger RNA (mRNA), with an 84–base pair (bp), 5′ untranslated region; a 2.2-kb open reading frame; and a 1.6-kb, 3′ untranslated region. The *F13A1*A contains 15 exons and 14 introns, is expressed primarily in cells of bone-marrow origin, and is present in platelets in large amounts. The average FXIII-A content of a single platelet was estimated to be (mean [SD]) 60 fg (10 fg), which corresponds to 3% of total platelet proteins.[4] Megakaryocytes synthesize FXIII-A and package it, together with its encoding mRNA, into newly formed platelets.[5] Factor XIII-A has also been detected in monocytes and in their bone-marrow precursor cells.[6] The expression of FXIII-A in monocytes is retained after malignant transformation and is unregulated in acute myelomonocytic, monocytic leukemias, and in chronic myelomonocytic leukemias.[7] Its detection by flow cytometry in leukemic cells is a useful intracellular marker in the classification of acute myeloid leukemias. Interestingly, FXIII-A becomes expressed in part of the lymphoblasts in acute B-cell lymphoid leukemia, whereas it is absent in mature lymphocytes, normal lymphoid precursors, and mature lymphocytes from B-cell chronic lymphoid leukemia.[8]

Factor XIII-B is a glycoprotein consisting of 641 amino acids with a molecular mass of approximately 80 kDa. The gene *F13B* is approximately 28 kb composed of 12 exons producing a 2.2 kb mRNA located at position 1q31–32.1.[9] FXIII-B is expressed in the liver and secreted by hepatocytes.

The activation of plasma FXIII (pFXIII) occurs in the final phase of the clotting cascade by the concerted action of thrombin and Ca^{2+}. In the initial step of the reaction, thrombin cleaves activation peptide FXIII from the NH_2 terminus of FXIII-A by hydrolyzing the peptide bond. Then, in the presence of Ca^{2+}, the inhibitory B subunits dissociate, which is a prerequisite for the truncated FXIII-A dimer to assume an enzymatically active conformation.[10] The primary, physiologic substrates of activated FXIII (FXIIIa) are fibrin

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and α_2 plasmin inhibitor. The FXIIIa substrate proteins are coagulation factors, components of the fibrinolytic system, adhesive and extracellular matrix proteins, and intracellular, cytoskeletal proteins.

Extensive cross-linking of fibrin by the action of FXIII increases the resistance of the fibrin clot to fibrinolysis.[11] A noncontrolled cross-linking by FXIIIa causes excessive cross-linked fibrin and extensive cross-linking of other plasma proteins to fibrin, which could lead to undesired, prolonged persistence of thrombi. Activated clotting factors are inactivated by 2 mechanisms.[12] Proteolytically active factors are inhibited by specific serine protease inhibitors, by serpins (like antithrombin III or tissue factor pathway inhibitor), or by less-specific protease inhibitors, like α_2 macroglobulin. The other process of inactivation of active factors is performed by highly specific proteolytic enzymes. As no plasma protein inhibitor of FXIIIa has been discovered, the proteolytic inactivation mechanism is likely responsible for degradation. Polymorphonuclear granulocytes become activated and release proteases, elastase, cathepsin G, and matrix metalloproteinase in the fibrin clot. These proteases proteolytically degrade both FXIII subunits and inactivate FXIIIa within the fibrin clot.[13] The time course for FXIIIa degradation by polymorphonuclear granulocytes suggests that the proteolytic degradation of FXIIIa by the released proteases does not interfere with the initial cross-linking events; however, it could prevent the formation of an excessive cross-linked plasma clot and facilitate the elimination of fibrin when it is no longer needed. Factor XIII influences fibrinolysis by the cross-linking fibrin α chains into high–molecular-weight polymers, rendering the clot more resistant to lysis. In addition, the binding of α_2 plasmin inhibitor acts by reducing the binding of plasminogen to cross-linked fibrin and prevents its prompt elimination by the powerful fibrinolytic system.[11]

In addition, intracellular and extracellular FXIII molecules also support immobilization and killing of bacteria, as well as phagocytosis by macrophages. Thus, FXIII may have a role in innate immunity.

CLINICAL FEATURES

Factor XIII deficiency is rare; its incidence is approximately 1 in 2 million, and it accounts for 6% of the rare bleeding disorders.[14] Congenital FXIII deficiency can be attributed to defects in both the FXIII-A and FXIII-B genes.[15] Inherited FXIII deficiency is an autosomal recessive trait; patients with severe disease are homozygotes or compound heterozygotes. Congenital FXIII-A deficiency may be a quantitative defect resulting from decreased synthesis of the protein (type I deficiency) or a qualitative defect (type II deficiency), characterized by a normal or near-normal antigen concentration of functionally defective FXIII-A. Untreated, severe congenital FXIII-A deficiency causes critical bleeding events in most cases, with intracranial hemorrhage being the major cause of death. Typically, delayed-type umbilical stump bleeding represents the first classic clinical sign of congenital FXIII-A deficiency. Muscle and subcutaneous soft tissues are also preferred sites of severe bleeding complications.[16] The FXIII-A deficiency is frequently associated with impaired wound healing and leads to abortion in pregnant women. Severe FXIII-B deficiency has been rarely reported.[17]

Factor XIII deficiency is considered severe when the factor level is less than 5%, moderate when it is between 5% and 10%, and mild when factor XIII levels are greater than 10%. In a study for the European Network of Rare Bleeding Disorders,[16] the distribution of the clinical bleeding severity in patients with FXIII deficiency varied, and 48.5% of patients had grade-3 bleeding, 6.1% had grade-2 bleeding, 6.1% had grade-1 bleeding, and 39.3% were asymptomatic, where *grade-1 bleeding* is bleeding that occurred after trauma or drug ingestion (antiplatelet or anticoagulant therapy), *grade 2* is spontaneous minor bleeding (bruising, ecchymosis, minor wounds, oral cavity bleeding, epistaxis, and menorrhagia), and *grade 3* includes spontaneous major bleeding (intramuscular hematomas requiring hospitalization, hemarthrosis, and central nervous system, gastrointestinal, and umbilical cord bleeding). The mean factor-activity level that was necessary for patients to remain asymptomatic was 31 U/dL (range, 10.83–51.31 U/dL). Undetectable FXIII levels were associated with grade-3 bleeding.

Acquired FXIII deficiency with significant reductions in FXIII levels has been reported in several medical conditions, such as major surgery, pulmonary embolism, stroke, leukemia, myelodysplastic syndrome, Crohn disease, ulcerative colitis, Henoch-Schönlein purpura, liver cirrhosis, sepsis, and disseminated intravascular coagulation. In these acquired FXIII-deficient states, FXIII-A subunit levels drop to 20% to 70%, caused by decreased synthesis or increased consumption. There are few cases of another autoimmune bleeding disorder, designated as *autoimmune/acquired hemorrhaphilia due to anti-FXIII/13 inhibitors* (AH13).[18] This AH13 must be distinguished from regular hemorrhagic acquired FXIII deficiency. The AH13 tends to be more severe than regular hemorrhagic acquired FXIII deficiency and requires both immunosuppressive therapy to eradicate autoantibodies and FXIII replacement therapy to stop the bleeding.[19]

LABORATORY STUDIES

The screening tests for coagulopathies—prothrombin time, activated partial thromboplastin time, and thrombin time—do not show prolongation in cases of FXIII deficiency. Traditionally, the solubility of the fibrin clot in concentrated urea, acetic acid, or monochloroacetic acid solution has been used to screen for FXIII deficiency. This qualitative method detects only very severe FXIII deficiency. The method is not standardized; its sensitivity depends on the fibrinogen level, the clotting reagent (thrombin and/or Ca^{2+}), and the solubilizing agent and its concentration. Depending on those variables, the detection limit of the clot solubility assay varies between less than 0.5% and 5% FXIII activity. The lack of sensitivity of this test may, in part, be the reason for the many undiagnosed or late-diagnosed FXIII deficiencies. Currently, the use of this method as the screening test for FXIII deficiency is not recommended.

A quantitative functional FXIII activity assay that detects all forms of FXIII deficiency should be used as a first-line screening test. Quantitative FXIII activity assays are based on either of 2 assay principles,[20] namely, the measurement of ammonia released during the transglutaminase reaction, or the measurement of labeled amine incorporated into a protein substrate. In both types of assays, FXIII in the

plasma is activated by thrombin and Ca^{2+}. In the ammonia-release assay, the fibrin formed by thrombin action is inhibited by the action of a polymerase inhibitor. The FXIIIa cross-links a low–molecular-weight substrate amine to a glutamine containing oligopeptide. The ammonia released is determined spectrophotometrically in a glutamate dehydrogenase–catalyzed, nicotinamide adenine dinucleotide–dependent reaction. The ammonia-release assays are quick, 1-step, true kinetic assays and can be easily automated; however, the sensitivity is low, and the limit of quantitation is between 3% and 5%. In addition, they require a parallel blank sample of plasma to enable correction for the FXIIIa-independent nicotinamide adenine dinucleotide–consuming and ammonia-producing reactions present in the plasma.[21]

The amine-incorporation assays are based on the covalent linkage of fluorescent, radiolabeled, or biotinylated amines to a glutamine residue in a protein substrate by FXIIIa. After the reaction has stopped, the unbound amines are removed from the protein-linked fraction, and the latter is quantitatively measured. The advantage of amine-incorporation methods is their high sensitivity. However, they are more time consuming than the ammonia-release assays and are difficult to standardize, and the separation step makes it difficult to design a true kinetic assay.

If plasma FXIII activity is decreased, the subtype of FXIII deficiency is established by either (1) measurement of the (FXIII-A2B2) antigen or the individual subunit concentration in the plasma, or (2) measurement of the FXIII activity and FXIII-A antigen in platelet lysate. Autoantibodies against FXIII subunits can be measured by (1) mixing studies for the detection of neutralizing antibodies against FXIII-A, or (2) binding assays for the detection of nonneutralizing antibodies against FXIII-A and FXIII-B. In addition, an evaluation of fibrin cross-linking by sodium dodecyl sulfate-polyacrylamide gel electrophoresis should be performed. Lastly, an analysis and detection of the molecular genetic defect is carried out.

In attempts at standardization, a World Health Organization reference plasma with assigned pFXIII activity and FXIII-A2B2 antigen values in international units (the *1st international standard for factor XIII, plasma*) was established.[22] Neutralizing autoantibodies against FXIII-A can be detected in a mixing study; in the mixture of patient and normal plasma, the FXIII activity of normal plasma will be inhibited by a neutralizing autoantibody. The diagnosis of nonneutralizing inhibitors requires binding assays, that is, the detection of the binding of patients' immunoglobulin (IgG/IgM) to purified pFXIII and purified FXIII subunits in enzyme-linked immunosorbent assays or dot-blot arrangements. The demonstration of accelerated clearance of administered FXIII concentrate from the circulation also helps in making the diagnosis.

TREATMENT AND PROPHYLAXIS

Fresh frozen plasma and cryoprecipitate are good sources of FXIII providing 1 and 3 unit/mL of coagulation FXIII respectively, and can be used successfully to treat FXIII deficiency. Fresh frozen plasma and cryoprecipitate are easily available but carry the risk of transmission of bloodborne viruses. Pasteurized FXIII concentrates are safer and have a higher titer of FXIII (about 240 U/vial) are, therefore, preferred.[20,23–25] In patients with acute bleeding episodes, 10 to 30 IU/kg of FXIII concentrates should be administered until the bleeding has stopped. Recently, new, recombinant FXIII manufactured in *Saccharomyces cerevisiae* (bakers' yeast) was shown to be effective and safe in a phase 3 trial for the prevention of bleeding in cases of congenital FXIII-A subunit deficiency. This recombinant FXIII-A subunit associates in plasma with the endogenous FXIII-B subunit to form a stable FXIII heterotetramer.[26]

Because of the long half-life of plasma FXIII (11–14 days) and because a factor XIII level greater than 3% to 5% is usually sufficient to prevent spontaneous bleeding, prophylaxis is the management strategy of choice. Lifelong prophylactic therapy every 4 to 6 weeks of 10 to 20 U/kg of FXIII is recommended in patients with severe FXIII deficiency to prevent life-threatening, spontaneous bleeding.[10] For major surgery, the plasma FXIII should be maintained at more than 5% until the wound healing is complete. Because of the risk of abortion and pregnancy loss, prophylaxis with a source of FXIII during pregnancy is essential and should be started as early as possible (ideally, before 5–6 weeks of gestation).[17]

ACQUIRED FXIII DEFICIENCY

Acquired FXIII deficiency can arise from decreased production or increased consumption of FXIII, as well as from the production of autoantibodies against FXIII subunits. Some diseases, including leukemia, liver disease, Crohn disease, disseminated intravascular coagulation, ulcerative colitis, inflammatory bowel disease, Henoch-Schönlein purpura, systemic lupus erythematosus, sepsis, pulmonary embolism, stroke, and major surgery, are reported to be associated with acquired FXIII deficiency.[20,27–29] In these patients, the plasma level of FXIII usually remains greater than 30% and rarely requires replacement therapy. Autoantibodies against FXIII subunits have been reported in some patients with autoimmune disease. These autoantibodies are either neutralizing or nonneutralizing types. Neutralizing autoantibodies affect the activation of FXIII or the activity of activated FXIII, whereas the nonneutralizing autoantibodies reduce FXIII subunits by forming an immune complex with them, where they are then cleared from the plasma by the reticuloendothelial system.[30] Plasmapheresis; immunosuppression with cyclophosphamide, cyclosporine, or combinations of cyclophosphamide and cyclosporine; anti-CD20, and intravenous γ globulin are tools that can be used for the elimination of autoantibodies against FXIII subunits.[20]

In summary, FXIII is an essential component of the clotting system; its deficiency causes severe bleeding diathesis. The main function of FXIII in hemostasis is the stabilization of newly formed fibrin. Factor XIII increases the rigidity and strength of the fibrin clot and protects it against shear stress in the circulation. Factor XIII also protects fibrin from prompt elimination by the fibrinolytic system. Congenital FXIII deficiency is a rare, lifelong bleeding tendency. Acquired FXIII deficiency is mainly caused by hyperconsumption or hyposynthesis, but these patients rarely bleed. In contrast, the less-prevalent AH13, which is caused by either anti-FXIII-A or anti-FXIII-B autoantibodies, results in life-threatening bleeding. Factor XIII deficiency, however rare, must be considered in a patient with bleeding of unknown cause. Treatment of AH13 includes immunosup-

pressive therapy together with administration of FXIII concentrates.

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