Published in final edited form as:

Transfus Apher Sci. 2018 December ; 57(6): 700–704. doi:10.1016/j.transci.2018.07.006.

State of the Art in Factor XIII Laboratory Assessment

Michael A. Durda¹, Alisa S. Wolberg², and Bryce A. Kerlin^{1,*},^{3,4}

¹The Ohio State University College of Medicine, Columbus, OH

²University of North Carolina at Chapel Hill, Department of Pathology and Laboratory Medicine, Chapel Hill, NC

³Nationwide Children's Hospital, Division of Hematology/Oncology/Blood and Marrow Transplantation, Columbus, OH

⁴The Research Institute at Nationwide Children's Hospital, Center for Clinical & Translational Research, Columbus, OH

* Department of Pediatrics, Columbus, OH

Abstract

Factor XIII, a heterotetrameric proenzyme, is converted to an activated transglutaminase by thrombin and calcium in the final phases of coagulation. Factor XIII catalyzes the formation of crosslinks between fibrin monomers and between α 2-antiplasmin and fibrin. These crosslinks mechanically stabilize fibrin against shear stress, enable red cell retention within the clot, and protect the clot from premature degradation. Congenital factor XIII deficiency is caused by autosomal recessive mutations, presenting early in life with a severe bleeding diathesis. Acquired deficiency may be caused by autoimmunity. Currently available assays for laboratory diagnosis of factor XIII deficiency include clot solubility assays, quantitative factor XIII activity assays, factor XIII antigen assays, and genetic testing. The International Society on Thrombosis and Haemostasis Scientific and Standardization Committee has recommended an algorithm for the laboratory diagnosis and differentiation of the different forms of factor XIII deficiency. However, implementation of this algorithm has been limited by technical and budgetary challenges associated with the currently available factor XIII-specific assays. The purpose of this review is to discuss the advantages and limitations of the currently available assays employed for the laboratory diagnosis of factor XIII deficiency.

Keywords

Rare bleeding disorder; factor XIII deficiency; assays; ammonia release; amine incorporation; isopeptidase; ELISA; clot solubility

Corresponding Author: Alisa S. Wolberg, PhD, 819 Brinkhous-Bullitt Building, CB #7525, Department of Pathology and Laboratory Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7525, Telephone: 919-962-8943, alisa_wolberg@med.unc.edu.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Introduction

Plasma Factor XIII (pFXIII) is a protransglutaminase consisting of two A-subunits (FXIII-A) and two B-subunits (FXIII-B) that circulates as a heterotetramer (FXIII-A₂B₂) [1, 2]. FXIII-A is produced in cells of bone marrow origin, whereas FXIII-B is produced in the liver by hepatocytes [1, 3]. In circulation, FXIII-B carries and protects FXIII-A from spontaneous activation and clearance that may occur spontaneously in the presence of plasma ionized calcium. FXIII also exists as an A-subunit homodimer within the cytoplasm of platelets, monocytes, histiocytes, and other cells. Thrombin and calcium activate pFXIII in the final phase of the coagulation cascade. Activation occurs following thrombin-mediated cleavage at arginine 37 of the A-subunits, resulting in the release of a 37-amino acid 'activation peptide' and subsequent dissociation of the B-subunits. The activated enzyme, FXIII-A₂*, catalyzes the formation of ε-N-(γ-glutamyl)-lysyl protein crosslinks [4]. The hemostatic function of FXIII-A₂* is to crosslink fibrin γ-chains and α-chains into γ-γ dimers and high molecular weight α-α and γ-α polymers. FXIII-A₂* also catalyzes the formation of crosslinks between α₂-antiplasmin (α₂-AP) and fibrin α-chains [1, 5, 6]. Fibrin crosslinking mechanically stabilizes the fibrin clot, protecting it from shear stress and enabling the retention of red blood cells during clot retraction, whereas α₂-AP crosslinking covalently incorporates this potent plasmin inhibitor into the forming clot, protecting the clot from premature degradation by the fibrinolytic system [1, 7–9]. In addition to these hemostatic functions, FXIII also plays important roles in wound healing, angiogenesis, and pregnancy maintenance [1, 10–15].

The clinical relevance of congenital FXIII deficiency was first described by Fanny Duckert and colleagues who observed a severe bleeding diathesis in a boy whose only coagulation abnormality was clot dissolution in 5 M urea, which could be corrected by mixing with normal plasma [1, 10]. Severe FXIII deficiency (<0.05 IU mL⁻¹) is associated with a severe bleeding phenotype, usually presenting early in life. However, recent studies have demonstrated a significant correlation between activated pFXIII (FXIIIa) activity and bleeding and have demonstrated that clinically significant bleeding episodes occur with FXIIIa activities up to 0.3 IU mL⁻¹ [16, 17]. The majority of clinical research has focused on severe congenital deficiency [1]. Umbilical stump bleeding within the first few days of life is a frequent presenting symptom and has thus become essentially pathognomonic for severe deficiency. Other clinical features and bleeding tendencies include ecchymoses, hematomas, prolonged bleeding following trauma and, rarely, hemarthroses and intramuscular hemorrhage.

The most concerning bleeding feature in severe deficiency is life-threatening intracranial hemorrhage, which occurs in up to 30% of untreated patients. Women with inherited FXIII deficiency often have recurrent spontaneous miscarriages. Congenital deficiency may occur from genetic defects in either the FXIII-A (*F13A1*) or FXIII-B (*F13B*) gene. In addition, acquired FXIII deficiency has been reported in a number of clinical conditions (e.g. disseminated intravascular coagulation, malignancy, inflammatory bowel disease), including autoantibodies against FXIII which predominantly occurs in elderly persons (sometimes called 'autoimmune hemorrhophilia') [18, 19].

The most commonly employed coagulation screening assays (i.e. prothrombin time, activated partial thromboplastin time, and fibrinogen) are unable to identify FXIII deficiency. Thus, timely and accurate diagnosis is dependent upon both a high index of clinical suspicion and FXIII-specific laboratory analysis. The currently available methods for clinical laboratory diagnoses of FXIII deficiency include clot-solubility assays, quantitative FXIIIa activity assays, FXIII antigen assays specific for the FXIII-A₂B₂ complex, FXIII-A₂, or FXIII-B₂, and genetic testing [3]. In 2011, the International Society on Thrombosis and Haemostasis Scientific and Standardization Committee (ISTH-SSC) recommended an algorithm for the laboratory diagnosis of Factor XIII deficiency to discriminate the various forms of the disease (Figure 1) [20]. The patient should first be screened with a quantitative FXIIIa activity assay capable of detecting all forms of FXIII deficiency (FXIII-A deficiency, FXIII-B deficiency, or anti-FXIII antibodies). For patients with low activity levels, FXIII-A vs. FXIII-B deficiency may be established by quantifying FXIII-A₂B₂, FXIII-A₂, and FXIII-B₂ or by determination of platelet lysate FXIII activity and FXIII-A₂ quantity (since platelet FXIII is predominantly FXIII-A₂). To evaluate for autoimmune-mediated, acquired deficiency, mixing studies and binding-assays should be considered for the detection of neutralizing and non-neutralizing antibodies, respectively. Evaluation of fibrin crosslinking may be analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) to verify FXIII-dependent formation of γ - γ dimers and high molecular weight α -chain-rich polymers. Finally, sequencing the FXIII-A (*F13A1*) and FXIII-B (*F13B*) genes may determine the cause of congenital disease at the molecular level and facilitate genetic counseling.

Unfortunately, many clinical laboratories do not offer the full repertoire of assays required to implement the ISTH-SSC-recommended algorithm [21, 22]. Challenges include lack of regulatory approval for some assays (which may vary from one regional regulatory authority to another), limited reagent availability, inadequate expertise with poorly standardized techniques, and inadequate sensitivity, specificity, and reproducibility of some assays. Moreover, some of these assay platforms are not routinely implemented or validated in clinical laboratories (i.e. SDS-PAGE or platelet-lysate testing). Thus, full implementation of the recommendations may require collaboration with a research laboratory that has established expertise with these specialized techniques. In this review, we will discuss the advantages and disadvantages of currently available FXIII assays.

Factor XIII Activity Assays

Clot Solubility Testing

Clot solubility assays evaluate the stability of crosslinked fibrin. Citrated plasma is first allowed to clot by the addition of calcium and/or thrombin, followed by exposure to a protein denaturing reagent, usually urea, acetic acid, or monoacetic acid [21]. Cross-linked fibrin is more stable and thus resistant to denaturation, whereas uncrosslinked fibrin denatures and re-dissolves into solution. Thus, this assay is highly attractive in both its simplicity and low-cost, especially in resource-limited geographic regions. Unfortunately, this qualitative assay can only detect severe FXIII deficiency, because exposure to low levels of FXIII for even brief timeframes is sufficient to crosslink and stabilize the fibrin clot

against the commonly used denaturing agents [20]. Moreover, this method is limited by a lack of inter-laboratory standardization, and its sensitivity is dependent on additional factors including the plasma fibrinogen level, choice of clotting reagent(s), denaturing agent and concentration, as well as incubation times used for the various steps [20]. Based on these variables, the detection limit of this assay is between $<0.005 \text{ IU mL}^{-1}$ and 0.05 IU mL^{-1} FXIIIa activity [20]. Despite the ISTH-SSC recommendation against the use of clot solubility assays, recent laboratory surveys conducted by both the United Kingdom National External Quality Assessment Scheme for Blood Coagulation (UK NEQAS) and the Prospective Rare Bleeding Disorders Database (PRO-RBDD) suggest that clot solubility methods continue to be employed by 25% to 43% of clinical laboratories [22].

Quantitative Activity Assays

Quantitative FXIIIa activity assays have been developed based on two actions of FXIII: the transglutaminase activity and the isopeptidase activity [20]. Briefly, the transglutaminase activity drives the formation of an isopeptide bond between an acyl group and a free amine group on a neighboring peptide (Figure 2) [23]. During an intermediate step of the reaction, a thioacyl complex is formed between the acyl donor peptide and the active site cysteine of FXIIIa, and an ammonia (NH_3) molecule is released. The isopeptidase activity is characterized by the hydrolysis of isopeptide bonds (Figure 3) [24, 25]. Assays based on these activities are described below.

Ammonia-release assays:

The most commonly employed FXIIIa activity assays in the clinical laboratory ('ammonia-release' assays) are based upon quantification of elaborated ammonia as an indirect measurement of transglutaminase activity. The ammonia released during the transglutaminase reaction is quantified in a side reaction using a spectrophotometer to detect ammonia-dependent reduction of NAD(P)H (Figure 2) [1]. The most commonly cited assays approved for clinical use are the Berichrom "FXIII Chromogenic" (Dade Behring, Marburg, Austria, Germany) and the Technochrom "FXIII Kit" (Technoclone, Vienna, Austria) which are based upon ammonia-dependent NADH and NADPH reduction, respectively [1, 26, 27]. The advantages of ammonia-release assays include that they are true one-step kinetic enzyme assays that are amenable to automation. Despite these advantages, they have relatively low sensitivities preventing reliable quantification in the severe deficiency range ($<0.05 \text{ IU mL}^{-1}$ [Berichrom]). Importantly, there are other ammonia-producing and NADH-consuming biochemical reactions normally found in human plasma [23, 28]. These reactions may interfere with the accurate determination of FXIIIa transglutaminase activity and thus must be corrected for by plasma blanking in the presence of iodoacetamide (a potent FXIII antagonist). In the absence of these blanking procedures, ammonia-release assays are prone to FXIIIa activity overestimation by as much as $0.02 - 0.15 \text{ IU mL}^{-1}$. A recent study demonstrated poor inter-laboratory correlation of ammonia-release assays, which may be due to variable implementation of the recommended blanking procedures [22]. Unfortunately, the FXIIIa ammonia-release activity assays are not currently approved in all parts of the world.

Amine-incorporation assays:

Amine-incorporation assays also quantify the transglutaminase-activity-dependent formation of isoamide bonds catalyzed by FXIIIa (Figure 2) [1, 29]. The amine substrate can be any of several labeled compounds such as biotinylated cadaverine (5-biotinamidopentylamine), and is paired with a glutamine-containing protein such as casein, modified casein, or fibrinogen. FXIIIa-dependent incorporation of the amine substrate is determined by measuring the residual unincorporated, labeled amine following a separation step using streptavidin-enzyme conjugate, in this example, or an alternative, label-appropriate method. These methods are highly sensitive, but are more time consuming and technically challenging than the ammonia-release assays. Moreover, the assays are difficult to standardize and the separation step prevents the determination of true enzyme kinetics, thus they are not dynamic activity assays [20]. Lastly, amine-incorporation may yield falsely high activity levels in subjects with the *F13A1* valine (Val) 34 → leucine (Leu) polymorphism if incorporation is determined too early after the addition of FXIII activators because Val34→Leu accelerates FXIII activation but does not affect final enzyme activity [20, 30]. Given these limitations, amine-incorporation assays are rarely performed in clinical laboratories.

Isopeptidase assay:

The FXIIIa-dependent hydrolysis of $\gamma:\epsilon$ isopeptide bonds was first described in 1997 [24]. Zedira GmbH (Darmstadt, Germany) has capitalized on this activity to formulate a FXIII substrate (A101) with a fluorophore and a fluorescent quencher that are linked by a $\gamma:\epsilon$ bond (Figure 3) [25]. In this assay, isopeptidase-dependent release of the quencher unmasks A101 fluorescence, which increases over time enabling dynamic measurement of enzyme activity with a spectrofluorometer. The published minimum limit of FXIIIa detection is 0.02 IU mL⁻¹ with a minimum limit of quantitation of 0.05 IU mL⁻¹ [25]. Comparison studies of the Berichrom ammonia-release and Zedira assays demonstrated good inter-assay correlation (0.95) when serial dilutions of pooled normal plasma were used, but only 0.77 using healthy donor plasmas. Unfortunately, the correlation coefficient between the two assays at FXIIIa levels below 0.4 IU mL⁻¹ was not studied. Thus, it is unclear how the Zedira assay compares to a 'gold standard' ammonia-release assay in the FXIII deficiency range. Nonetheless, because this assay should be easily automated and not subject to interfering enzyme activities, it may offer a promising improvement over ammonia-release methods. The transglutaminase and isopeptidase activities can be considered inverse reactions; the transglutaminase activity catalyzes the formation of isopeptide bonds whereas the isopeptidase activity splits isopeptide bonds. However, the relevance of the isopeptidase activity to hemostasis remains unknown and may thus be less physiologically relevant than the transglutaminase activity [9, 25, 31]. The fluorogenic method for measuring isopeptidase activity is not yet available for clinical application.

Factor XIII Quantitative Antigen Assays

Approximately 40% of UK NEQAS centers and 25% of PRO-RBDD centers use FXIII antigen assays as either second line or follow-up tests as recommended by the ISTH-SSC [22, 32]. Antigen assays used for the detection and classification of FXIII deficiencies and

for monitoring response to therapy include immunoassays measuring FXIII-A₂, FXIII-B₂, and the FXIII-A₂B₂ complex. The HemosIL FXIII-A₂ latex immunoassay is approved for use by the United States Food and Drug Administration [1, 3]. The product insert reports a lower detection limit of 0.025 IU mL⁻¹ and coefficient of variation (CV) of 3.5 – 5.5%. However, a recent External quality Control of diagnostic Assays and Tests (ECAT) study suggested that the HemosIL has substantial inter-laboratory variability: 13.4% CV for samples with >0.7 IU mL⁻¹, 27.5% CV with 0.1–0.7 IU mL⁻¹, and 57% CV at levels below 0.1 IU mL⁻¹ FXIII-A₂ [3]. A highly sensitive FXIII-A₂B₂ ELISA (R-ELISA FXIII, Reanal-ker, Budapest, Hungary) has also been reported [33]. This assay employs an anti-FXIII-A primary antibody and anti-FXIII-B secondary antibody, essentially eliminating interference from free FXIII-B subunits and fibrinogen. The R-ELISA is able to determine FXIII concentrations as low as 0.001 IU mL⁻¹ with CV <5%. Several other FXIII immunoassays are less commonly utilized in clinical laboratories, with commercial availability and regulatory approval varying by nation [3, 21].

FXIII Genotyping

The gene encoding FXIII-A (*F13A1*) has been mapped to chromosome 6 (6p24–25). It contains 15 exons which produce a 3.9-kb mRNA. *F13B* (1q31–32.1) has 12 exons producing a 2-kb mRNA encoding the FXIII-B sequence [1]. Congenital FXIII deficiency follows an autosomal recessive inheritance pattern. Severe disease may be caused by either homozygous or compound heterozygous mutations at either locus. Over 95% of severe cases are due to FXIII-A deficiency, whereas FXIII-B deficiency has been described in fewer than 20 families [1, 34, 35]. More than 153 mutations within the FXIII-A gene have been published to date, most of which are missense or nonsense point mutations with no mutational hotspots being recognized [36, 37]. *F13B* mutations have been described in all but 2 of the 12 exons, also with no mutational hot spots. A systematic approach to sequencing the coding regions and exon-intron boundaries is thus required to fully evaluate potential disease-causing mutations. In the United States, Prevention Genetics (Marshfield, WI), Claritas Genomics (Cambridge, MA), and Fulgent Genetics (Temple City, CA) offer Clinical and Laboratory Improvement Amendments (CLIA)-approved mutation analysis for both *F13A1* and *F13B*. ARUP Laboratories (Salt Lake City, UT) offers genetic testing for only the FXIII-A Val34→Leu variant. In geographic regions with a high incidence of consanguinity, most notably Southeast Iran, persistence of founder mutations increases the prevalence of FXIII deficiency [37–39]. In this setting, since the most prevalent, and thus most likely, mutation(s) is known, a more restricted approach to genetic diagnosis may be feasible. For example, in the Iranian population, targeted genotyping using a combination of polymerase chain reaction and restriction fragment length polymorphism detection, has been successfully employed [37–39]. Sequencing of a short specific DNA segment limited to one or two exons containing the most prevalent mutations has also accurately discriminated healthy vs. deficient subjects in Iran [39].

Summary

FXIII deficiency is a rare bleeding disorder resulting from the failure to adequately stabilize fibrin within the hemostatic plug [37]. Accurate diagnosis of FXIII deficiency is challenging

due to both the rarity of the condition and limitations of currently available FXIII-specific assays. ISTH-SSC guidelines for the laboratory diagnosis of FXIII deficiency have not been uniformly employed in clinical laboratories because these assays are not always readily available for clinical use, and because of a lack of adequate inter-laboratory standardization. The rarity of FXIII deficiency leads to low assay volume even in larger reference laboratories, leading to proficiency maintenance challenges and unfavorable return on investment accounting assessments. Nonetheless, implementation of these guidelines may improve the accurate and timely diagnosis of FXIII deficiency, with the potential to improve patient outcomes [37]. Because the testing algorithm begins with accurate FXIIIa activity measurement, future discovery and development of simplified, reliable, high-throughput methods to directly measure FXIIIa transglutaminase activity may facilitate broader application of the ISTH-SSC recommendations.

Abbreviations:

FXIII	Factor XIII
pFXIII	Plasma Factor XIII
FXIIIa	Activated form of blood coagulation FXIII
FXIII-A	Factor XIII A-subunit monomer
FXIII-A₂	Factor XIII A-subunit dimer
FXIII-B	Factor XIII B-subunit monomer
FXIII-B₂	Factor XIII B-subunit dimer
FXIII-A₂B₂	Subunit structure of plasma FXIII
FXIII-A₂*	Active A subunit dimer
α2-AP	α 2-antiplasmin

References

- [1]. Karimi M, Berczky Z, Cohan N, Muszbek L. Factor XIII Deficiency. *Seminars in thrombosis and hemostasis*. 2009;35:426–38. [PubMed: 19598071]
- [2]. Muszbek L, Ariens RA, Ichinose A, Isth Ssc Subcommittee On Factor X. Factor XIII: recommended terms and abbreviations. *Journal of thrombosis and haemostasis : JTH*. 2007;5:181–3. [PubMed: 16938124]
- [3]. Hsu P, Zantek ND, Meijer P, Hayward CP, Brody J, Zhang X, et al. Factor XIII Assays and Associated Problems for Laboratory Diagnosis of Factor XIII Deficiency: An Analysis of International Proficiency Testing Results. *Seminars in thrombosis and hemostasis*. 2014;40:232–8. [PubMed: 24497117]
- [4]. Lorand L. Factor XIII: structure, activation, and interactions with fibrinogen and fibrin. *Annals of the New York Academy of Sciences*. 2001;936:291–311. [PubMed: 11460485]
- [5]. Kimura S, Aoki N. Cross-linking site in fibrinogen for alpha 2-plasmin inhibitor. *The Journal of biological chemistry*. 1986;261:15591–5. [PubMed: 2877981]
- [6]. Sakata Y, Aoki N. Cross-linking of alpha 2-plasmin inhibitor to fibrin by fibrin-stabilizing factor. *The Journal of clinical investigation*. 1980;65:290–7. [PubMed: 6444305]

- [7]. Byrnes JR, Wilson C, Boutelle AM, Brandner CB, Flick MJ, Philippou H, et al. The interaction between fibrinogen and zymogen FXIII-A2B2 is mediated by fibrinogen residues gamma390–396 and the FXIII-B subunits. *Blood*. 2016;128:1969–78. [PubMed: 27561317]
- [8]. Muszbek L, Bagoly Z, Bereczky Z, Katona E. The involvement of blood coagulation factor XIII in fibrinolysis and thrombosis. *Cardiovascular & hematological agents in medicinal chemistry*. 2008;6:190–205. [PubMed: 18673233]
- [9]. Fraser SR, Booth NA, Mutch NJ. The antifibrinolytic function of factor XIII is exclusively expressed through alpha(2)-antiplasmin cross-linking. *Blood*. 2011;117:6371–4. [PubMed: 21471521]
- [10]. Duckert F, Jung E, Shmerling DH. A hitherto undescribed congenital haemorrhagic diathesis probably due to fibrin stabilizing factor deficiency. *Thrombosis et diathesis haemorrhagica*. 1960;5:179–86. [PubMed: 13724728]
- [11]. Seitz R, Duckert F, Lopaciuk S, Muszbek L, Rodeghiero F, Seligsohn U. ETRO Working Party on Factor XIII questionnaire on congenital factor XIII deficiency in Europe: status and perspectives. Study Group. *Seminars in thrombosis and hemostasis*. 1996;22:415–8.
- [12]. Inbal A, Lubetsky A, Krapp T, Castel D, Shaish A, Dickneite G, et al. Impaired wound healing in factor XIII deficient mice. *Thrombosis and haemostasis*. 2005;94:432–7. [PubMed: 16113836]
- [13]. Dardik R, Loscalzo J, Inbal A. Factor XIII (FXIII) and angiogenesis. *Journal of thrombosis and haemostasis : JTH*. 2006;4:19–25. [PubMed: 16129024]
- [14]. Inbal A, Muszbek L. Coagulation factor deficiencies and pregnancy loss. *Seminars in thrombosis and hemostasis*. 2003;29:171–4. [PubMed: 12709920]
- [15]. Koseki-Kuno S, Yamakawa M, Dickneite G, Ichinose A. Factor XIII A subunit-deficient mice developed severe uterine bleeding events and subsequent spontaneous miscarriages. *Blood*. 2003;102:4410–2. [PubMed: 12933578]
- [16]. Menegatti M, Palla R, Boscarino M, Bucciarelli P, Muszbek L, Katona E, et al. Minimal factor XIII activity level to prevent major spontaneous bleeds. *Journal of thrombosis and haemostasis : JTH*. 2017.
- [17]. Peyvandi F, Palla R, Menegatti M, Siboni SM, Halimeh S, Faeser B, et al. Coagulation factor activity and clinical bleeding severity in rare bleeding disorders: results from the European Network of Rare Bleeding Disorders. *Journal of thrombosis and haemostasis : JTH*. 2012;10:615–21. [PubMed: 22321862]
- [18]. Ichinose A, Japanese Collaborative Research Group on AH. Autoimmune acquired factor XIII deficiency due to anti-factor XIII/13 antibodies: A summary of 93 patients. *Blood reviews*. 2017;31:37–45.
- [19]. Biswas A, Ivaskevicius V, Thomas A, Oldenburg J. Coagulation factor XIII deficiency. Diagnosis, prevalence and management of inherited and acquired forms. *Hamostaseologie*. 2014;34:160–6. [PubMed: 24503678]
- [20]. Kohler HP, Ichinose A, Seitz R, Ariens RA, Muszbek L, Factor X, et al. Diagnosis and classification of factor XIII deficiencies. *Journal of thrombosis and haemostasis : JTH*. 2011;9:1404–6. [PubMed: 22946956]
- [21]. Jennings I, Kitchen S, Woods TA, Preston FE, Uk N. Problems relating to the laboratory diagnosis of factor XIII deficiency: a UK NEQAS study. *Journal of thrombosis and haemostasis : JTH*. 2003;1:2603–8. [PubMed: 14675096]
- [22]. Jennings I, Kitchen S, Menegatti M, Palla R, Walker I, Makris M, et al. Detection of Factor XIII deficiency: data from multicentre exercises amongst UK NEQAS and PRO-RBDD project laboratories. *International journal of laboratory hematology*. 2017;39:350–8. [PubMed: 28406553]
- [23]. Ajzner E, Muszbek L. Kinetic spectrophotometric factor XIII activity assays: the subtraction of plasma blank is not omissible [corrected]. *Journal of thrombosis and haemostasis : JTH*. 2004;2:2075–7. [PubMed: 15550059]
- [24]. Parameswaran KN, Cheng XF, Chen EC, Velasco PT, Wilson JH, Lorand L. Hydrolysis of gamma:epsilon isopeptides by cytosolic transglutaminases and by coagulation factor XIIIa. *The Journal of biological chemistry*. 1997;272:10311–7. [PubMed: 9092583]

- [25]. Oertel K, Hunfeld A, Specker E, Reiff C, Seitz R, Pasternack R, et al. A highly sensitive fluorometric assay for determination of human coagulation factor XIII in plasma. *Analytical biochemistry*. 2007;367:152–8. [PubMed: 17582378]
- [26]. TECHNOCHROM®FXIIIPackageInsert.https://diapharma.com/wp-content/uploads/2017/04/5360010_Technochrom_FXIII_REV3019090-005_08-2016_insert.pdf; diapharma; 2018.
- [27]. Berichrom Factor XIII Chromogenic Package Insert. Available from: <https://www.healthcare.siemens.com/hemostasis/systems/bcs-xp-system/assays>; Siemens Healthineers; 2018.
- [28]. Lawrie AS, Green L, Mackie IJ, Liesner R, Machin SJ, Peyvandi F. Factor XIII--an under diagnosed deficiency--are we using the right assays? *Journal of thrombosis and haemostasis : JTH*. 2010;8:2478–82. [PubMed: 20727071]
- [29]. Song YC, Sheng D, Taubenfeld SM, Matsueda GR. A microtiter assay for factor XIII using fibrinogen and biotinylcadaverine as substrates. *Analytical biochemistry*. 1994;223:88–92. [PubMed: 7695107]
- [30]. Wilmer M, Rudin K, Kolde H, Poetzsch B, Lenz W, Moessmer G, et al. Evaluation of a sensitive colorimetric FXIII incorporation assay. Effects of FXIII Val34Leu, plasma fibrinogen concentration and congenital FXIII deficiency. *Thrombosis research*. 2001;102:81–91. [PubMed: 11323018]
- [31]. Rijken DC, Abdul S, Malfliet JJ, Leebeek FW, Uitte de Willige S. Compaction of fibrin clots reveals the antifibrinolytic effect of factor XIII. *Journal of thrombosis and haemostasis : JTH*. 2016;14:1453–61. [PubMed: 27148673]
- [32]. Kohler HP, Ariens RA, Whitaker P, Grant PJ. A common coding polymorphism in the FXIII A-subunit gene (FXIIIVal34Leu) affects cross-linking activity. *Thrombosis and haemostasis*. 1998;80:704. [PubMed: 9798996]
- [33]. Katona E, Haramura G, Karpati L, Facht J, Muszbek L. A simple, quick one-step ELISA assay for the determination of complex plasma factor XIII (A2B2). *Thrombosis and haemostasis*. 2000;83:268–73. [PubMed: 10739385]
- [34]. Kerlin B, Brand B, Inbal A, Halimeh S, Nugent D, Lundblad M, et al. Pharmacokinetics of recombinant factor XIII at steady state in patients with congenital factor XIII A-subunit deficiency. *Journal of thrombosis and haemostasis : JTH*. 2014;12:2038–43. [PubMed: 25263390]
- [35]. World Federation of Hemophilia Report on the Annual Global Survey 2012 Montreal, QC, Canada2013.
- [36]. Biswas A, Ivaskevicius V, Seitz R, Thomas A, Oldenburg J. An update of the mutation profile of Factor 13 A and B genes. *Blood reviews*. 2011;25:193–204. [PubMed: 21640452]
- [37]. Karimi M, Peyvandi F, Naderi M, Shapiro A. Factor XIII deficiency diagnosis: Challenges and tools. *International journal of laboratory hematology*. 2018;40:3–11.
- [38]. Dorgalaleh A, Assadollahi V, Tabibian S, Shamsizadeh M. Molecular Basis of Congenital Factor XIII Deficiency in Iran. *Clinical and applied thrombosis/hemostasis : official journal of the International Academy of Clinical and Applied Thrombosis/Hemostasis*. 2016;1076029616680473.
- [39]. Eshghi P, Cohan N, Lak M, Naderi M, Peyvandi F, Menegatti M, et al. Arg77His and Trp187Arg are the most common mutations causing FXIII deficiency in Iran. *Clinical and applied thrombosis/hemostasis : official journal of the International Academy of Clinical and Applied Thrombosis/Hemostasis*. 2012;18:100–3. [PubMed: 22156982]

Figure 1: International Society on Thrombosis and Haemostasis Scientific and Standardization Committee-recommended laboratory diagnostic algorithm for Factor XIII deficiency.

The algorithm steps are illustrated in the flowchart and the purpose of each step is described to the right.

Figure 2: Ammonia-release assays are based upon the transglutaminase reaction catalyzed by activated factor XIII.

(**A**) FXIIIa catalyzes the formation of an isopeptide bond. A free ammonia molecule is generated with the formation of each bond. (**B**) Ammonia levels serve as an indirect measure of transglutaminase activity in an ammonia-consuming side reaction producing NAD(P), which is detected with a spectrophotometer by absorbance at the appropriate wavelength (340 nm). (Q: glutamine with its side-chain; X: any other amino acid; R: peptide; FXIIIa: activated plasma Factor XIII; NH_3 : ammonia; NAD(P)H: nicotinamide adenine dinucleotide (phosphate))

Figure 3: The isopeptidase reaction catalyzed by activated factor XIII may be measured fluorometrically.

The isopeptidase activity of FXIIIa hydrolyzes isopeptide bonds, represented here by the hydrolysis of an isoamide bond linking a glutamine residue to a synthetic fluorescent quencher. FXIIIa-dependent removal of the quencher unmasks fluorescence of a fluorophore linked to a synthetic glutamine-containing peptide, which can be detected spectrofluorometrically using the indicated excitation (λ_{Ex}) and emission (λ_{Em}) wavelengths. (Q: glutamine with its side-chain; X: any other amino acid; H₂O: water; FXIIIa: activated plasma factor XIII)