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Factor XIII deficiency diagnosis: Challenges and tools

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Abstract

Factor XIII deficiency (FXIIID) is a rare hereditary bleeding disorder arising from heterogeneous mutations, which can lead to life-threatening hemorrhage. The diagnosis of FXIIID is challenging due to normal standard coagulation assays requiring specific FXIII assays for diagnosis, which is especially difficult in developing countries. This report presents an overview of FXIIID diagnosis and laboratory methods and suggests an algorithm to improve diagnostic efficiency and prevent missed or delayed FXIIID diagnosis. Assays measuring FXIII activity: The currently available assays utilized to diagnose FXIIID, including an overview of their complexity, reliability, sensitivity, and specificity, as well as mutational analysis are reviewed. The use of a FXIII inhibitor assay is described. Diagnostic tools in FXIIID: Many laboratories are not equipped with quantitative FXIII activity assays, and if available, limitations in lower activity ranges are important to consider. Clot solubility tests are not standardized, have a low sensitivity, and are therefore not recommended as routine screening test; however, they are the first screening test in almost all coagulation laboratories in developing countries. To minimize the number of patients with undiagnosed FXIIID, test quality should be improved in less well-equipped laboratories. Common country-specific mutations may facilitate diagnosis through targeted genetic analysis in reference laboratories in suspected cases. However, genetic analysis may not be feasible in every country and may miss spontaneous mutations. Centralized FXIII activity measurements should also be considered. An algorithm for diagnosis of FXIIID including different approaches dependent upon laboratory capability is proposed.

KEYWORDS

factor XIII, factor XIII deficiency, factor XIII deficiency diagnosis, FXIII assays, laboratory assays

1 | INTRODUCTION

Inherited factor XIII deficiency (FXIIID) is a rare bleeding disorder affecting the final stage of the coagulation system and resulting in a bleeding diathesis.[1] The worldwide incidence of FXIIID, inherited as an autosomal recessive disorder, is approximately one per 1-3 million people. Its prevalence depends on geographic region and is higher in areas in which consanguineous marriage is common; however, there is no difference worldwide in affected ethnicity or race. The molecular defects in FXIIID arise from heterogeneous mutations, which can be country-specific, facilitating methods to confirm FXIIID diagnosis in such areas.[2-4]

Signs and symptoms of FXIIID range from life-threatening hemorrhage such as intracranial hemorrhage to mild forms, including skin bleeding. Umbilical cord bleeding and soft tissue hematoma are the most common and often the first symptom of FXIIID.[3,5,6] In women with severe FXIIID, recurrent miscarriage is common. Heterozygous carriers may show a bleeding tendency upon provocation such as traumatic injury or invasive procedures, and in some cases, umbilical cord bleeding, menorrhagia, miscarriages, or postpartum bleeding.[7-9]

The diagnosis of FXIIID is challenging due to the rarity of the disorder and because standard coagulation screening tests, including prothrombin time, activated partial thromboplastin time, thrombin time, platelet count, or bleeding time, are normal; therefore, specific FXIII

assays are required. FXIIID remains one of the most underdiagnosed rare bleeding disorders.[10-12] Although comprehensive guidelines for FXIIID diagnosis have been published by the International Society on Thrombosis and Haemostasis Scientific and Standardization Committee (ISTH-SSC), these have not been implemented uniformly worldwide due to insufficient laboratory assay resources.[13] However, they have lead to a significant improvement in diagnostic efficiency in parts of the world in which they are used for diagnosis. Therefore, novel approaches are required for the diagnosis of FXIIID to address the gap between expected prevalence and established diagnoses.

A report on the Annual Global Survey of the worldwide distribution of rare bleeding disorders by the World Federation of Hemophilia published in October 2015 revealed 1327 patients with FXIIID registered from 65 counties.[14] This survey revealed that half of available data originated from Europe, underscoring the need for increased efforts to establish accurate diagnosis and improve worldwide data collection systems. Based upon this survey, many developing countries did not report patients with FXIIID, emphasizing the difficulty in diagnosis in resource-poor settings. The data further underscore the need to improve diagnostic tools and develop novel approaches to diagnose patients with FXIIID.

The aim of this report was to present a comprehensive and updated overview of FXIIID diagnosis, including diagnostic tools and challenges, and to propose an algorithm that may be considered to improve diagnostic efficiency and prevent missed or delayed FXIIID diagnosis, thereby reducing morbidity and mortality of patients with this disease.

2 | RESULTS

2.1 | Assays used to measure FXIII activity

Assays to measure FXIII activity are based on the transglutaminase reaction catalyzed by FXIII after its activation, which is described below.

2.1.1 | Factor XIII activation process

FXIII is a proenzyme, protransglutaminase, which circulates in an inactive form as a heterotetramer consisting of two catalytic A subunits and two carrier/protective B subunits (FXIII-A_2B_2).[13] FXIII is activated in three steps: initially, the activation peptide A, consisting of 37 amino acids, is cleaved from subunit A in the presence of fibrin and thrombin. Subsequently, subunit B is dissociated from subunit A in the presence of calcium and fibrin. Lastly, in the presence of calcium, the cleaved FXIII-A_2 undergoes conformational changes exposing active-site cysteine residues, which react with fibrin monomers catalyzing their cross-linking.[3,12,15]

2.1.2 | Transglutaminase reaction catalyzed by activated FXIII

Activated FXIII (FXIIIa) acts in the final stage of coagulation to stabilize the fibrin clot. In a first step, the cysteine residue of activated subunit A reacts with a glutamine residue of a gamma chain fibrin molecule, forming a binary complex, a process during which ammonia is released (Figure 1A). In a second step, this binary complex can undergo two different subsequent reactions: (i) the primary amine group of a lysine residue from another gamma chain fibrin molecule breaks the binary complex thioester bond in a transglutaminase reaction and reacts with the glutamine residue, forming a glutaminyl-lysyl bond (Figure 1B); (ii) a free amine group reacts with the binary complex, resulting in an isopeptide glutamine-amine bond (Figure 1C). These reactions, including ammonia release, transglutaminase reaction, and incorporation of a free amine group into the glutamine-FXIIIa complex, result in gamma chain fibrin to fibrin dimerization and cross-linking, and form the fundamental basis for laboratory assays measuring FXIII activity via specific products released during these steps.[16]

In addition to cross-linking gamma chain fibrin molecules, activated FXIII can attach the fibrinolytic inhibitor (α_2 antiplasmin) to alpha chain fibrin molecules to prevent premature degradation of the formed clot by fibrinolysis via plasmin.[10,16]

2.2 | Diagnostic tools in congenital FXIII deficiency

Complete diagnosis of FXIIID is based upon identification of characteristic symptoms, a detailed patient history, including bleeding at birth, a thorough clinical evaluation, and a variety of specialized laboratory tests. Umbilical cord bleeding at birth is observed in more than 80% of affected severely deficient cases and is therefore almost pathognomonic and highly suggestive of FXIIID.[17] Other hemorrhages, such as subcutaneous bleeding, which occurs in 57% of patients with FXIIID, also indicate specific deficiencies, including FXIIID.[3,6,17,18] Clinical symptoms may also manifest in some heterozygous carriers, which should prompt further diagnostic investigation and clinical risk management.[7,9] Delayed diagnosis is associated with increased morbidity and mortality; as such, timely diagnosis of FXIIID by clinical evaluation, patient and family history, and laboratory tools is crucial. A simple rule with which to approach laboratory evaluation is to ensure that it is flexible, readily available, reliable, and accurate. In vitro assays to confirm diagnosis include the following: (i) clot solubility test, (ii) FXIII activity assay, (iii) FXIII antigen assay, (iv) inhibitor assay, and (v) molecular diagnosis and thromboelastography (TEG). TEG is currently not standardized and therefore not further discussed in this review. Advantages and disadvantages of the other assays are discussed below.

2.2.1 | Clot solubility test (CST)

The first case of FXIIID described was diagnosed in 1960 using this method.[19] Its sensitivity to FXIII activity levels depends on fibrinogen level, clotting (thrombin and/or Ca^{2+}), and solubilizing agents and their concentration (2% acetic acid, 1% monochloroacetic [MCA] acid, or 5 mol L^{-1} urea) (Table 1). As hypofibrinogenemia and dysfibrinogenemia can cause false-positive results using the 5 mol L^{-1} urea solubility test, functional assays should be performed in case of a positive 5 mol L^{-1} urea test to rule out hypo- or dysfibrinogenemia patients.[10] Hypofibrinogenemia and dysfibrinogenemia can be confirmed or excluded using thrombin time, reptilase time, and/or a fibrinogen antigen

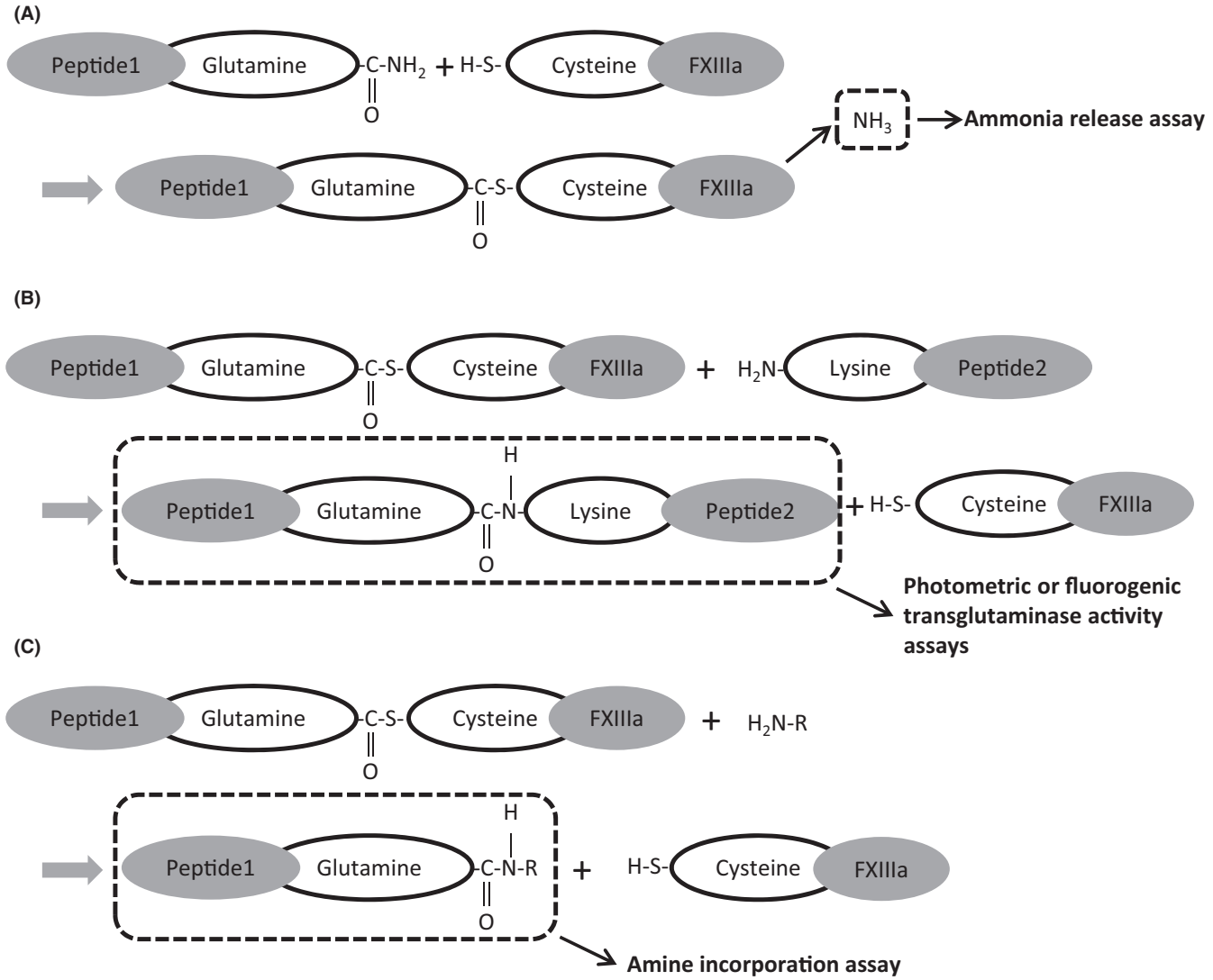

FIGURE 1 Transglutaminase reaction and assay principles for measurement of FXIII activity. The dashed boxes indicate the products measured with the respective assay

TABLE 1 Clotting and solubilizing agents: important factors that determine the sensitivity of the clot solubility test [adapted from[2]]

Clotting agent	Solubilizing agent	Sensitivity to FXIII levels (%)	Comment
$CaCl_2$	Urea	<0.5-5	More specific than acetic acid-based assay
Thrombin	Acetic acid	10	Sensitive and rapid, but less specific than urea-based assay
$CaCl_2$	Acetic acid	0-3	Less sensitive than urea-based assay

assay in conjunction with a fibrinogen activity assay. High pepsinogen levels, as they may occur in patients with gastric diseases or a helicobacter pylori infection, can also lead to a false-positive CST when using acetic acid-based assays.[20]

The advantages of the CST are that it is easy to perform, inexpensive, and without specific instrumentation requirements, so that it can be established in most laboratories. However, disadvantages include a lack of standardization and low sensitivity (inability to detect mild or moderate deficiency, including heterozygous carriers). The CST therefore underestimates the number of FXIIID cases, may lead to missed or delayed diagnosis, and cannot be used for monitoring prophylactic treatment.[4,10,13] Although it is not recommended as a routine screening test for the aforementioned reasons, the CST is often the first screening test utilized in many coagulation laboratories in developing countries as well as in 20% of developed countries.[10] Apart from resource issues, the lack of regulatory approval for some commercially available quantitative assays may present a barrier to more widespread adoption and use. Therefore, these assays cannot be

discarded as screening tests, especially in developing countries due to the lack of alternative assays with better sensitivity to moderate and mild deficiency states; for this reason, the quality of the test should be optimized to minimize the number of patients with undiagnosed FXIIID. Although international recommendations or guidelines do not exist for the use of the CST, an optimized strategy may include the utilization of two different CSTs with 5 mol L^{-1} urea or acetic acid as solubilizing agents and $CaCl_2$ or thrombin as clotting agents, respectively, in parallel.[2] FXIIID should be considered if one of the two CSTs is abnormal. The establishment of a local cutoff solubility time may also be of advantage to optimize this strategy.

2.2.2 | Quantitative assays (functional activity assays)

Quantitative FXIII activity assays are recommended as first-line screening tests whenever possible. Three main methods are used to measure FXIII activity levels, all currently based upon the transglutaminase reaction in the cross-linking process: (i) ammonia release assay, (ii) amine incorporation assay, and (iii) isopeptidase assay.[13,16] There are challenges and limitations to each of the quantitative methods used for FXIIID diagnosis, the major disadvantages being variation in the limit of quantitation (<1%-10%) and low sensitivity. The details of these three methods, including advantages and disadvantages, are described below and in Table 2.[2,10,13,16,21,22]

- The ammonia release assay is the most common and convenient method due to the short time required for performance (10 minutes). It measures FXIII activity via photometric absorbance at 340 nm wavelength, which is decreased in the presence of FXIII activity by a glutamate dehydrogenase-mediated indicator reaction in which one molecule, NADH or NADPH, combined with one molecule of ammonia is converted to NAD^+ or NADP. Increased activity for levels <10% may be observed, thereby causing overestimation.[3,16] The cause of this increased activity is FXIIIa-independent ammonia release, which adds to FXIII-dependent ammonia release. Therefore, the use of a plasma blank is required, in which FXIIIa is inhibited by 1 mmol L^{-1} iodoacetamide and only released ammonia not related to FXIIIa activity is measured. The subtraction of a plasma blank from patient plasma is absolutely required to avoid incorrect results in the low activity range (<5%-10%), and its application ensures a more reliable detection of FXIIID patients with levels <5%.[23] Failure to subtract the plasma blank may result in serious clinical consequences if patients with very low FXIII activity appear to have acceptable levels and are not correctly diagnosed and managed accordingly. Three commercial ammonia release assay kits are currently available, some of which supply a reagent for measurement of a plasma blank; if a plasma blank is not supplied, this should be prepared by the local laboratory.
- In the amine incorporation assay, fluorescent, radiolabeled, or biotinylated amines covalently bound to a glutamine residue of a substrate are measured after free unbound amines have been separated from the protein-bound fraction. Although amine incorporation assays are more sensitive than the ammonia release assay, they are more difficult to standardize, have difficulty with reproducibility, and are time-consuming. Moreover, certain types of this assay that measure transglutaminase activity at the initial stage of FXIII activity are sensitive to the Val34Leu FXIII polymorphism, resulting in falsely increased FXIII activity when present, whereas those assays measuring the transglutaminase activity of the completely activated enzyme are not affected.[13]
- The isopeptidase assay is based on the isopeptidase activity of FXIIIa under certain conditions, which results in the gradual removal of primary amines bound to a glutamine residue in an oligopeptide. This gradual release of amines containing a quencher results in increased fluorescence of a peptide substrate, which is labeled with a fluorophore.[16] Although overestimation does not occur in this assay, the limit of quantitation is around 5% normal FXIII activity; therefore, it is less sensitive than other FXIII activity assays.[16]

2.2.3 | Immunological assays (FXIII antigen assays)

A FXIII antigen assay is required for the classification of FXIII deficiency. If FXIII activity is decreased, the deficiency subtype, FXIII-A or FXIII-B, is determined using an immunological FXIII antigen assay to assure appropriate classification and treatment. Several FXIII antigen assays are available, including immunoassays to measure FXIII-A, FXIII-B, and the FXIII-A_2B_2 complex. Clearly, one limitation to antigen assays is that they may fail to identify rare qualitative (type II) deficiencies, in which the FXII-A enzyme is present but functionally defective.[12] Although assays for the measurement of the different FXIII subunits are available, some centers may only use the most frequently employed FXIII-A ELISA, thereby not complying with current recommendations and potentially missing the rarer FXIII-B subunit deficiency.

Electroimmunoassays (EIA), radioimmunoassays (RIA), latex-enhanced immunoprecipitation assays, or enzyme-linked immunosorbent assays (ELISA) are four common immunoassay methods; with ELISA considered superior to other methods. Several types of ELISA are in use, including highly sensitive chemiluminescence FXIII antigen ELISAs and R-ELISA FXIII (Reanal-ker, Budapest, Hungary).[3,10,16] The FXIII antigen ELISA method has a limit of detection of 0.014 μg/L (FXIII-A), 0.019 μg/L (FXIII-B), and 0.016 μg/L (FXIII-A_2B_2), laboratory imprecision of <12%, and an estimated total error of <25%.[24] The R-ELISA FXIII method is a one-step sandwich ELISA with good sensitivity (as low as 0.1%) and suitable for monitoring substitution therapy.[16,24] Other methods, including EIA and RIA, are used infrequently due to lower sensitivity, laborious procedures, or because they may produce artifacts.[25]

2.2.4 | Inhibitor (autoantibody) assays

Detected low factor activity can be attributed to congenital factor deficiency or development of an autoantibody, leading to autoimmune

TABLE 2 Different methods to measure FXIII activity

Method	Kit	Detection limit (%)	Reference range (U/dL)	Advantages	Disadvantages
Ammonia release assay (Photometric measurement of ammonia release by glutamate dehydrogenase-mediated NADH/NADPH-dependent indicator reaction at 340 nm wavelength)	Berichrom® FXIII (Dade-Behring, Marburg, Germany) REA chrom FXIII (Reanal-ker, Budapest, Hungary) Technochrom® FXIII (Technoclone, Vienna, Austria)	<5 <5 <5	70-140 70-140 70-140	• Well standardized • Easily automated on blood coagulation analyzer • One-step, quick • True kinetic assay with good reproducibility • Insensitive to FXIII-A Val134Leu polymorphism • Slight modification of assay can decrease its detection limit to <1%	• Activity overestimated if plasma blank measurement is not used
Amine incorporation assay (Fluorescence, photometry, or liquid scintillation measurement of covalent binding of a labeled amine to a glutamine residue of a protein by FXIIIa)	[Pefakit® FXIII (Pentapharm, Basel, Switzerland)][a]	0.1-1	70-140	• Highly sensitive	• Difficult to standardize • Time-consuming, cumbersome • Cannot be automated • Sensitive to FXIII-A Val134Leu polymorphism, causing increased FXIII activity
Isopeptidase assay	FXIII-Assay Kit (Zedira GmbH, Darmstadt, Germany)	<5	70-140	• Direct, simple • Independent of subsequent enzymatic steps	• Poor correlation with the FXIII-A subunit ELISA compared to photometric assay

NADH, nicotinamide adenine dinucleotide; NADPH, nicotinamide adenine dinucleotide phosphate hydrogen; FXIII-A, factor XIII subunit A; FXIII, factor XIII; FXIIIa, activated factor XIII; ELISA, enzyme-linked immunosorbent assay.

[a]Pefakit® FXIII is no longer commercially available.

FXIII deficiency; these cannot be differentiated using quantitative assays. Inhibitor assays are performed to determine whether FXIIID in a patient is hereditary or due to formation of an autoantibody recognizing FXIII. The development of a FXIII autoantibody is a very rare event, with less than 100 cases worldwide reported to date[26]. Approximately 50% of cases are idiopathic, whereas underlying diseases related to an imbalance of the immune system, such as autoimmune disorders, solid neoplasms, myeloproliferative and lymphoproliferative diseases, prolonged drug use, and pregnancy, are also associated with the development of autoantibodies against FXIII. Autoimmune FXIII deficiency occurs mainly in older adults and can result in drastically decreased FXIII levels, with potentially life-threatening bleeding. Clinical symptoms cover a large range from common multiple mucocutaneous or intramuscular bleeds to life-threatening internal hemorrhages.[26] Older adults with a recent and spontaneous onset of bleeding symptoms lacking previous bleeding symptoms, even when undergoing surgery or invasive procedures, and with no family history of FXIIID should be considered and FXIII antigen and inhibitor assays performed. There are two types of autoantibody: (i) neutralizing autoantibodies against FXIII-A, which can be measured using mixing studies in which FXIII activity in normal plasma is inhibited by a neutralizing autoantibody in a mixture of patient and normal plasma, and (ii) non-neutralizing inhibitors, which are determined using binding assays to detect the binding of a patient's IgG (IgM) to purified plasma FXIII and purified FXIII subunits in a non-commercially available ELISA by adding anti-human IgG or in dot blot arrangements.[10,13] The use of an inhibitory assay is age-dependent and not recommended for FXIIID evaluation when diagnosed at birth, as the development of a FXIII autoantibody is unlikely in neonates or babies who have not been exposed to a FXIII product. The effect of the presence of FXIII autoantibodies on FXIII activity and antigen levels are summarized in Table 3.

2.2.5 | FXIII molecular (genetic) analysis

Molecular analysis requires amplification and sequencing of FXIII-A and/or FXIII-B genes. The A subunit gene is located on chromosome 6 and contains 15 exons and 14 introns. To date, 153 mutations have been reported, of which missense mutations occur in more than half of cases and represent the most common type of genetic defect.[27] Severe and spontaneous bleeding episodes occur in both homozygous and heterozygous patients, the latter more commonly under hemostatic stress situations.[7-9]

FXIII subunit B deficiency accounts for less than 5% of recognized FXIIID cases. The B subunit gene is located on chromosome 1 and contains 12 exons and 11 introns; 16 mutations, mostly missense, have been reported.[28] The absence of FXIII subunit B leads to shorter plasma half-life of FXIII subunit A. FXIII subunit B deficiency causes a less severe phenotype due to the remaining 5%-10% FXIII activity present in the plasma.[6,27]

The *FXIII-A* gene is highly polymorphic, and a number of molecular variants have been characterized. FXIII-specific activity in normal individuals is related to specific polymorphisms in the *FXIII-A* gene. More than 1000 noncoding polymorphisms have been observed in both FXIII subunits. Five common coding polymorphisms have been detected in the *FXIII-A* gene and affect either FXIII activity or plasma level: Val34Leu (the most common; accelerated FXIII activation), Tyr204Phe (decreased FXIII plasma level and activity), Pro564Leu (decreased FXIII plasma level and increased FXIII activity), Glu651Gln, and Val650Ile (low to normal FXIII activity).[27] The frequency of polymorphisms has been observed to be dependent on ethnicity. The spectrum of common *FXIII-A* gene mutations in a few selected countries, including both developed and developing countries, is shown in Table 4.[27] In some countries, molecular analysis can facilitate diagnosis if population-specific mutations are known. Based on this knowledge, genetic analysis in some countries may aid diagnosis, especially in less well-equipped coagulation laboratories; in this circumstance, after a CST, targeted genetic analysis could be performed in reference laboratories prior to more specific FXIII activity and antigen assays. This strategy involves a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay or the sequencing of a short specific DNA segment limited to one or two exons, both of which require knowledge of specific primers; this type of testing requires less than 24 hours.[2] This strategy may be useful in countries in which highly specific recurrent mutations are common; it will not detect spontaneous novel mutations. An example where this strategy has been employed is Iran, where nearly one-third of all patients with FXIIID worldwide have been reported. Two mutations are found commonly, of which one (c.562T>C) was detected in 95% of affected patients.[29,30] Using this approach, identified FXIIID cases increased more than twofold within 2 years from 2014 (217 registered patients, annual report of WFH) to 2016 (483 patients).[29,31] In addition, FXIII-targeted genetic analysis

TABLE 3 FXIII activity and antigen levels in the presence of autoantibody [adapted from[13]]

FXIII- autoantibody	Plasma FXIII activity	Plasma FXIII-A_2B_2 antigen	Plasma FXIII-A antigen	Plasma FXIII-B_2 antigen	Platelet FXIII activity	Platelet FXIII-A antigen
A						
Neutralizing antibody	Severe decrease (<3%)	NL or slight decrease	NL or slight decrease	Decrease (>30%)	NL	NL
Non-neutralizing antibody	Severe decrease (FXIII-A)	Severe decrease	Severe decrease	Decrease (>30%)	NL	NL
B						
Non-neutralizing antibody	Severe decrease (<3%)	Severe decrease	Severe decrease	Severe decrease	NL	NL

FXIII, factor XIII; FXIII-A, factor XIII subunit A; FXIII-A_2B_2, factor XIII A_2B_2-heterotetramer; FXIII-B_2, factor XIII B_2-homodimer; NL, normal level.

TABLE 4 Commonly reported FXIII-A gene mutations in different countries [extracted from[27]]

	Developing or emerging country		Developed country		
	Iran	Tunisia	Pakistan	Finland	The netherlands
Mutation(s)	Trp187Arg Arg77His	c.869insC	Ser296Arg c.2045G>A IVS11+1G>A	Arg661X	Arg326Gln Gly262Glu Val316Phe

with these founder mutations has allowed for genetic counseling of relatives of affected patients, many of whom are likely to be heterozygous carriers, with an option for antenatal diagnosis.

3 | DISCUSSION

The diagnosis of FXIIID represents a significant challenge and may require a variety of approaches based on geographic area and laboratory facility availability. Issues include normal routine coagulation screening tests, necessitating advanced laboratory techniques, which often are not available in a considerable number of developing countries. As a result, FXIIID is one of the most underdiagnosed rare coagulation disorders.[10-12] Family and patient history along with clinical evaluation are important to raise suspicion and early diagnosis; as such, awareness of clinical symptoms indicative of this disorder is paramount to ensure timely diagnosis, including abnormal umbilical cord bleeding, delayed umbilical detachment, intracranial bleeding in a full-term newborn, and subcutaneous bleeding. In addition, women with FXIIID experience a high rate of miscarriage.[32] Heightened clinical suspicion and referral of suspected cases to comprehensive hemophilia centers at an early age is crucial to minimize delayed diagnosis and increased

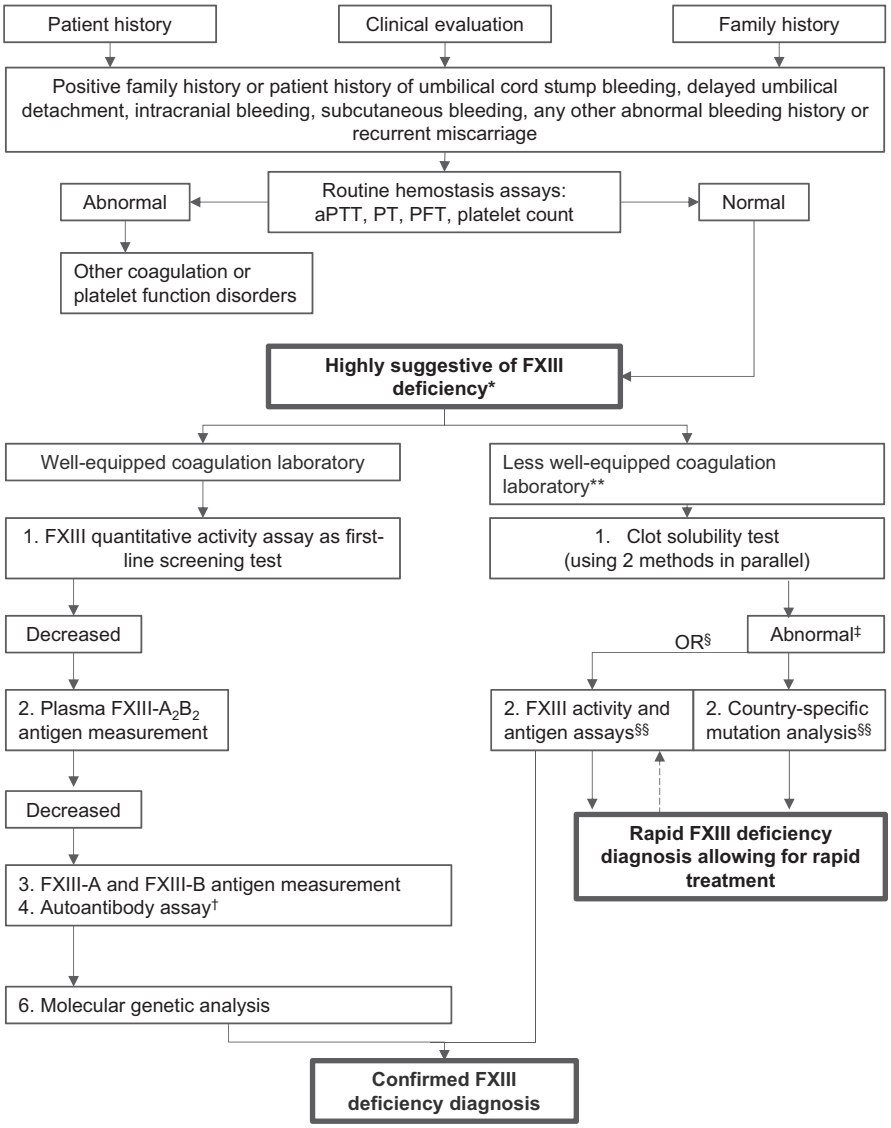

FIGURE 2 Algorithm to guide the diagnosis of FXIII deficiency. *Alpha2 antiplasmin deficiency and other rare deficiencies should be considered if FXIII assays negative. **Without capacity to perform quantitative assays. †Performance of assay is age-dependent; not recommended for neonates. ‡If clot solubility test is normal, but clinical symptoms support FXIIID, sample should be sent to reference laboratory for FXIII activity measurement. §Mutation analysis performed in countries with specific common and recurrent mutations. §§Send sample to central, national, or international reference laboratory if not locally available. aPTT, activated partial thromboplastin time. PT, prothrombin time. PFT, platelet function test. FXIII, factor XIII

rates of morbidity and mortality. False-positive platelet function tests may contribute to missed or delayed diagnosis of FXIIID. Symptoms of severe umbilical cord bleeding, intracranial hemorrhage, and hemarthrosis are unusual in patients with platelet function disorders.[17]

Specific common and recurrent mutations in geographic areas due to founder effects may facilitate diagnosis through establishment of targeted genetic testing; if targeted genetic tests are available in reference laboratories, diagnosis may be established more rapidly, especially if specific functional tests are not readily available. This strategy cannot be implemented in all countries if causative mutations are not consistent or vary within a country. For example, mutation Trp187Arg exists almost exclusively in southeast Iran, where the largest global population of patients with FXIIID has been identified; performing targeted genetic analysis after a positive CST greatly improved identification of affected cases in this area.[2] In geographic regions with a known elevated incidence of FXIIID, and particularly among families with affected homozygous FXIIID individuals, the targeted diagnosis and appropriate management of heterozygous carriers may be prudent in the context of premarital or family planning counseling, planned invasive procedures, bleeding symptoms, miscarriage, or menorrhagia.[7-9]

The establishment of a national reference laboratory for analysis is ideal in countries with less well-equipped local coagulation laboratories. Countries with a wide variety of causative mutations do not lend themselves to use of targeted genetic analysis, and hence confirmation of FXIIID through specific FXIII activity assays in a central, national, or international laboratory is required. Access to a reference laboratory that can perform FXIII activity assays is crucial for the diagnosis of symptomatic milder FXIIID and heterozygous carriers, who cannot be identified using CSTs alone. In such areas, the CST quality should also be optimized through use of two simultaneous different CST methods. Therefore, an algorithm for diagnosis of FXIIID reflecting a variety of approaches based upon local coagulation laboratory availability and capability for FXIII assays is proposed (Figure 2).

The first step in the diagnostic approach to FXIIID requires detailed patient and family history, and a clinical evaluation, with attention to bleeding episodes typical of the disorder. In suspected cases, the proposed algorithm may be utilized. There exist limitations to existing quantitative FXIII activity assays, including the most commonly utilized ammonia release assay, even in well-equipped laboratories, most specifically in the low activity ranges; the use of a plasma blank minimizes this effect and allows differentiation of severe cases. Less well-equipped laboratories, without the capacity to perform quantitative assays, may use CST in conjunction with country-specific centralized targeted mutation analysis or FXIII activity measurement to ensure timely diagnosis. Using this combination of approaches may result in improved diagnostic capacity and case identification.

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CONFLICT OF INTEREST

M. Karimi, M. Naderi, and A. Shapiro stated that they have no interests which might be perceived as posing a conflict or bias. F. Peyvandi has received consulting fees from Freeline, Kedrion Biopharma, LFB, and Octapharma and speaker fees from Ablynx, Bayer, Grifols, Novo Nordisk, and Sobi and has been member of advisory boards organized by Ablynx and F. Hoffmann-La Roche Ltd.

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