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CASE REPORT

Autoimmune factor XIII deficiency with unusual laboratory and clinical phenotype

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Abstract

Hemorrhagic diathesis due to anti-factor XIII (FXIII) autoantibody is a rare but severe disorder. Challenges of the diagnosis and treatment is demonstrated by the case of a 67-year-old female without previous bleeding history, who suffered a huge muscular hematoma. Without blank subtraction 18% plasma FXIII activity was measured; however, after correction for blank the activity was below the limit of detection and the lack of fibrin cross-linking in the patient's plasma confirmed the latter result. FXIII-A_2 antigen was not detectable by enzyme-linked immunosorbent assay (ELISA); however, it was well detected by western blotting. The autoantibody showed high affinity toward FXIII-A_2. Its considerable inhibitory activity was demonstrated by high titer in Bethesda units and the low immunoglobulin G concentration required for inhibition. The main biochemical effect was the inhibition of Ca^{2+}-induced activation. Eradication therapy was only partially successful. Four months after the last hemorrhagic event the patient suffered deep vein thrombosis complicated by pulmonary embolism.

KEYWORDS

autoimmune disease, blood coagulation, factor XIII, factor XIII deficiency, hemorrhagic disorder

Blood coagulation factor XIII (FXIII) plays a key role in the final step of coagulation cascade. It is of tetrameric structure consisting of two potentially active A subunits and two protective/inhibitory B subunits (FXIII-A_2B_2). A sequential activation by thrombin and Ca^{2+} is needed for its transformation into an active transglutaminase (TG). Thrombin cleaves off an activation peptide from FXIII-A then in the presence of Ca^{2+} FXIII-B_2 dissociates and FXIII-A assumes an enzymatically active configuration (FXIII-A*; FXIIIa).[1] The main task of FXIIIa is to cross-link fibrin γ-, and α-chains and to attach α_2-plasmin inhibitor to fibrin through ε(γ-glutamyl)lysyl isopeptide bonds. This way FXIIIa mechanically stabilizes the fibrin clot and protects it from fibrinolytic degradation. In addition, FXIII is essential for carrying out pregnancy, it is involved in wound healing and angiogenesis, and very likely it might also be implicated in several other cellular functions.[2]

The severe bleeding diathesis of patients with inherited FXIII-A deficiency clearly indicates the importance of FXIII in maintaining hemostasis.[3] In the general population FXIII-A deficiency is among the rarest inherited coagulation disorder (one in two million), but in countries with a high frequency of consanguineous marriages,

Boglárka Hurják and Julien Bovet contributed equally to this work.

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particularly if it is combined with a founder mutation, the frequency is much higher.[4] Autoantibodies formed against either of the FXIII subunits also result in severe, frequently life-threatening hemorrhagic complications with a mortality rate around 25%.[5] It is also a rare condition, in a most recent review 48 well-established published cases, 47 with anti-FXIII-A and 1 with anti-FXIII-B antibody, were collected.[6] The autoantibody might be related to autoimmune disease; however, particularly in elderly patients it is frequently idiopathic. The autoantibody might interfere with the activation of FXIII, might inhibit the TG activity of FXIIIa and by forming immune-complex with the protein might accelerate its clearance from the circulation. A classification of anti-FXIII antibodies based on the above criteria has been proposed.[6] The classical method used for the diagnosis and for measuring the inhibitory strength of anti-FXIII-A autoantibodies is based on Bethesda-Nijmegen assay.[7] We proposed to supplement this assay by the determination of the patient's immunoglobulin G (IgG) concentration required for 50% inhibition of FXIII activation/activity and by the determination of the binding affinity between FXIII-A and the patient's IgG.[6]

In the present study, a patient with anti-FXIII-A demonstrating unusual laboratory and clinical features was investigated. The results allowed us to point out difficulties in the diagnostic process and to test the recommended novel approach to the antibody characterization with the aim of introducing these techniques into laboratory practice. The described unusual clinical complication could draw clinicians' attention for such a possibility.

A 67-year-old female, during an intended brief hospitalization for cortisone injection in her osteoarthritic knees, was accidentally hurt and huge hematomas developed at the posterior side of both thighs. No previous history of spontaneous bleeding and post-surgical hemorrhagic complication were recorded. Despite eight transfusions of red blood cells hemoglobin concentration remained low. She was hospitalized for 45 days with several misdiagnoses. After this period, medical consultation at the Hemophilia Care Center, University Hospital of Dijon suspected FXIII deficiency and 17% FXIII activity was measured using the Berichrom assay (Dade Behring) without blank compensation. As such an extent of FXIII deficiency does not explain the severity of bleeding,[8] FXIII activity measurement was repeated by the ammonia release assay without and with blank compensation[9] (Technochrom FXIII assay: Technoclone, Vienna, Austria). Correction for blank revealed that the real FXIII activity was below the limit of detection (Figure 1A). Such undetectable FXIII activity was confirmed by the complete lack of fibrin cross-linking in the clot of the patient plasma (Figure 1A). No mutation was found in the *FXIIIA1* gene by bidirectional sequencing of exons and flanking intronic regions. The presence of inhibitory anti-FXIII autoantibody was revealed by mixing study and 74 Bethesda unit (BU) was measured by Bethesda-Nijmegen assay.[7] No FXIII-A_2B_2 and FXIII-A_2 antigen were detected in the patient's plasma by enzyme-linked immunosorbent assay (ELISA),[10,11] while FXIII-B antigen was in the reference interval (Figure 1B). However, the fact that a considerable amount of FXIII-A was detected in the plasma by western blotting suggested that the autoantibody interfered with the binding of monoclonal anti-FXIII-A antibodies used in the ELISAs (Figure 1B).

Essentials

- The diagnosis and treatment of factor XIII (FXIII) deficiency due to anti-FXIII autoantibody is challenging.
- Blank correction of FXIII activity and excluding interference with immunoassays are important.
- Binding assay and half maximal inhibitory concentration determination are important for complete evaluation.
- Anti-FXIII autoantibody does not provide protection against thromboembolism.

Most recently, further techniques were proposed for the more precise characterization of anti-factor autoantibodies in general, and anti-FXIII autoantibodies in particular.[6] In our case the affinity of the autoantibody to recombinant FXIII-A_2 (a kind gift of Dr E Olsen, Novo Nordisk, Måløv, Denmark) was determined by surface plasmon resonance (SPR) using Biacore 3000 instrument (GE Healthcare, Little Chalfont, UK; Figure 1C). As expected the antibody showed high affinity toward FXIII-A_2 with a K_D of $2.77 \pm 0.66 \times 10^{-9}$ mol/L.

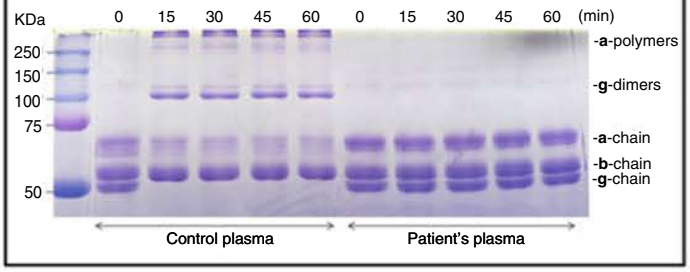
We also determined 50% inhibitory concentration (IC_{50}) of the patient's IgG, which, in our opinion, is a more accurate measure of the autoantibody's inhibitory power than the Bethesda unit. Fifty percent inhibition was achieved at 74 ± 8.6 μg/mL patient's IgG concentration (Figure 1D), while normal IgG, even in the highest concentration, had no effect on FXIII activity.

The autoantibody might interfere with the cleavage of FXIII-A by thrombin, with the Ca^{2+} induced structural changes and with the transglutaminase activity of FXIIIa. It may also exert a combined effect. To properly classify the inhibitory effect of the autoantibody we tested these possibilities separately. The effect of the patient's IgG on the proteolytic cleavage of the FXIII-A by thrombin was studied by western blotting. Comparison of the time course of FXIII-A truncation in the presence of normal and patient's IgG suggests that the removal of the activation peptide by thrombin was not prevented by the antibody (Figure S1A in supporting information).

We also tested the combined effect of patient's IgG on Ca^{2+} induced FXIII activation and on the activity of FXIIIa. In this set-up the patient's IgG exerted a close to total (92.3%) inhibition (Figure S1B). Finally, we investigated the effect of patient's IgG on fully activated FXIII. In this case the inhibition of FXIIIa by the patient's IgG was moderate: 44% of the transglutaminase activity was inhibited by 300 μg/mL IgG (Figure S1C) suggesting that the inhibition of transglutaminase activity only partially contributed to the combined inhibition of Ca^{2+} induced activation and FXIIIa activity. According to the proposed classification the neutralizing anti-FXIII-A autoantibody is of combined type (type IV).[6]

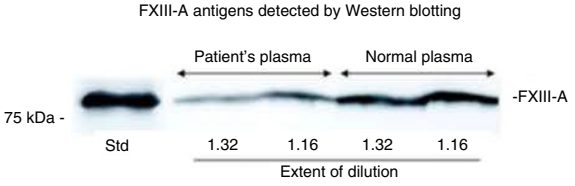
A

FXIII activity		
Berichrom®assay	Technochrom®assay	Technochrom®assay
Without blank correction		With blank correction
17%	18.5%	<1%

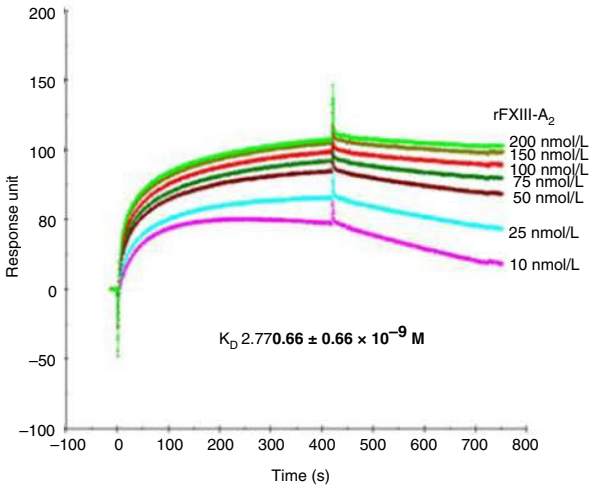


B

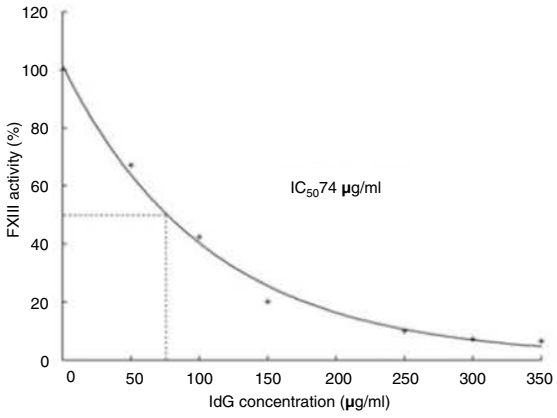
FXIII antigens measured by ELISA		
FXIII-A_2B_2 antigen	FXIII-A_2 antigen	FXIII-B_2 antigen
<0.5 %	<0.5 %	116%



C



D



E

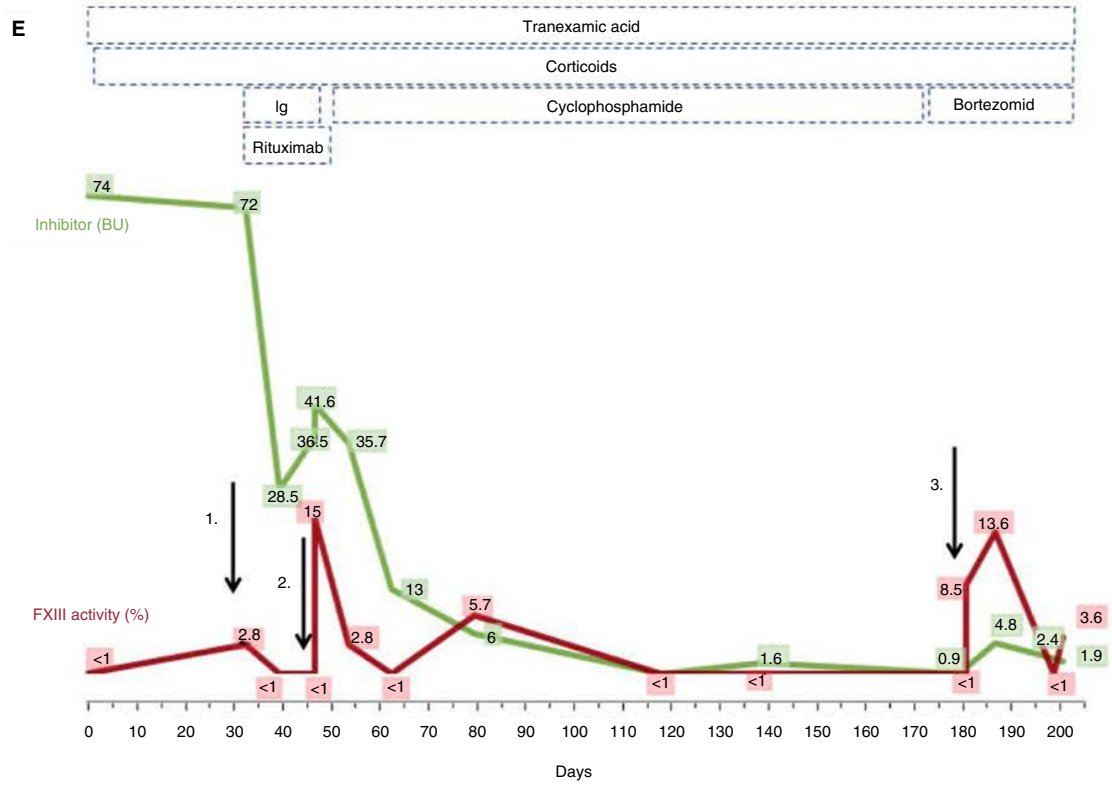


FIGURE 1 Autoantibody against factor XIII (FXIII) A subunit; its effects and binding characteristics. A, FXIII activity in the patient's plasma measured by the ammonia release assay with and without blank subtraction (upper part) and by fibrin cross-linking (lower part). The crosslinking of fibrin was evaluated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). In the patient's sample the crosslinking of fibrin γ-, and α-chains was completely blocked. B, FXIII antigens in the patient's plasma as determined by enzyme-linked immunosorbent assay (ELISA) and Western blotting. C, The binding affinity of the autoantibody to FXIII-A_2 as determined by surface plasmon resonance technique. D, Concentration dependent inhibition of FXIII activation/activity by the patient's immunoglobulin G (IgG). The IgG concentration required for 50% inhibition (IC_{50} value) was calculated. E, The time course of FXIII activity (in red) and the titer of inhibitory autoantibody (in green). The actual numerical values are shown above or below the lines. Arrow 1: hematoma of the right diaphragm pillar and the administration of 44 IU/kg Fibrogammin®. Arrow 2: left diaphragm pillar hematoma with a large rectus abdominis muscle hematoma, 22 IU/kg Fibrogammin® supplementation. Arrow 3: left femoral deep vein thrombosis complicated by pulmonary embolism. A filter was placed in the inferior vena cava under 22 IU/kg of Fibrogammin® protection

The clinical course, therapeutic modalities, changes in FXIII activity, and the inhibitor titer starting from the time of the diagnosis are shown in Figure 1E. The patient received tranexamic acid and corticoids throughout the observed period. She suffered major bleeding complications at two occasions, first right diaphragma pillar hematoma, then left diaphragma pillar hematoma together with a large rectus abdominis muscle hematoma. Supplementation with plasma derived FXIII concentrate (Fibrogammin®, CSL Behring) could not control the bleedings and only minor temporary elevation of FXIII activity was observed. The bleedings were finally stopped by embolization using an interventional radiological technique. After the first bleeding episode, an eradication strategy was initiated, which included Rituximab, immunoglobulin, and later cyclophosphamide or bortezomid. The eradication considerably decreased the inhibitor titer, but the inhibitor was never eliminated; its lowest titers were in the range of 0.9-4.0 BU and FXIII activity remained below the limit of detection. Unexpectedly, 4 months after the last hemorrhagic episode, she presented left femoral deep vein thrombosis complicated by pulmonary embolism. A filter was placed in the inferior vena cava due to contraindication of anticoagulant treatment. Evolution was favorable with a good recanalization. Thrombotic complications are a rarity in patients with anti-FXIII autoantibody; only a few cases have been reported,[12-15] and some of them were attributed to vein compression by a huge hematoma.[13,15] Similarly to our case two of the reported cases were also complicated by pulmonary embolism.[13,14] It is to be noted that, as in our case, relatively quick recanalization was reported, probably due to the enhanced lysis of non-crosslinked fibrin.[12,14]

The diagnosis and clinical management of acquired FXIII deficiency due to anti-FXIII-A autoantibody is rather challenging. The case presented here demonstrates that unexpected irregularities in the laboratory evaluation could make the diagnosis even more difficult and unforeseen clinical events might complicate the clinical course. From the case presentation the following conclusions can be drawn: (a) FXIII activity measurement without blank compensation might be seriously misleading in grading the severity of the deficiency. (b) The anti-FXIII-A autoantibody might interfere with the antibody used in the immunoassay resulting in gross underestimation of the FXIII-A_2 and FXIII-A_2B_2 antigen levels. (c) In addition to the Nijmegen-Bethesda assay, determination of IC_{50} and the dissociation constant are also useful for proper autoantibody characterization. (d) Identification of the mechanism by which the autoantibody interferes with the activation/activity of FXIII is required for proper classification. (e) The inhibitory anti-FXIII-A autoantibody does not protect the patient from thromboembolic complications.

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CONFLICTS OF INTEREST

The authors state that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

JB and ED conducted the clinical evaluation, monitoring and treatment of the patient; BH performed FXIII activity measurements, IC50 determination, SDS PAGE and western blotting experiments; É.K. measured FXIII antigen levels; determination of dissociation constant was carried out by KP; LM designed and controlled the laboratory evaluation; the draft of the manuscript was written by LM; JB and BH were also involved in preparing the final version of the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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