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-A2B2). A sequential activation by thrombin and Ca2+ is needed for its transformation into an active transglutaminase (TG). Thrombin cleaves off an activation peptide from FXIII-A then in the presence of Ca2+ FXIII-B2 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 α2-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, the incidence is significantly higher.

**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-A2 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-A2. 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 Ca2+-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

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The severe bleeding diathesis of patients with inherited FXIII-A deficiency clearly indicates the importance of FXIII in maintaining hemostasis. 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,
were collected. 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 FXIIla 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. The classical method used for the diagnosis and for measuring the inhibitory strength of anti-FXIII-A autoantibodies is based on Bethesda-Nijmegen assay. 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.

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 severe transfusions of red blood cells hemoglobin concentration. At this time, the patient had no symptoms of bleeding. Eight transfusions of red blood cells hemoglobin concentration remained low. She was hospitalized for 45 days with severe transfusions of red blood cells hemoglobin concentration. At this time, the patient had no symptoms of bleeding.

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, FXIII activity measurement was repeated by the ammonia release assay without and with blank compensation. As such an extent of FXIII deficiency does not explain the severity of bleeding, FXIII activity measurement was repeated by the ammonia release assay without and with blank compensation.

The autoantibody might interfere with the cleavage of FXIII-A by thrombin, with the Ca²⁺ 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. In particular, in our case the affinity of the autoantibody to recombinant FXIII-A₂ (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-A₂ with a KD of 2.77 ± 0.66 × 10⁻⁹ mol/L.

We also determined 50% inhibitory concentration (IC₅₀) 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 and FXIII-B. For each of the autoantibody we tested these possibilities separately. In particular, in our case the affinity of the autoantibody to recombinant FXIII-A₂ (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-A₂ with a KD of 2.77 ± 0.66 × 10⁻⁹ mol/L.

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A

<table>
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<tr>
<th>FXIII activity</th>
<th>Berichrom/assay</th>
<th>Technochrom/assay</th>
<th>Technochrom/assay</th>
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<tbody>
<tr>
<td></td>
<td>Without blank correction</td>
<td>With blank correction</td>
<td></td>
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<tr>
<td>FXIII activity</td>
<td>17%</td>
<td>18.5%</td>
<td>&lt;1%</td>
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B

<table>
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<tr>
<th>FXIII antigens measured by ELISA</th>
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<tbody>
<tr>
<td>FXIII-A2B2 antigen</td>
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<td>&lt;0.5%</td>
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C

D

E

fxIII activity (%) vs Days

Inhibitor (BU)

Tranexamic acid

Corticoids

Rituximab

Bortezomib

IC50 74 µg/ml

Kd 2.770.66 ± 0.66 x 10^-9 M
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 bortezomib. 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, and some of them were attributed to vein compression by a huge hematoma. Similarly to our case two of the reported cases were also complicated by pulmonary embolism. 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.

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-A₂ and FXIII-A₂B₂₂ antigen levels. (c) In addition to the Nijmegen-Bethesda assay, determination of IC₅₀ and the dissociation constant are also useful for proper autoantibody characterization. (d) Identification of the mechanism by which the autoantibody interferes with the activation/}

**REFERENCES**


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