Congenital Factor XIII Deficiency With the Presence of Inhibitor: A Case Study

Serap Karaman, Emre Akkaya, MD,† Sema Genc,† Fuat Bilgili,‡ Alper S. Kendirci, MD,‡ Deniz Tugcu,* Aysegul Unuvar,* Zeynep Karakas,* Demet Demirkol,§ Zuhal Bayramoglu, MD,‖ and Beyhan Omer†*

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Summary: Coagulation factor XIII (FXIII) is a fibrin-stabilizing factor with additional roles in wound healing and interactions between the decidua and fetus. Congenital FXIII deficiency is rare bleeding disorder. Inhibitor development against FXIII in inherited FXIII deficency is also uncommon, but may cause severe, life-threatening bleeding. FXIII is the last step in the coagulation cascade with normal coagulation paramaters (PT, aPTT), the detection of inhibitor to FXIII is quite difficult. The treatment of inhibitor-positive congenital FXIII deficiency is challenging due to the lack of a role of by-pass agents such as FVII. The best known ways of treatment in these cases are the use of high-dose FXIII concentrates and immunosuppression. Herein, we report the management of postoperative bleeding diathesis in a patient with FXIII deficiency who developed inhibitors, and to follow the clinical course of the disease with FXIII concentrate and immunosuppression.

Key Words: congenital FXIII deficiency, laboratory diagnosis, inhibitor treatment

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Factor XIII (FXIII) is crucial in the formation of cross-linked fibrin clots and is the last enzyme in the clotting cascade. It is a protransglutaminase that consists of 2 A subunits and 2 B subunits.[1] The A subunit includes a catalytic site, and the B subunit functions as a carrier protein, which facilitates the cross-linking of fibrin monomers by leading to the formation of the complex between the proenzyme form of FXIII, fibrinogen, and activator thrombin.[2] FXIII-A is also produced by monocytes, macrophages, megakaryocyte/platelets, chondrocytes, and osteoblast/osteocyte lineages.[3,4]

FXIII deficiency causes a rare type of bleeding disorder, and can be congenital or acquired. Congenital FXIII deficiency is mostly due to a mutation of genes encoding A and B subunits.[5] Patients with FXIII-A deficiency have life-long bleeding diathesis, and the severity and the frequency of bleeds are directly related with residual FXIII activity.[6,7] However, severe FXIII-B deficiency is a less common form, and FXIII-A antigen concentrations are also low in these patients and the bleeding type complies with mild-to-moderate FXIII deficiency.[2,8] The classification of the severity of bleeding is as follows: grade 1 bleeding occurs after trauma or antiplatelet or anticoagulant therapy, grade 2 represents minor bleeds, and grade 3 occurs with spontaneous major bleeds with undetectable plasma FXIII level.[9] Nevertheless, the acquired form of FXIII deficiency occurs due to inhibition of FXIII activation/activity or acceleration of the elimination of FXIII from the circulation because of the autoimmune or neoplastic diseases such as leukemias, severe liver disease, inflammatory bowel disease, and systemic lupus erythematosus.[10–12] FXIII also has some other roles in wound healing, bone extracellular matrix stabilization, and the interaction between the embryo and decidua of the uterus.[13,14]

In this study, we aimed to present the management of postoperative bleeding diathesis in a patient with FXIII deficiency who developed inhibitors, and to follow the clinical course of the disease with FXIII concentrate and immunosuppressive therapy.

CASE PRESENTATION

An 8-year-old boy with FXIII deficiency was admitted to the orthopedics clinic with severe pain and progressive decrease in the range of motions of bilateral hip joints due to bilateral dysplasia. Bilateral osteotomy was planned. During follow-up for cerebral palsy, epilepsy, and hydrocephaly with VP-shunt, he was first referred to the hematology clinic at the age of 3 years for the investigation of coagulopathy due to a history of subdural and intracranial bleeding on the fifth postnatal day. The patient was diagnosed as having congenital FXIII deficency. The FXIII level was measured using an FXIII chromogenic assay from Siemens and was reported as 9%. During the patient's follow-up, he underwent some minor interventions and procedures such as circumcision, orcihopexy, and small interventions for muscle spasity. FXIII concentrate plasma-derived was administered before each operation and only tranexamic acid and local fibrin-sealant were used for these interventions with no significant bleeding. However, the frequency of gingival bleeding, epistaxis, and infections had increased in last 6 months. The day before surgery, a FXIII concentrate 500 U (20 U/kg) with tranexamic acid (10 mg/kg, dose x3/d) was administered. On the day of surgery, a 500 U FXIII infusion was administered again, and the surgery was completed without any complications. Before surgery, the patient's hemoglobin (Hb) concentration (13.4 g/dL), hematocrit (Htc) (40.2%), white blood cells (WBC) (7.7×103), prothrombin time (PT) (11.37 s), and activated partial thromboplastin time (aPTT) (21.9 s) were in normal ranges. FXIII activity was 15.5% (70 to 140), the inhibitor level against FXIII was negative, and C-reactive protein concentration was 1.76 mg/L (0 to 5).

After surgery, the Hb and Htc values decreased rapidly to 7.7 g/dL and 22.4%, respectively, and the platelet count was 134,000/μL. Bleeding from operation lodge and incision sites was detected (Fig. 1). There were no major vascular leakages observed in imaging. The bleeding was persistent, so FXIII concentrate was administered at 1250 U for the first day, and 500 U in the following 5 days, and for vascular stabilization, corticosteroid therapy (2 mg/kg/d) was started. After the infusion of the concentrate, the factor level was found as 10%, the PT and aPTT concentrations were still within reference

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From the Departments of *Pediatric Hematology-Oncology; †Biochemistry; ‡Orthopedics and Traumatology; §Child Intensive Care Unit; and ‖Pediatric Radiology, Faculty of Medicine, Istanbul University, Istanbul, Turkey.
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Reprints: Sema Genc, Department of Biochemistry, Istanbul Faculty of Medicine, Capa, Istanbul 34390, Turkey (e-mail: nsgenc@hotmail.com).
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FIGURE 1. Bleeding from operation lodge.

ranges, and the fibrinogen level was 483 mg/dL. However, the C-reactive protein concentration rose to 96.2 mg/L on the day of surgery, and increased to 198 mg/L on the second day. He had high fever and cough and was diagnosed as having pneumonia, and antibiotherapy was started. In the sixth day after surgery, the FXIII level decreased to 7.3%, and the D-dimer level was 947.3 µg/L (normal level, <550 µg/L), the mixing test was positive for inhibitor with a titration of 8 Bethesda units (BU). Because FXIII concentrate was obtained with special permission from Turkish Health Authorities from European countries, the following 20 days, the cryoprecipitate infusion and replacement therapy with erythrocyte suspension on demand basis were given due to the uncontrolled bleeding. The bleeding was not controlled on the fifth day of the cryoprecipitate therapy; therefore, 1250 U FXIII was administered again. Corticosteroid therapy was pursued with IVIG (2 g/kg-total dose) for immunosuppression to eradicate inhibitor because ~3 weeks are required to ensure factor concentrate. Bleeding continued for 2 weeks with the risk of developing compartment syndrome. Even though the inhibitor titer was decreased to 5 BU, anti-CD 20 monoclonal antibody (rituximab) was added to treatment (375 mg/m^2/wk). After the first week of anti-CD20 monoclonal antibody (postoperative third week), the bleeding and transfusion requirement of the patient were slightly decreased. The infusion of high-dose FXIII concentrate (1250 U) was given for the treatment of inhibitor positivity as soon as the preparation was obtained. During immunotherapy, 1250 U FXIII concentrates were administered once a week for 2 weeks. After the fourth dose of the rituximab therapy, the inhibitor was still positive with a titration of 1.4 BU. Accordingly, another immunosuppressive agent, sirolimus was added to treatment (0.8 mg/m^2, dose x2/d) with a high-dose FXIII concentrate (1250 U), and rituximab and corticosteroid were stopped. The inhibitor titer was negative on 10th day of the sirolimus therapy and FXIII activity rose to 41.9% (Fig. 2). Trimetoprim-sulfometaxasole was used for the prevention of Pneumocystis pneumonia infection and IVIG due to the long period of immunsupression. On the seventh day of the sirolimus treatment, he had high fever and vomiting, and Leifsonia aquatica was detected in the blood and catheter cultures; meronem and vancomycine were started. After meronem and vancomycine therapy, the blood culture was sterile. However, on the 19th day of the sirolimus therapy, respiratory distress developed and the patient was immediately transferred to the pediatric intensive care unit. Despite of all supportive therapy, he died of acute respiratory distress syndrome(Fig. 3).

During the monitoring of the patient, laboratory analysis was performed as follows: complete blood counts were analyzed using a LH 780 hematology analyzer from Beckman Coulter. All coagulation tests including PT, aPTT, D-dimer, and fibrinogen levels were measured using a BCS-XP device from Siemens. FXIII activity was measured using a CS-2500 coagulation analyzer from Sysmex, based on an ammonia release assay during the transglutaminase reaction of FXIII. Released ammonia is measured photometrically via an NADH-dependent reaction.

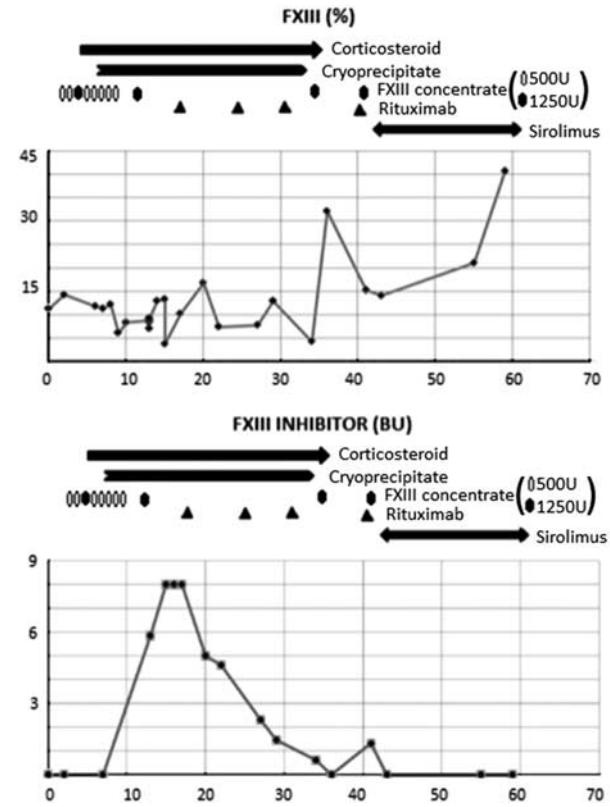


FIGURE 2. Alterations of FXIII activity (%) and FXIII inhibitor level (BU) over the course of patient's hospital admission. BU indicates Bethesda units; FXIII, factor XIII.

The mixing test is performed to screen the presence of inhibitor. In the mixing test, the patient's plasma is mixed with a similar volume of normal plasma with 100% FXIII activity (1:1). Correction of the result suggests FXIII deficiency, whereas nonrecovered results indicate the presence of inhibitor.

Most laboratories use a classic Bethesda assay to quantify inhibitory antibody. In this method, the 1:1 mixture of normal and FXIII deficient plasma is compared with the activity of a mixture of normal and patient's plasma. A various dilutions of patient's plasma were used for the determination of antibody titer. The presence of the FXIII inhibitors should be considered if the FXIII



FIGURE 3. Widespread inflamation in the lungs.

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activity of the patient plasma plus NPP is less than 10% of the FXIII activity of the normal and factor insufficient plasma mixture.[15,16] Inhibitor detection was also analyzed through the incubation of patient plasma at 56°C for 30 minutes.[17]

DISCUSSION

Congenital FXIII deficiency can manifest at any age, but mostly diagnosis is made in infancy. However, acquired FXIII deficiency can occur due to the neutralizing inhibitors against FXIII. In a systematic review, Franchini et al[18] reported that most inhibitor-positive cases were seen in the elderly, and slightly more frequently in women than in men.

Although clinical findings suggest FXIII deficiency, laboratory diagnosis is very important. For laboratory diagnosis, a qualitative method that investigates the solubility of fibrin clots in concentrated urea, acetic acid, and monochloracetic acid has been used traditionally. However, this method is not standardized and the sensitivity depends on the fibrinogen, thrombin, and Ca+2 levels, and only severe FXIII deficiency can be detected using qualitative methods.[9]

A quantitative functional FXIII activity assay is used as a first-line screening test, which depends on the measurement of ammonia released by transglutaminase, or the determination of amine-incorporated protein substrate. However, studies have reported that ammonia release and NADH-consuming reactions independent of FXIIIa activity may cause overestimation of FXIII activity. The subtraction of a plasma blank is recommended to avoid overestimation of FXIII activity.[19–21] In our study, the lack of a plasma blank in the Berichrom reagent used for the measurement of FXIII activity may have resulted in low sensitivity of this measurement and cause the most important limitation of our study. Therefore, measurement of FXIII activity is reported to alter between 3% and 10%, which is particularly important in the diagnosis of severe cases and varies depending on the instrument used.[22] In our case, all FXIII activity measurements were performed using the same method. The quality control of FXIII results were ensured by the establishment of both internal and external quality control studies (EQA). Although reproducibility studies were low at normal, low levels, the EQA were also acceptable ranges (SDIs were between 0.4 and 1.0 at both levels).

In our case, after hip surgery, the bleeding was not controlled despite the use of FXIII concentrate. The development of inhibitors against FXIII is suggested. FXIII inhibitor was detected in the mixing study and a classic Bethesda assay was performed to quantify inhibitory antibodies. The inhibitor titer was also studied using the deactivation method of Naderi et al,[17] and after deactivation, the FXIII level increased. Although neutralizing antibodies against either FXIII-A or FXIII-B subunits may inhibit FXIII activity, non-neutralizing antibodies may create immune complexes with FXIII-A and B subunits and expedite the removal of FXIII from circulation.[23] In our case, although a high FXIII concentrate was administrated to the patient, low FXIII activity may be the reason of the increased clearance of factor from circulation.

There are several methods used for the evaluation of the interaction between FXIII antibodies and FXIII-A and FXIII-B subunits. Quchterlony gel diffusion, enzyme-linked innunosorbent assay (ELISA), and immunochromatographic assays are but a few.[24,25] In previous studies, only 4 cases with antibodies against FXIII have been reported, in one against the FXIII-B subunit and in another against the FXIII-A subunit.[26–29] Only 2 of these cases were congenital FXIII deficiency.[28,29] Also, in 2 cases, FXIII deficieny was diagnosed at the age of 3 years as in our case, and FXIII activities varied between 1% and 10%, similar to our findings.[28,29] However, the FXIII antigen levels of these cases were between 0.1% and 2.0%, unfortunately, neither antigen level nor binding/neutralizing test were performed in our case.

The treatment of FXIII deficiency in a patient who is inhibitor-positive is highly difficult. The purpose of inhibitor treatment is to control bleeding and to remove inhibitors from the circulation. A variety of treatment options have been using such as plasmapheresis, immunoadsorption, and immunosupresive therapy.[11,24] Recent studies have shown that immunosuppression using cyclophosphamide and/or corticosteroid treatment reduced the inhibitor titer and restored FXIII activity.[11,29] There are studies showing reduced levels of FXIII inhibitor and clinical improvement in response to infusion of immunoglobulins and antiCD20 antibody.[11,25,29] In our case, corticosteroid therapy and intravenous immunoglobulin were primarily used for immunosuppression. Two weeks after the operation, despite the inhibitor titer being decreased to 5 BU, rituximab (anti-CD 20 monoclonal antibody) was added to treatment due to bleeding. After immunosuppresion plus the fourth rituximab therapy, the FXIII inhibitor was still positive with a titration of 1.4 BU. Accordingly, another immunosuppressive agent, sirolimus, was added to treatment with the FXIII concentrate. Inhibitor was negative after 10th day of sirolimus treatment and the FXIII level rose to 41.9%. Success associated with the use of anti-CD 20 monoclonal antibody and sirolimus have also been demonstrated, in accordance with our findings.[11,29,30]

In conclusion, although FXIII deficiency is a rare bleeding disorder, precise and timely detection of inhibitors against FXIII is important for the timely initiation of appropriate treatment to remove inhibitors from the circulation to control bleeding and improve patient prognosis. In this respect, coagulation laboratories have important responsibilities for the examination of factor activity and inhibitor levels using highly accurate and precise methods in the contribution to patient treatment.

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