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Challenges in Diagnosis and Management of Acquired Factor XIII (FXIII) Inhibitors

Joan D. Beckman*, Raj S. Kasthuri*, Alisa S. Wolberg†, and Alice D. Ma*

*Department of Medicine, Division of Hematology/Oncology, University of North Carolina at Chapel Hill, Chapel Hill, United States

†Department of Pathology and Laboratory Medicine, University of North Carolina at Chapel Hill, Chapel Hill, United States

Factor XIII (FXIII) is a thrombin-activated protransglutaminase that crosslinks fibrin α - and γ -chains and antifibrinolytic substrates to the fibrin network to improve clot mechanical and biochemical stability.[1] FXIII consists of two A subunits and two carrier B subunits. With an incidence of 1 per 2–3 million individuals, congenital FXIII deficiency, due to insufficient A or B subunits, is a rare disorder that manifests with soft-tissue bleeding and increased risk for spontaneous intracranial bleeding.[2] Acquired FXIII deficiency, due to anti-FXIII-A or anti-FXIII-B antibodies, is rarer still, with 93 cases reported in the largest systematic review.[3] However, due to diagnostic test variability and underreporting, the actual incidence of both congenital and acquired FXIII deficiency may be greater.[4]

Acquired FXIII deficiency manifests when anti-FXIII antibodies decrease FXIII activity by neutralizing activated FXIII, increasing FXIII clearance, or interfering with FXIII binding to fibrin.[1, 5] Eliminating anti-FXIII antibodies is challenging, and often requires multiple prolonged courses of immunosuppression.[5] However, prolonged immunosuppression places patients at risk for severe complications and death. Furthermore, despite therapy, patients often experience refractory bleeding, which can be difficult to treat due to paucity of guidelines and lack of real-time laboratory monitoring.

This letter details the challenges encountered during treatment of acquired FXIII deficiency with refractory bleeding. Specifically, we discuss the limitations of available diagnostic tests in predicting response to therapy. Furthermore, highlighting potential strategies to predict FXIII therapeutic response, we describe correlation between clinical tests and an

Corresponding Author: Alice D. Ma, Department of Medicine, Division of Hematology/Oncology, 170 Manning Drive, CB 7305, Chapel Hill, NC 27599-7305, alice_ma@med.unc.edu.

Authorship Contributions

JDB designed and performed experiments, analyzed results, and wrote the manuscript. RSK analyzed results and wrote the manuscript. ASW supervised experiments and data analysis and wrote the manuscript. ADM analyzed results and wrote the manuscript.

Disclosures of Conflicts of Interest

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experimental whole blood clot contraction assay. Finally, we describe successful FXIII inhibitor eradication using mycophenolate mofetil (MMF). Collectively, this report illustrates the necessity of close collaboration between coagulation laboratories and clinicians in diagnosis and management of rare bleeding conditions.

An eighty-four year old male with past medical history of oral lichen planus, chronic bronchitis, hypothyroidism, hypertension and heart disease was transferred to our facility due to refractory bleeding (Figure 1a). Three months prior, he presented with a spontaneous large right posterior leg hematoma treated with packed red blood cells (pRBC) transfusion and vessel cauterization. He re-presented several weeks later with hypotension, worsening anemia, gross hematuria, and an enlarging right posterior scapular hematoma (Figure 1b). Primary evaluation revealed normal PT/INR, aPTT, fibrinogen, and plasma coagulation factors. A FXIII activity was sent to a reference lab (Esoterix, Phoenix, AZ) and returned with a FXIII activity of <5% (normal range 50–150%). An anti-FXIII antibody with a titer of 1:40 was detected. For immunosuppression, the patient was started on steroids (30 mg prednisone twice daily) and received two doses of IVIg (1 gram kg⁻¹) plus four weekly doses of rituximab (375 mg m⁻²). However, despite immunosuppression and treatment with recombinant factor VIIa (5 mg every 8 hours), he continued to experience significant blood loss requiring ~22 units of pRBC.

On assuming care of the patient, urea clot lysis time was noted to be consistently <2 hours (Figure 1d). Repeat coagulation labs revealed low fibrinogen levels, which were most likely secondary to pRBC transfusions and readily corrected with 10 units cryoprecipitate. Despite treatment with cryoprecipitate, a one-time dose of 20 units kg⁻¹ plasma-derived FXIII concentrate, and continued immunosuppression (Figure 1a), the patient continued to have refractory bleeding (Figure 1c), undetectable FXIII activity (Figure 1d,e) and elevated anti-FXIII titers. Therefore, available strategies to manage the patient's FXIII inhibitor and refractory bleeding were critically assessed.

As FXIII deficiency is rare, the United States does not have a FDA-approved clinical FXIII activity assay; therefore most clinical laboratories rely on referral testing or urea clot solubility testing. However, in the presence of mild-to-moderate FXIII activity, urea clot solubility testing is not a reliable predictor of treatment efficacy due to false-negative results. [4] FXIII activity assays are more sensitive to decreased FXIII activity, but send-out testing limits utility during real-time treatment monitoring. Furthermore, congenital FXIII patients with FXIII activity levels between 5–20% have variable bleeding rates [6] and acquired FXIII deficiency patients exhibit no correlation between residual FXIII activity and bleeding. [3] Consequentially, in FXIII inhibitor patients FXIII concentrate use is generally reserved for life-threatening bleeds with the frequency and dose of FXIII concentrates left to the discretion of physicians who lack readily available and reliable laboratory testing.

Due to our patient having refractory bleeding, we initiated bi-weekly plasma-derived FXIII concentrates at 40 unit kg⁻¹. Given that urea clot lysis time and FXIII activity were available, we evaluated the ability of the selected dosing strategy to normalize the real-time (urea clot lysis time) and referral (FXIII activity) laboratory values. Post-FXIII infusion urea clot lysis times improved (Figure 1d), as did FXIII activity (Figure 1e). Importantly, after

starting bi-weekly FXIII concentrate, the patient's hemoglobin stabilized and his clinical status improved (Figure 1c).

As FXIII is essential for whole blood RBC clot retention [7, 8], we also performed an experimental whole blood clot contraction assay to assess if *ex vivo* FXIII concentrates could improve and/or normalize whole blood clot composition. Briefly, phlebotomy was performed on consenting subjects in accordance with the Declaration of Helsinki and the University of North Carolina Institutional Review Board into 0.105 M sodium citrate, pH 5.5 (10% v/v, final concentration). The citrated whole blood was pretreated for 30 minutes at 37°C with either buffer (20 mM N-2-hydroxyethylpiperazine-N'-2-euthanesulfonic acid [HEPES] pH 7.4, 150 mM NaCl), or plasma-derived FXIII concentrate (Corifact, CSL Bering, King of Prussia, PA, USA, at final concentration of 0.5, 1, and 2 IU mL⁻¹). Clotting was triggered in recalcified (10 mM, final) whole blood via addition of tissue factor (Innovin, diluted 1:12000, 1 pM, final, Dade Innovin, B4212-40 Siemens Healthcare, Erlangen, Germany). Clot contraction, carried out in triplicate, proceeded at 37°C for 120 minutes in siliconized multiwell plate. Retracted clots were removed and photographed; after clot removal, the percent RBCs in serum (%RBCs) was quantified using spectrophotometry to measure the serum absorbance at 575 nm compared to a standard curve of diluted whole blood as previously described.[7, 8] Compared to healthy blood, FXIII inhibitor blood formed clots that were loose and pliable; addition of FXIII concentrate to the FXIII inhibitor blood resulted in more firm, stiff clots (Figure 1f). Reflecting inhibition of FXIII, compared to healthy blood, FXIII inhibitor blood had increased % RBC in serum ($9.1 \pm 1.6\%$ versus $17 \pm 0.1\%$, mean $\pm$ standard deviation, Figure 1g). At the highest FXIII concentration tested (2.0 U mL⁻¹), addition of FXIII concentrates to the FXIII inhibitor blood reduced the %RBC in serum to levels comparable to healthy control ($17 \pm 0.1\%$ RBC untreated FXIII inhibitor blood versus $9.4 \pm 0.1\%$ RBC FXIII inhibitor blood + 2.0 U mL⁻¹ FXIII, Figure 1g), which suggests that 2.0 U mL⁻¹ FXIII may be sufficient to compete with the anti-FXIII antibodies present. Importantly, these data support the clinical pre- and post-FXIII infusion urea clot lysis time and FXIII activity levels and suggest that whole blood clot contraction assays are a potential option to predict effectiveness of FXIII treatment.

Despite achieving control of the refractory bleeding with bi-weekly FXIII infusions in our patient, FXIII inhibitor elimination remained an issue. Anti-FXIII antibodies are challenging to treat, with 27% of patients achieving a partial remission and 45% of patients achieving full remission; however, within one year 16% of FXIII inhibitor patients experience a relapse- leaving inhibitor-related bleeding a significant cause of mortality.[9] Prednisone, followed by cyclophosphamide and rituximab, is the most common form of immunosuppression.[3, 9] However, 8–37% of FXIII inhibitor patients treated with prolonged immunosuppression experience serious infections and/or death. [3, 9] One previous report treated a refractory FXIII inhibitor patient with MMF and achieved both a partial remission of the anti-FXIII inhibitor and cessation of spontaneous bleeding, suggesting MMF is a viable steroid-sparing option. [10]. Given that our patient was medically frail and unresponsive to steroids, MMF at 1000 mg twice daily was started. Due to GI intolerance, the dose was decreased to 500 mg twice daily. As bleeding stabilized, the patient was discharged to a skilled nursing facility on bi-weekly FXIII concentrate infusions

and MMF. Six months after discharge his hemoglobin remained stable and his FXIII inhibitor titer decreased, prompting a dose reduction in his bi-weekly FXIII infusions to 20 units kg⁻¹. At twelve months, the patient's pre-infusion FXIII activity normalized and FXIII infusions were stopped (Figure 1e); MMF therapy was discontinued after an additional six months. As of submission the patient has been off all therapies for >6 months without evidence of recurrence.

In conclusion, acquired FXIII deficiency is a challenging diagnosis with limited case series to guide therapy. This report highlights the limitations of available diagnostic tests in guiding and monitoring patient response to FXIII therapy. Furthermore, we report the novel observation that an experimental whole blood clot contraction assay and available clinical tests correlated to patient response to FXIII therapy. In addition to predicting sensitivity to FXIII treatment, the clot contraction assay might also be used to monitor patient response to FXIII treatment. Finally, we describe FXIII inhibitor eradication using MMF as a steroid-sparing agent. Collectively, this case illustrates the necessity of close collaboration between coagulation laboratories and clinicians in diagnosis and management of patients with rare bleeding conditions.

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Figure 1: Clinical and laboratory analysis for acquired FXIII inhibitor patient.

(A) Timeline of patient's treatment course. Note care between Sept 2015-Jan 2016 (bold line) occurred as inpatient. (B) CT scan demonstrating a $18.2 \times 7.6 \times 28.6$ cm fluid collection in the right posterior chest wall. (C) Hemoglobin values for patient. (D) Urea clot lysis time values from patient without FXIII (closed circle, solid line) and within 1–24 hours after FXIII infusion (open circle, dashed line). Normal value is 24 hours. (E) FXIII activity (%) from patient without FXIII (closed circle, solid line) and 1–24 hours after FXIII infusion (open circle, dashed line). (F) Whole blood was obtained from a healthy subject and the

FXIII inhibitor patient. Sample from the FXIII inhibitor patient was treated with FXIII-A₂B₂ for 30 minutes at 37°C following initiation of clot contraction with recalcification (10 mM, final) and tissue factor (1 pM, final). After 120 minutes, clots were removed and photographed. Bars in image relative to size of single well from a 96 multiwell plate. **(G)** Percent (%) RBC in serum after clot removal was quantitated by comparing absorbance at 575 nm of serum to standard curve of dilution whole blood. Values mean ± standard deviation for n=3 technical replicates per subject/condition.