



Characterization of anti-factor VIII antibody in a patient with acquired hemophilia A

Jisu Oh¹, Yeongmin Lim¹, Moon Ju Jang¹, Ji Young Huh², Midori Shima³, Doyeun Oh¹

Departments of ¹Internal Medicine, ²Laboratory Medicine, CHA University School of Medicine, Seongnam, Korea, ³Department of Pediatrics, Nara Medical University School of Medicine, Nara, Japan

p-ISSN 2287-979X / e-ISSN 2288-0011
<http://dx.doi.org/10.5045/br.2013.48.1.58>
Blood Res 2013;48:58-62.

Received on January 8, 2012
Revised on June 23, 2012
Accepted on February 4, 2013

Correspondence to

Doyeun Oh, M.D., Ph.D.
Midori Shima, M.D., Ph.D.
D.O., Department of Internal Medicine,
CHA Bundang Medical Center and CHA
University, Yatapro-59, Bundang-gu,
Seongnam 463-712, Korea
M.S., Department of Pediatrics, Nara
Medical University School of Medicine,
840 Shijo-cho, Kashihara, Nara 634-8522,
Japan
Tel: D.O., +82-31-780-5217
M.S., +81-74-422-3051
Fax: D.O., +82-31-780-5208
M.S., +81-74-424-9222
E-mail: D.O., doh@cha.ac.kr
M.S., mshima@naramed-u.ac.jp

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Abstract

Acquired hemophilia A (AHA) is a bleeding disorder caused by the development of an autoantibody against endogenous factor VIII (FVIII). In this study, the epitope of the autoantibody was identified in a 67-year-old female patient with AHA. A prolonged activated partial thromboplastin time (77.4 s) that failed to correct in an incubation mixing test (68.2 s), a decreased FVIII activity, and a high FVIII inhibitor (14.6 Bethesda units/mL) were observed. Enzyme-linked immunosorbent assay demonstrated that the antibody belonged to the immunoglobulin G4 subclass. An immunoblotting assay revealed the light chain (A3/C1/C2 domain) of FVIII as the binding region of the antibody. The bleeding experienced by our patient resulted from the interference of FVIII binding to both FIX by anti-A3 antibodies and phospholipids and von Willebrand factor by anti-C2 antibodies. To the best of our knowledge, this is the first study in Korea characterizing an autoantibody in the context of AHA.

Key Words Acquired hemophilia A, Factor VIII autoantibody, Epitope

INTRODUCTION

Acquired hemophilia A (AHA), a bleeding disorder that occurs in patients without a family history of hemophilia, is caused by the development of autoantibodies against endogenous factor VIII (FVIII). The incidence of AHA is 1.3–1.5 cases per million people per year [1]. Up to 85% of affected individuals are more than 60 years of age [2]. The age distribution of AHA is typically biphasic, with a small peak between 20 and 30 years of age and the major peak between 68 and 80 years of age [3]. In approximately 50% of AHA cases, FVIII autoantibodies are associated with the postpartum period, autoimmune diseases, underlying hematologic or solid cancers, infections, vaccination, or the use of certain medications [4–6]. Clinical manifestations of AHA

include bleeding at any location in the body; however, hemorrhage, the hallmark of congenital hemophilia, is uncommon in AHA. Diagnosis of AHA requires identifying hemophilia with inhibitors and excluding congenital hemophilia. The two major therapeutic strategies for AHA are the control of bleeding and the eradication of autoantibodies [7].

Antibodies against FVIII may either block the function of FVIII or promote the clearance of factor VIII from circulation [8, 9]. Identification of the FVIII epitopes to which inhibitor antibodies bind is crucial for understanding the mechanisms of inhibitor activity [9]. In this study, we attempted to identify the epitope of the autoantibody against FVIII in a patient with AHA.

CASE REPORT

A 67-year-old female, experiencing 12 h of acute abdominal and right flank pain, was admitted to our hospital through the emergency department. Her medical history was unremarkable, except for hypertension controlled with amlodipine. The patient had no family history of abnormal bleeding. One week prior to hospital admittance, she developed bruises on her left lower extremity and gross hematuria. She did not receive medical care, and these conditions resolved spontaneously.

Initial examination revealed pale conjunctiva and both soft-tissue swelling and bruising on her left lower extremity. Tenderness to palpation over the lower abdomen and rebound tenderness were noted. Her vital signs were within normal ranges. Complete blood cell count indicated hypochromic anemia with normal white blood cell counts (hemoglobin, 8.2 g/dL; hematocrit, 23.9%; white blood cell counts, $9.1 \times 10^9/L$; platelet counts, $342 \times 10^9/L$). The patient's prothrombin time (PT, 12.5 s; international normalized ratio [INR], 1.11) and thrombin time (TT, 14.3 s) were normal. Her activated partial thromboplastin time (APTT, 77.4 s) was prolonged and not corrected by incubation mixing test (APTT, 68.2 s).

Abdominal computed tomography scan demonstrated multifocal fluid collections throughout the abdomen and pelvis, localized mainly on the right side. An enlarged psoas muscle, intramuscular hematomas in the right iliopsoas, and a paravertebral muscle with active bleeding were noted. Multiple calcifications within the pancreas and atrophy of the pancreatic parenchyma suggested chronic pancreatitis. The spleen, liver, and gall bladder appeared normal. Angiography of the abdominal arteries demonstrated suspicious extravasation from a right lumbar artery. Gelfoam embolization of the right second and third lumbar arteries was performed. The patient was immediately treated with recombinant FVIIa (90 IU/kg every 6 to 8 h; NovoSeven) for 2 days because of active bleeding. The FVIII level was

2%, and the titer of FVIII inhibitor was 14.6 Bethesda units (BU)/mL. The levels of FIX (121%), FXI (90%), and FXII (94%) were all within normal ranges. The patient was treated with oral prednisolone (30 mg/d) and azathioprine (150 mg/d) to eradicate the autoantibody. The tests for anti-nuclear antibody, lupus anticoagulant, hepatitis B virus surface antigen, hepatitis C virus antibody, and human immunodeficiency virus antigen and antibody were all negative, with the exception of anti-Ro antibody (3+). After 2 days of immunosuppressive treatment, the patient's flank pain and hematuria resolved and, 2 weeks later, the hematoma disappeared. On the 45th hospital day, the patient was discharged on oral prednisolone (20 mg/d) and azathioprine

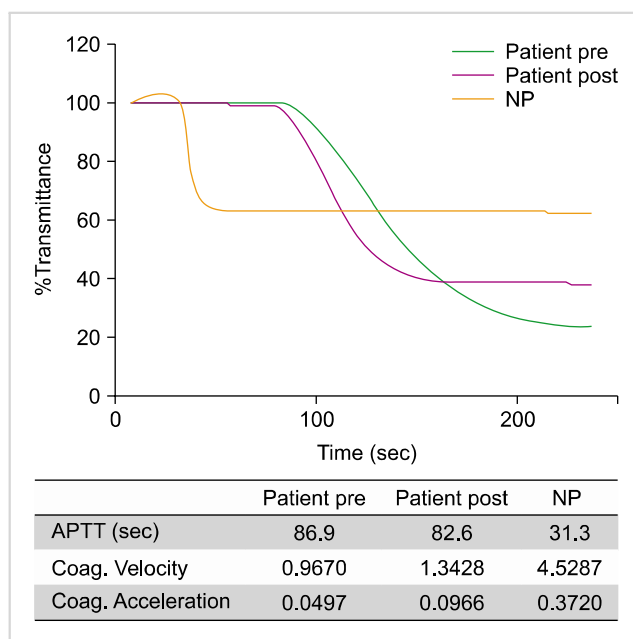


Fig. 2. The APTT waveform analysis of the patient's plasma. Minor hemostatic effect by infusion. Abbreviations: Patient Pre, Pre-treatment; Patient Post, Post-treatment; NP, normal pooled plasma; Coag, coagulation.

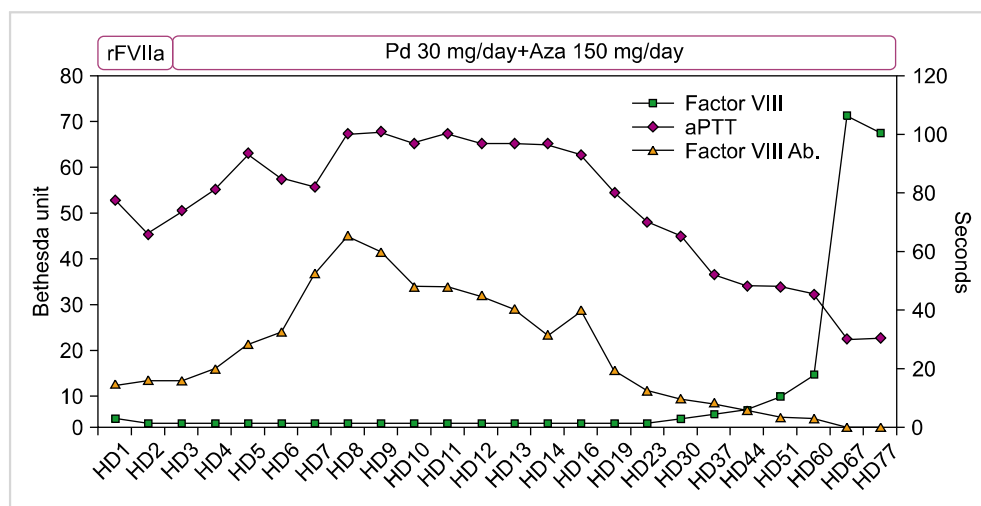


Fig. 1. Clinical course of our patient. This graph shows FVIII (%), aPTT (s), and FVIII Ab (BU, Bethesda unit) levels. Abbreviations: rFVIIa, recombinant activated factor VII; Pd, prednisolone; Aza, azathioprine; HD, hospital day.

(150 mg/d), without further complications. The prednisolone treatment was discontinued approximately 6 months later. The patient's APTT normalized; her FVIII level reached the upper 50%, and the inhibitor was undetectable (Fig. 1).

APTT waveform analysis (MDA series; Organon, Technika) of the patient's plasma showed a delayed clotting pattern compared to normal pooled plasma (Fig. 2). Thrombin generation assay (Thermo Fisher Scientific, Waltham, MA, USA) showed decreased production of thrombin in the patient's plasma compared to normal plasma. Virtually no hemostatic effect occurred after the infusion of plasma. Slight improvement in the waveform analysis, but not in the thrombin generation test, was noted (not shown). Enzyme-linked immunosorbent assay demonstrated that the patient's antibody belonged to the immunoglobulin (Ig) G4 subclass. Immunoblotting assays demonstrated that the light chain (A3/C1/C2 domain) of FVIII is the binding region of the autoantibody (Figs. 3, 4).

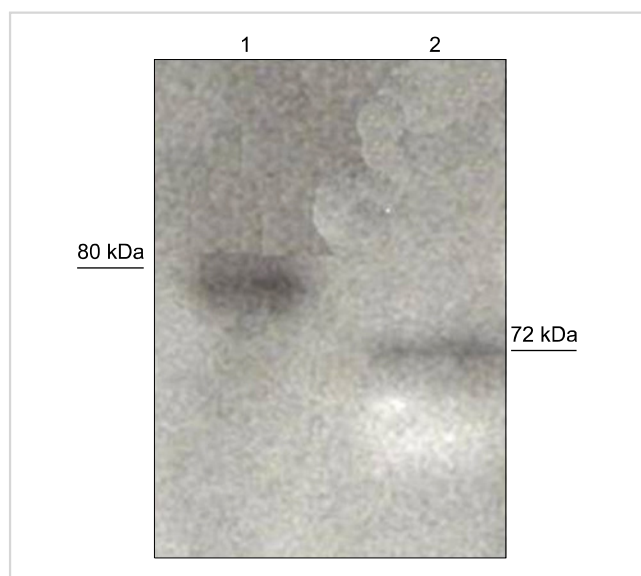


Fig. 3. Immunoblotting assay. Lane No.1 shows an 80-kDa immune complex band (indicating that the antibody against a light chain of FVIII exists in the patient's serum). Lane No.2 shows a cleaved 72-kDa band after activation by thrombin.

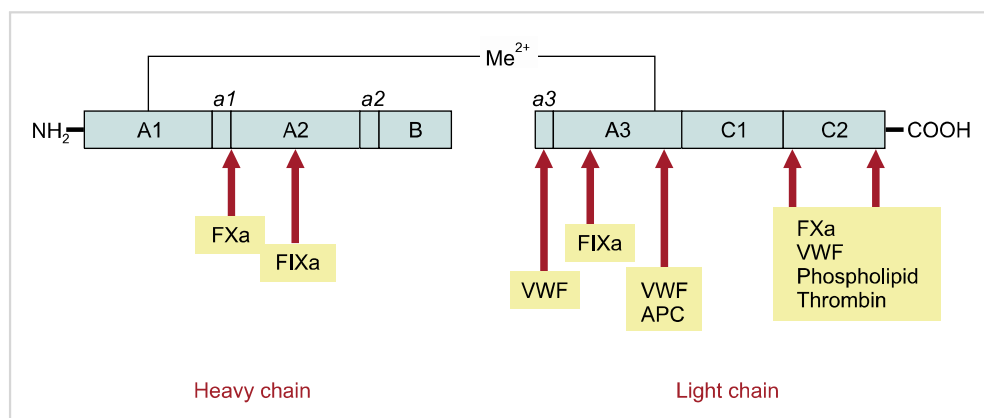


Fig. 4. Structure of factor VIII and reported epitopes of factor VIII inhibitors. Epitope regions are indicated by arrows. In this case, the dominant IgG subclass is IgG4, and the binding region is the 72-kDa A3/C1/C2 domain of the light chain. FIXa and FXa indicate activated factor IX and X, respectively. Abbreviations: VWF, von Willebrand factor; APC, activated protein C.

DISCUSSION

FVIII functions as a cofactor for FIXa in the tenase complex, and deficiency of FVIII reduces the generation of thrombin on the surface of activated platelets. Synthesized as a 330-kDa precursor protein with an A1-a1-A2-a2-B-a3-A3-C1-C2 domain structure, the single-chain FVIII protein is proteolytically processed to yield a series of amino-terminal heavy-chain fragments of approximately 92–200 kDa and a carboxy-terminal light chain of 80 kDa [8]. Most acquired FVIII inhibitors are IgG1 and IgG4 autoantibodies, directed toward epitopes within the A2, A3, and C2 domains of the FVIII molecule. The IgG subclass of inhibitor in our case was also IgG4, and the binding region was the 72-kDa A3/C1/C2 domain of the light chain (common in AHA). Depending on the location of the target epitope, different mechanisms of action for the anti-FVIII antibodies have been reported. Anti-A3 antibodies neutralize the procoagulant activity of FVIII by preventing its interaction with FIXa. Anti-C2 antibodies disrupt the binding of FVIII to phospholipid and von Willebrand factor (VWF), whereas antibodies to A2 and A3 interfere with FVIII binding to factor X and factor IXa. The role of the C1 domain has not been discovered [9–12]. The bleeding that occurred in our patient developed from the interference of FVIII binding to both FIX, by anti-A3 antibodies, and phospholipids and VWF, by anti-C2 antibodies.

Several studies have investigated FVIII-specific CD4⁺ T cells as a plausible source of the observed inhibitor production in AHA [13, 14]. First, Bray *et al.* [13] observed that the FVIII inhibitors may spontaneously disappear in conjunction with an HIV-associated decline in CD4⁺ T cell counts. Reding *et al.* [14] demonstrated that a strong positive correlation exists between inhibitor titer and the proportion of Th2-driven IgG4 anti-FVIII antibodies, and that Th1 cells may be involved in the long-term maintenance of anti-FVIII antibody synthesis. Those results could lead to the assumption that FVIII-specific CD4⁺ T cells are crucial for the development of AHA. Based on these results, immunosuppressive treatments targeting CD4⁺ T cells might effectively prevent the synthesis of anti-FVIII antibody.

Table 1. Cases of acquired hemophilia A reported in the Korean journals.

No.	Gender	Age	Clinical Sx.	FVIII (%)	Inhibitor (BU)	Tx. of active bleeding	Long-term strategy	Etiology	Outcome	Relapse
1 [16]	F	61	Hemarthropathy	1	10	Greenmono [®]	Cyclophosphamide (150 mg/d) +prednisolone (65 mg/d)	Total knee arthroplasty	Good	No
2 [17]	F	65	Whole body purpura, hemoperitoneum	2	850	F VIII (7,500 µ/d)	Cyclophosphamide (100 m/d) +prednisolone (125 mg/d)	Influenza vaccination	Dead	No
3 [18]	M	67	Intra-abdominal hematoma	28	0.1	Greenmono [®] +NovoSeven [®]	Prednisolone (50 mg)	Operation	Good	No
4 [19]	M	65	Blood oozed from his tracheostomy site	<1	5.4	Greenmono [®] +steroid	Steroid	Clopidogrel	Good	No

Abbreviations: M, male; F, female; F VIII, factor 8; IVIG, intravenous immunoglobulin.

Patients with AHA are at high risk of bleeding-related mortality, and this is likely due to the delay in the diagnosis of AHA [1, 2]. Thus, AHA should be suspected by the physician immediately for patients especially those who are elderly, have an autoimmune disease, are pregnant, or have a malignancy, or experiencing recent onset of bleeding with isolated prolongation of APTT that is not corrected in mixing studies. We speculate that the cause of the present case is autoimmune disease because of positive results with the anti-Ro antibody; yet, the patient had no other symptoms. Therefore, we deduce that the cause of our case is idiopathic. Most AHA patients have hemorrhaging of the skin, muscles, or soft tissues and mucous membranes (e.g., epistaxis, gastrointestinal and urological bleeds, and retroperitoneal hematomas), whereas hemarthrosis, a typical feature of congenital FVIII deficiency, is uncommon. We summarized all reported cases of AHA in the Korean journals (Table 1). If a physician suspects AHA, laboratory tests, including specific factor assays and the Bethesda assay for the quantification of inhibitors, should be performed for confirmatory diagnosis. Recently, the Korean Society of Thrombosis and Hemostasis (KSTH) proposed guidelines for the management of acquired hemophilia [15]. In summary, this is the first demonstration of epitope localization for a FVIII inhibitor in a patient with AHA in Korea. More studies are necessary for further clarification of the pathophysiology of AHA.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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