INTERNAL MEDICINE

□ CASE REPORT □

Cross-reacting Material-positive Hemophilia A Diagnosed in a Patient with a Spontaneous Thigh Hemorrhage

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Abstract

A 53-year-old man, who had been diagnosed with mild hemophilia A (HA) at 35 years of age, was hospitalized with a thigh hematoma. His bleeding continued despite the administration of recombinant factor VIII (FVIII). The results of an FVIII/von Willebrand factor binding assay were normal. The patient's FVIII coagulant activity (FVIII:C) was low, but his FVIII antigen levels were within the normal limits, suggesting FVIII protein dysfunction. The FVIII:C measurements obtained by one-stage clotting and chromogenic assays were different. An FVIII gene analysis revealed a missense mutation p.Ser308Leu, which is rare in Japan. This case highlights that gene analyses and chromogenic assays are necessary to interpret the discrepancies between FVIII:C and the bleeding phenotype of patients with mild HA.

Key words: mild hemophilia A, dysfunctional factor VIII protein

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Introduction

Hemophilia A (HA), which is the most common inherited bleeding disorder, is caused by coagulation factor VIII (FVIII) deficiency. According to the Japan Foundation for AIDS Prevention, 4,986 HA patients were registered in Japan in 2015. The phenotypic severity of HA is correlated with the reduction in FVIII coagulant activity (FVIII:C), which is classified as severe (<1%), moderate (1-5%), or mild (5-40%) (1). Patients with mild HA seldom bleed severely and tend to only be diagnosed when they are injured or require surgery. In approximately 45% of HA patients, the FVIII:C level is reduced in comparison to the FVIII antigen (FVIII:Ag) level. In rare (<5%) cases, patients have normal levels of FVIII:Ag, termed cross-reacting material (CRM)-positive HA (2, 3), which suggests FVIII dysfunction. Plasma tests in CRM-positive patients often show discrepancies between one-stage (FVIII:C-1st) and classic two-stage clotting (FVIII:C-2nd) or chromogenic two-stage assays (FVIII:C_{chro}) (3). We herein report the case of a CRM-positive HA patient who presented with a discrepancy between his FVIII:C-1st and FVIII:C_{chro} results, and who was subsequently shown to have a gene mutation that is rarely seen in Japan.

Case Report

A 53-year-old man (body weight 60 kg) was hospitalized with a spontaneous right-thigh hematoma. He reported no family history of hemorrhagic disease. He had experienced repeated episodes of bleeding in the tissues of both thighs and knee joints every few years since his early teens, but had rarely received FVIII infusions. He was diagnosed with mild HA at 35 years of age, with a FVIII:C value of 30-40%. On presentation at our hospital, his right thigh was markedly swollen and he reported spontaneous pain. An intramuscular hemorrhage of the right quadriceps femoris muscle was detected by contrast computed tomography (CT) (Fig. 1A). His prothrombin time (PT) and activated partial thromboplastin time (APTT) were normal on admission (Table). However, because of his history, 2,000 units of recom-

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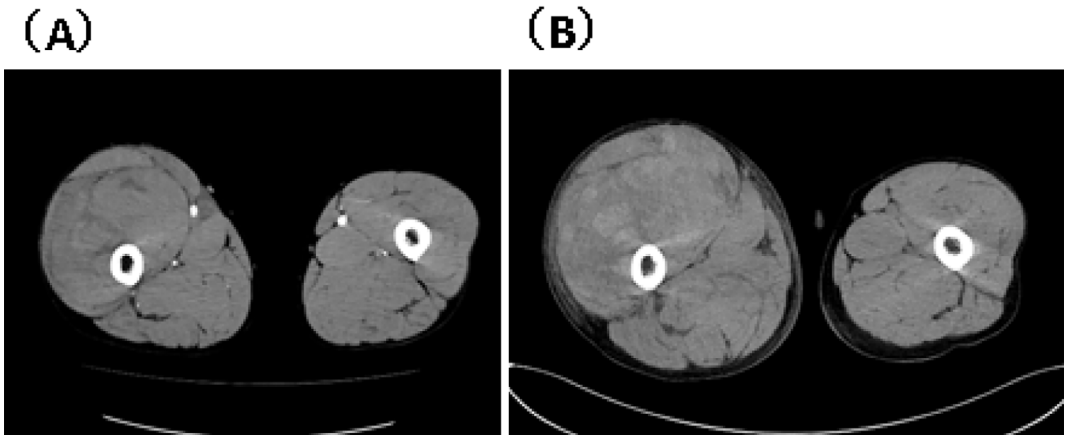

Figure 1. Computed tomography of an intramuscular hemorrhage of the right quadriceps femoris muscle in a 53-year-old male patient with a spontaneous right-thigh hematoma and mild hemophilia A. An image taken at admission (A) and an image taken on day 5, which shows the worsening of the hemorrhage (B).

Table. The Laboratory Data at Hospital Admission for a 53-year-old Man with a Spontaneous Right-thigh Hematoma and Mild Hemophilia A.

[Peripheral blood]		[Blood chemistry]		[Coaglation]	
WBC	8,800 /μL	T-Bil	1.4 mg/dL	PT(INR)	1.10
Neu	80.0 %	AST	51 U/L	APTT	31.4 sec
Lym	14.8 %	ALT	72 U/L	(25-35 sec)	
Mo	5.0 %	LDH	174 U/L	Fbg	221 mg/dL
Eo	0.1 %	(115-245 U/L)		D-dimer	0.4 μg/mL
Ba	0.2 %	Alb	4.0 g/dL		
Hb	13.4 g/dL	BUN	21.6 mg/dL		
Plt	18.7×10^4 /μL	Cre	0.60 mg/dL		
Ret	8 ‰	CK	158 U/L		
		(57-197 U/L)			
		CRP	0.1 mg/dL		
		(0-0.3 mg/dL)			

APTT: activated partial thromboplastin time, Alb: albumin, ALT: alanine aminotransferase, AST: aspartate phosphatase, Ba: basophils, BUN: blood urea nitrogen, CK: creatine kinase, Cre: creatinine, CRP: C-reactive protein, Eo: eosinophils, Fbg: fibrinogen, LDH: lactate dehydrogenase, Lym: lymphocytes, Mo: monocytes, Neu: neutrophils, Plt: platelets, PT-INR: prothrombin time international normalized ratio, Ret: reticulocytes, T-bil: total bilirubin

binant FVIII (ADVATE®) were administered to elevate his FVIII procoagulant activity to 80% (Fig. 2). The patient's FVIII:C was not investigated because he was admitted at night. The swelling and pain gradually improved after admission, and the hemorrhage was therefore considered to have been arrested and the recombinant FVIII infusion was suspended. However, he developed severe anemia (hemoglobin: 4.9 g/dL) on day 5 after admission, and CT revealed a further intramuscular hemorrhage (Fig. 1B). The patient's inadequate response to FVIII infusion led us to suspect that the continued bleeding had another cause. The patient's von Willebrand factor antigen (VWF:Ag) level (341%) and ristocetin cofactor (R.Cof) activity (190%) were elevated, but we considered these elevations to have nonspecific causes, including exercise load. We did not investigate his FVIII:C level at this time. Type 2N von Willebrand disease (VWD) was suspected, and the patient received an injection of 1,500 units of VWF/FVIII complex (Confact F®) on day 6. He also required daily red blood cell transfusions from days 5 to 7. The bleeding finally stopped on day 8.

On day 33, when the Confact F® was fully eliminated from the patient, a FVIII/VWF binding assay (4), FVIII:C-1st, FVIII:C_{chro} and FVIII:Ag measurements were performed. The FVIII/VWF binding assay showed the normal binding of FVIII to VWF (Fig. 3), ruling out a diagnosis of VWD type 2N. The patient's FVIII:C-1st value was 44.8% but his FVIII:Ag value was normal (97%), suggesting a FVIII protein dysfunction (CRM-positive). The FVIII:C-1st result was greater than the FVIII:C_{chro} result (18.0%). Such discrepancies between FVIII:C results are usually caused by mutations at or close to the interface of the A1, A2, and A3 domains of FVIII. We therefore performed an FVIII (*F8*) gene

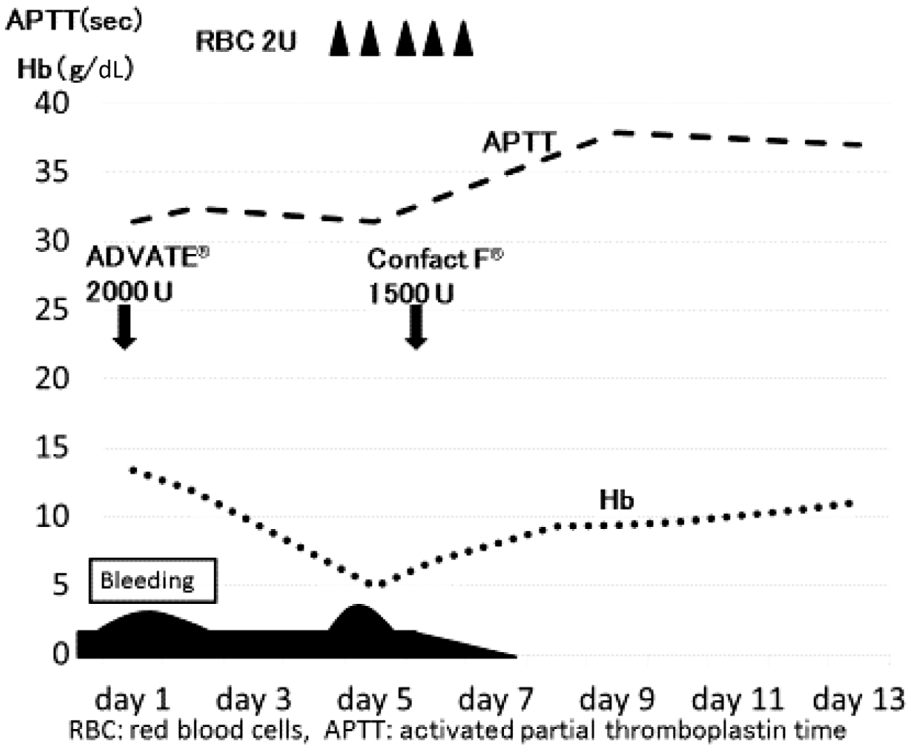

Figure 2. **The clinical course after hospitalization of the 53-year-old male patient with a spontaneous right-thigh hematoma and mild hemophilia A. The activated partial thromboplastin time (APTT) on admission was normal. Active thigh bleeding was detected by computed tomography on day 5, and red blood cell (RBC) transfusion was required on days 5-7.**

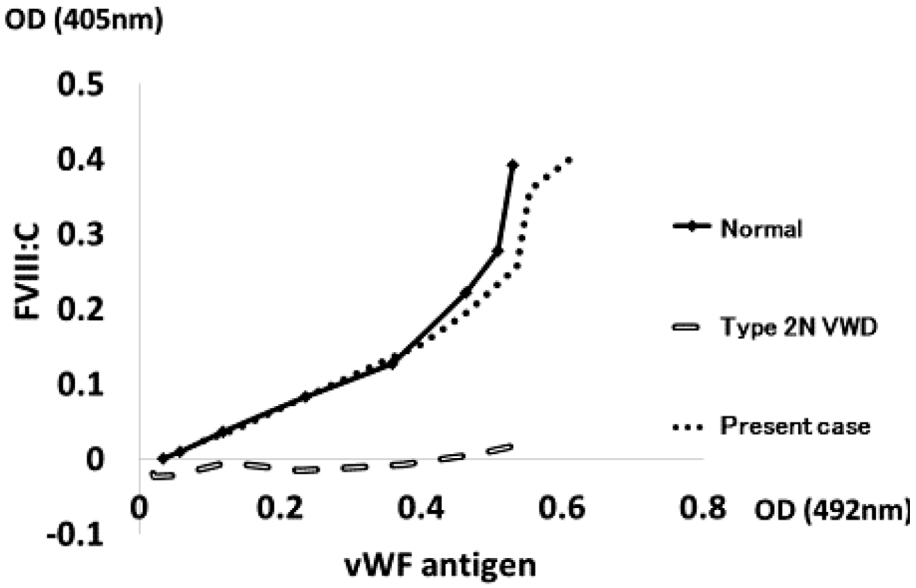

Figure 3. **Factor VIII/von Willebrand factor (FVIII/VWF) binding assay results in a 53-year-old male patient. A binding assay was performed in a solid-phase system using a polystyrene 96-well microtitration plate, as described previously (4). The wells were coated with monoclonal antibody to VWF. Serial dilutions of normal pooled plasma, plasma from type 2N von Willebrand disease, and the plasma from the present patient were incubated in the wells with equivalent amounts of purified FVIII. The amount of FVIII that bound to immobilized VWF was determined by measuring the FVIII coagulant activity (FVIII:C) using a chromogenic assay. The chromogenicity of FVIII:C was estimated at an optical density (OD) of 405 nm and the level of VWF binding to the coated monoclonal antibody was determined by an enzyme-linked immunosorbent assay at OD 492 nm. The FVIII:C value of the patient's plasma increased with an increase in VWF, demonstrating the normal FVIII-binding capacity of VWF.**

analysis and identified a missense mutation c.923C>T, p.Ser308Leu (Ser289Leu) in the A1-A2 domain interface. This mutation decreased the stability of FVIII:C, resulting in mild HA.

Discussion

The current case highlights three important clinical issues: 1) alternative assays should be considered if there is a discrepancy between the clinical bleeding tendency and the FVIII:C-1st results; 2) an analysis of the *F8* gene is useful for understanding the disease-producing mechanisms responsible for mild HA; and 3) type 2N VWD should be excluded before making a diagnosis of mild HA.

In the current case, the FVIII:C-1st result (44.8%) was more than twice that of the FVIII:C_{chro} result (18.0%). Although the severity of HA is assessed by the FVIII:C-1st assay in most laboratories, because it is easily automated, significant discrepancies between the FVIII:C-1st and FVIII:C-2nd or FVIII:C_{chro} results are observed in approximately 30% of patients with mild HA (5, 6). In most of these cases, the FVIII:C-1st value is at least twice as high as the FVIII:C-2nd or FVIII:C_{chro} value (7). FVIII:C-2nd and FVIII:C_{chro} are composed of two separate enzymatic phases, with a prolonged incubation time during the first phase, which tends to reduce the FVIII:C level in some patients with mild HA. At least 18 genetic mutations associated with assay discrepancies have been registered in the Factor FVIII Variant Database (http://www.factorviii-db.org/). Several groups have previously reported that, in comparison to the FVIII:C-1st value, the FVIII:C-2nd or FVIII:C_{chro} values were more strongly associated with the severity of bleeding in patients with mild HA (8, 9). However, these assays do not provide a full evaluation of the clinical severity of the disease, which is not always associated with FVIII coagulant activity alone. Several laboratory tests, such as the thrombin generation test, have recently been developed to provide a more comprehensive evaluation of the clotting function (10).

A genetic analysis of *F8* helps to clarify the disease-producing mechanisms responsible for mild HA. FVIII activity is regulated via proteolytic activation by thrombin *in vivo*. Thrombin-activated factor FVIII (FVIIIa) is a metal-ion-stabilized complex of A1, A2, and A3-C1-C2 subunits, and the dissociation of the A2 subunit is correlated with the inactivation of FVIIIa (11). In the present case, the detected mutation, c.923C>T, p.Ser308Leu (Ser289Leu), was located in the A1-A2 domain interface of the FVIII protein. The A2 subunit dissociation of the mutant was three-fold faster than that of the wild type of FVIIIa (12). In 1993, this missense mutation was first reported among the spectrum of mutations in CRM-positive HA (13). Since then, 28 cases have been registered in the FVIII Variant Database; only one of which was registered in Japan (13).

Type 2N VWD should be excluded before making a diagnosis of mild HA. VWF:Ag and R.Cof are almost normal in type 2N VWD, but VWF does not stabilize FVIII, resulting in low FVIII levels (14). The knowledge of the pattern of heredity (recessive or X-linked recessive) and the use of the FVIII/VWF binding assay help in distinguishing between HA and type 2N VWD in these patients. There was no evidence of a pattern of inheritance in the current patient, who was suspected of having type 2N VWD based on his diminished FVIII:C result and his poor response to FVIII infusion. However, the FVIII/VWF binding assay ruled out a diagnosis of type 2N VWD. In this case, we suspended the infusion of recombinant FVIII after the remission of the pain and swelling of the thigh. However, our evaluation of the response to FVIII infusion was premature and, in retrospect, the recombinant FVIII infusion should have been continued until the bleeding had been completely arrested. We considered the cause of incomplete hemostasis to have been the insufficient infusion of FVIII. The patient was ultimately diagnosed with mild HA.

In conclusion, basic coagulation tests such as PT and APTT do not always contribute to the diagnosis of mild HA, and an FVIII:C assay is crucial. However, as noted above, FVIII:C-1st, which is based on the APTT, is inadequate for making an accurate evaluation of mild HA, and FVIII:C_{chro}, FVIII:Ag, and a genetic analysis of FVIII are important assays for assessing the pathogenesis and clinical bleeding tendency of patients with mild HA. Furthermore, an FVIII/vWF binding assay should be used to rule out type 2N vWD before making a final diagnosis of mild HA.

The authors state that they have no Conflict of Interest (COI).

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