



Long-term treatment course of a patient with mild haemophilia A who developed a high titre factor VIII inhibitor

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The activity of coagulation factor VIII (FVIII:C) is reported to be between 5 and 40% in patients with mild haemophilia A (HA) [1]. This disease is often diagnosed in adulthood due to bleeding episodes after trauma or surgery that require replacement therapy with FVIII concentrates (FVIIIc). Although FVIII inhibitors develop less frequently in mild HA patients than in those with severe HA, the development of inhibitors may become a major clinical problem because the inhibitor may inhibit both exogenous and endogenous FVIII, thereby converting the bleeding phenotype from mild to severe [1]. We herein described the long-term treatment course of a patient with mild HA who developed a high titre FVIII inhibitor.

The male patient was first diagnosed with mild HA at 76 years of age when he developed a postoperative bleeding complication following transurethral resection of bladder tumour (TUR-BT). A conventional coagulation test showed a prolonged activated partial thromboplastin time of 46.2 s (control value, 29.1 s), his FVIII:C was 11%, and no inhibitor was detected. During the subsequent 7 months, he safely underwent a second TUR-BT and coronary artery bypass graft surgery for myocardial infarction with the continuous infusion of FVIIIc (Confact F®). However, postoperative bleeding persisted in the bladder when he underwent a third TUR-BT for a refractory lesion, in spite of intensive replacement therapy with FVIIIc. FVIII:C decreased below 1% and the Bethesda assay revealed the development of an inhibitor, the level of peaked at 160 Bethesda units per millilitre (Fig. 1). Frequent bleeding episodes were observed in the subcutaneous tissue, muscle and mucosa of the urogenital tract after the development of the inhibitor, which necessitated the repeated administration of the bypassing agent, recombinant activated factor VII (NovoSeven®;

Novo Nordisc Ltd., Tokyo, Japan). He subsequently underwent reconstructive free-flap surgery following surgical removal of a subcutaneous haematoma associated with overlying skin necrosis of the dorsum of the left hand.

We administered immunosuppressive treatment, which included prednisolone, oral cyclophosphamide and two cycles of rituximab ($375 \text{ mg m}^{-2} \text{ week}^{-1} \times 4$ doses); however, even though its titre declined gradually, the inhibitor persisted for over 30 months (Fig. 1). FVIII:C finally became detectable approximately 3 years after the inhibitor developed, and bleeding episodes no longer developed thereafter. We are notified that his grandson is treated for haemophilia at another medical centre; however, further information is unavailable.

Genetic analysis of the *F8* gene was performed after obtaining informed consent from the patient. An intron 22 inversion and intron 1 inversion were screened by long-distance polymerase chain reaction (PCR) and multiplex PCR, respectively. Twenty-six exons and their flanking regions were amplified by PCR using specific primers and analysed by conformation-sensitive gel electrophoresis followed by nucleotide sequencing. This analysis revealed the deletion of four nucleotides on the 3' splice site of intron 1 (c.144-11 TCTT del), a missense mutation, c.3780C>G; p.D1241E in the B-domain and a mutation, c.*1672A>G in the 3' untranslated region of exon 26.

In contrast to patients with severe HA with FVIII inhibitors, for whom immune tolerance induction (ITI) is indicated to restore responsiveness to FVIII, patients with mild HA who develop inhibitors have been shown to respond poorly to ITI [2]. Therefore, immune suppression therapy to eradicate inhibitors should be considered in patients with bleeding patterns similar to acquired haemophilia, as with the present case. A search of the literature found case reports or small case series of the successful use of rituximab, an anti-CD20 antibody, alone or in combination with high-dose FVIII for mild HA with inhibitors [3–6]. Franchini *et al.* summarized published cases and reported a high response rate to rituximab in patients with mild/moderate HA [6]. However, when the relationship between the rituximab treatment and

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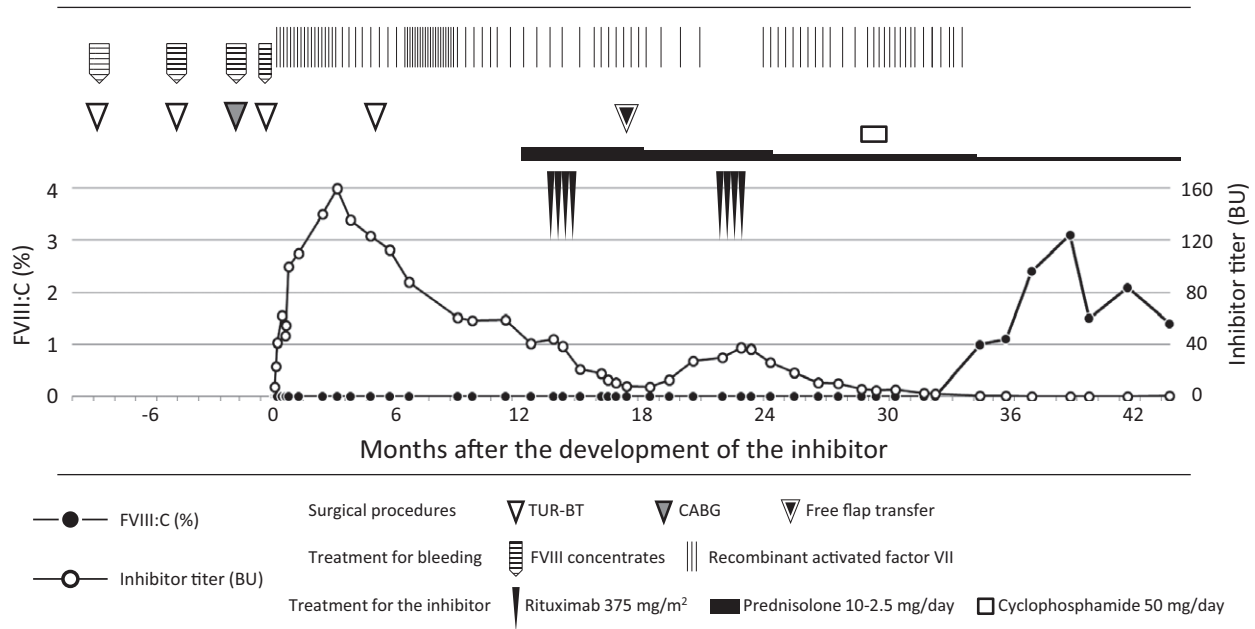


Fig. 1. Treatment course showing FVIII coagulant activity (FVIII:C) (%) and the inhibitor titre (Bethesda units per millilitre, BU mL⁻¹). The treatment for bleeding consisted of replacement therapy with FVIII concentrates and the administration of recombinant activated factor VII before and after the development of the inhibitor respectively.

response obtained in the present case was assessed, the effect of rituximab appeared to be limited even though the inhibitor titre transiently declined after the first cycle of rituximab. It may be more likely that the inhibitor declined and disappeared spontaneously by avoiding the administration of FVIIIc.

Molecular genetic studies of HA have identified a wide variety of mutations; a total of 2107 unique mutations, including 158 splice-site mutations, are listed in the Haemophilia A Mutation Database [7]. The type of mutation in the *F8* gene has generally been associated with not only the severity of HA, but also the risk of the formation of inhibitors [8]. Large deletions, inversions and nonsense mutations are associated with severe HA and the highest risk of inhibitor development, while missense mutations have accounted for approximately 90% of reported cases of mild HA, in which inhibitor development was an uncommon complication [1,7,9]. c.3780C>G; p.D1241E and c.*1672A>G, which were identified in

the present case, are included in the list of polymorphic nucleotide substitutions detected in the *F8* gene of both normal subjects and HA patients [7], even though previous studies have shown that p.D1241E is associated with lower FVIII:C and that p.D1241E-containing haplotypes contribute to the high incidence of inhibitors [8,10]. On the other hand, c.144-11 TCTT del has not been registered in the database. This small deletion, which is located adjacent to the splice acceptor site of intron 1, may have affected structural and/or functional alterations in the *F8* transcripts, thereby leading to the low FVIII:C observed in this case. Nevertheless, the mechanisms responsible for the development of inhibitors in patients with splicing mutations remain to be elucidated.

Disclosures

The authors stated that they had no interests which might be perceived as posing a conflict or bias.

References

- Franchini M, Favaloro EJ, Lippi G. Mild haemophilia A. *J Thromb Haemost* 2010; 8: 421–32.
- Collins PW, Chalmers E, Hart DP *et al*. Diagnosis and treatment of factor VIII and IX inhibitors in congenital haemophilia: (4th edition). UK Haemophilia Centre Doctors Organization. *Br J Haematol* 2013; 160: 153–70.
- Dunkley S, Kershaw G, Young G *et al*. Rituximab treatment of mild haemophilia A with inhibitors: a proposed treatment protocol. *Haemophilia* 2006; 12: 663–7.
- Dolnicar MB, Rajic V, Kitanovski L, Debeljak M. Rituximab for the treatment of high titre inhibitors in mild haemophilia A. *Blood Transfus* 2014; 12(Suppl. 1): s345–7.
- Carcao M, St Louis J, Poon MC *et al*. Rituximab for congenital haemophiliacs with inhibitors: a Canadian experience. *Haemophilia* 2006; 12: 7–18.
- Franchini M, Mengoli C, Lippi G *et al*. Immune tolerance with rituximab in congenital haemophilia with inhibitors: a systematic literature review based on individual patients' analysis. *Haemophilia* 2008; 14: 903–12.
- Kemball-Cook G. The Haemophilia A Mutation, Structure, Test and Resource Site. Available at <http://hadb.org.uk/>. Accessed February 2014.
- Viel KR, Ameri A, Abshire TC *et al*. Inhibitors of factor VIII in black patients with hemophilia. *N Engl J Med* 2009; 360: 1618–27.
- Bogdanova N, Markoff A, Eisert R *et al*. Spectrum of molecular defects and

mutation detection rate in patients with mild and moderate hemophilia A. *Hum Mutat* 2007; 28: 54–60.

10 Scanavini D, Legnani C, Lunghi B, Mingozzi F, Palareti G, Bernardi F. The factor VIII D1241E polymorphism is associated

with decreased factor VIII activity and not with activated protein C resistance levels. *Thromb Haemost* 2005; 93: 453–6.

Thromboembolic incidence with transiently elevated levels of coagulation factors in patients with von Willebrand disease treated with VWF:FVIII concentrate during surgery

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In the general population, persistently elevated levels of coagulation factor VIII (FVIII) above 150 U/dL (%) are a recognized risk factor for venous thromboembolic (VTE) events [1]. Available plasma-derived products for von Willebrand factor (VWF) replacement therapy in patients with von Willebrand disease (VWD) also contain FVIII, thereby increasing both VWF and FVIII levels [2]. Patients with VWD, particularly those who receive repeated and frequent dosing with VWF/FVIII concentrates to control bleeding, can develop transient but marked elevations in FVIII levels [3]. Thus, there is the potential for VTE in patients with VWD undergoing surgery and being treated with VWF/FVIII concentrates. There have indeed been reports of VTE in patients with VWD in the literature, and as noted in a review by Franchini *et al.* [4], these events appear to be rare and usually occur in patients with either elevated levels of FVIII (>150%) and/or other thromboembolic risk factors (e.g. advanced age, surgery, immobility, obesity, history of thrombotic episodes or hormone replacement therapy). The thrombogenic potential of VWF elevations is less clear, but there is limited evidence that elevated VWF levels may contribute to the risk of thrombosis [2]. However, to reduce potential thrombogenicity during VWF replacement treatment for surgery, current VWD treatment guidelines recommend levels remain below 250% for FVIII and 200% for VWF ristocetin cofactor activity (VWF:RCo) [2]. In contrast to the evidence of increased VTE risk with persistent FVIII elevation, the risk of VTE during transient elevations of FVIII or VWF:RCo, such as those experienced by patients treated with VWF/FVIII concentrate

for surgery, has not been established. In addition, the actual incidence of thrombotic complications in patients with VWD who receive VWF/FVIII concentrate therapy remains unknown. Using data from two clinical trials [5,6] of VWF/FVIII concentrate (Humate-P® and Haemate P®, CSL Behring, King of Prussia, PA, USA and Marburg, Germany) treatment in subjects with VWD undergoing surgery, we report the incidence of VTE in subjects who experienced transient elevations in FVIII and/or VWF:RCo above 150% (Table 1).

In the first study [5], VWF:RCo and/or FVIII activity $\geq 150\%$ was observed at least once in 29 of the 35 subjects. Several subjects experienced FVIII and/or VWF levels $\geq 300\%$, yet no VTE events occurred in this study population. In general, subjects who experienced elevated coagulation factor levels tended to be slightly older, received less frequent dosing regimens, underwent major surgery and received treatment for a longer period of time.

In the second study [6], VWF and/or FVIII activity $\geq 150\%$ was observed at least once in 24 of the 27 subjects who underwent surgery. In general, subjects in this study who experienced elevated coagulation factor levels tended to receive less frequent dosing regimens, underwent major surgery and received treatment for a longer period of time. Two postoperative VTE events occurred in this study (Table 1): a mild thrombophlebitis of the lower leg was reported in a 51-year-old woman 6 days after hysterectomy, and pulmonary embolism occurred in an 81-year-old woman 10 days after undergoing bilateral knee replacement. Thus, across the two studies, VTE was reported in two of 53 subjects (3.8%) with elevated FVIII and/or VWF:RCo levels.

The subject with pulmonary embolism had several VTE risk factors (advanced age, thrombocytosis, type A blood group, recent major orthopaedic surgery), and did not receive any antithrombotic prophylaxis. Her plasma FVIII level was 450% the day before the diagnosis of pulmonary embolism. The embolism was successfully resolved with heparin therapy.

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