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Characterization of an Anti-Factor VIII Inhibitor Related To P.Arg2178Cys Mutation in A Patient with Mild Haemophilia A

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Abstract

In patients with inherited mild haemophilia treated with clotting factor concentrates, the risk of inhibitor development is lower than in severe haemophilia. We report the observation of a 38-year-old man, with mild haemophilia A, previously treated for mucosal bleeding, who presented no major immunization risk factors except an *exon 23 missense mutation p.Arg2178Cys in the F8 gene in the C1 domain.* He developed an inhibitor against Octocog alfa. The implication of the mutation in this particular immunization process is well known. Characterization of this inhibitor has been extended as a type I inhibitor with classical epitope mapping and solely directed against exogenous factor VIII. Due to the occurrence of an anamnestic response associated with a high titre inhibitor, we had recourse to Eptacog alfa during a critical bleeding situation.

Keywords: Mild haemophilia A; Factor VIII concentrate (Octocog alfa); Inhibitor; Epitope mapping; *F8* gene mutation

Essentials

Unexpected anti-factor VIII inhibitor in a patient with mild haemophilia A.

Inhibitor (initially and after anamnestic response) directed only against exogenous factor VIII.

Specificity of the inhibitor against domains A1, A2 and C1.

Genetic abnormality: *exon 23 missense mutation p.Arg2178Cys* of the C1 domain.

Case report

A 38-year-old white European man, with mild haemophilia A (FVIII at 0.11 IU/dL), carrying the *exon 23 missense mutation p.Arg2178Cys in the F8 gene (legacy numbering: Arg2159Cys or R2159C) in the C1 domain*, was followed in our centre. An intravenous desmopressin therapeutic test performed at 18 years showed a satisfactory response (FVIII: 0.19 IU/dL at T0H, 0.70 IU/dL at T1H, 0.43 IU/dL at T4H). His vWF levels and platelet functions were normal. His blood type was A.

At the age of 30 years, a kite surfing accident occurred resulting in a subcutaneous haematoma extending over the entire right lower limb, associated with a first left metacarpal hand fracture, for which he underwent surgical repair under Octocog alfa (Advate®) prophylaxis. This represented our patient's first contact with a FVIII concentrate (5 Exposure Days (EDs)). One month later, he required prophylactic Octocog alfa administration (3 EDs) for the removal of the osteosynthesis material.

At the age of 35 years, he had a nosebleed unresponsive to desmopressin and antifibrinolytic agent at recommended therapeutic dose, requiring nasal packing under Octocog alfa prophylaxis (2 EDs). At the age of 36 years, he underwent the avulsion of three impacted wisdom teeth, under Octocog alfa prophylaxis (2 EDs) and antifibrinolytic agent. Eight days later, he was administered the same treatment, for bleeding due to an open pressure sore.

At the age of 38 years, he had three nosebleeds over a 3-week period, unresponsive to desmopressin and antifibrinolytic agent, requiring Octocog alfa (ADVATE®) injections with a satisfactory response.

Before the last Octocog alfa treatment, biological monitoring showed an Activated Partial Prothrombin Time (APTT) of 1.44, fibrinogen at 3.25g/l. Later, and for the first time a low titre positive FVIII inhibitor at 0.94 BU/ml was detected using

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Table 1: Clinical and Biological chronology.

Bleeding context / notable event	Date	APTT (ratio)	Fibrinogen (g/l)	FVIII (IU/dL) before treatment	Anti-FVIII inhibitor (BU/mL)	TGA (ETP nM.min)	Eds to rFVIII	BPAs
Kite surfing Accident*	4/7/2013	1.65	3.72	0.14	< 0.6		5	
Removal of osteosynthesis material	19/08/2013	1.48	3.52	0.13			3	
One month stay in Brazil	15/02/2015							
Epistaxis resistant Desmopressin and antifibrinolytic drugs / nasal packing	3/12/2015	1.46	3.43	0.26	< 0.6		2	
Routine follow-up	7/1/2016	1.51	3.3	0.26	< 0.6	Hypocoagulability profile (998)		
Avulsion of 3 impacted wisdom teeth	2/2/2016						2	
Pressure sore fall	10/2/2016						2	
Immunization againt Flavivirus (Zika virus)	13/08/2016							
ER consultation for isolated headaches (no haemorraging)	27/06/2018	1.51	3.4	0.1				
Epistaxis resistant to Desmopressin and antifibrinolytic drugs	27/08/2018	1.49	3.57	0.12	Infuffcient blood sample		1	
Epistaxis resistant to Desmopressin and antifibrinolytic drugs	11/9/2018						1	
Epistaxis resistant to Desmopressin and antifibrinolytic drugs	18/09/2018	1.44	3.25	0.28	0.94	Hypocoagulability profile	2	
Close follow-up	24/09/2018	1.47	4	0.2	5			
Close follow-up	28/09/2018							
Bilateral nasal cauterization	2/10/2018	1.54	3.45	0.16	11			1x5 mg and 2x2 mg
Routine follow-up	6/2/2020	1.45	3.46	0.25	1	Hypocoagulability profile (582)		
Routine follow-up	9/6/2023	1.47	3.07	0.2	0.7			

*subcutaneous haematoma extending over the entire right lower limb, first left metacarpal hand fracture
TGA = Thrombin Generation Assay
ETP = Endogenous thrombin potential
ED = Exposure Day
rFVIII = Octocog alfa (Advate®)
BPA = By Pass Agents = Eptacog alfa (Novoseven®)

the Nijmegen-Bethesda assay, with concomitant FVIII at 0.28 IU/dL few hours after desmopressine nasal administration (Table 1). A week after the last Octocog alfa injection, the inhibitor increased to 5 BU/ml. Within the next days, recombinant activated factor VII concentrate (Eptacog alfa Novoseven®) was administered for a complementary bilateral nasal cauterization procedure.

The inhibitor persisted over time, with a peak at 11 BU/ml with concomitant FVIII at 0.16 IU/dL, at day 15 of its first detection (i.e. at day 10 of the last Octocog alfa injection), before gradually decreasing over a period of more than 4 years of follow-up, without complete disappearance and with no eradication attempt. The inhibitor titre was then at 0.7 BU/ml with concomitant FVIII at 0.20 IU/dL, fibrinogen at 3.07 g/l and APTT at 1.47 (Table 1).

We performed a Thrombin Generation Assay retrospectively on three different samples: one during routine follow-up before immunization and two in the presence of the inhibitor, the results showed a hypocoagulability profile whether the inhibitor is present or absent, but more marked in the presence of the inhibitor. Indeed, without inhibitor and with 0.26 IU/dL level of FVIII, the patient had a normal endogenous thrombin potential (ETP: 998 nM.min). In the presence of 1 IU Bethesda inhibitor and 0.25 IU/dL level of FVIII, a decrease of ETP (582 nM.min) was observed.

Epitope mapping revealed a FVIII specific antibody characterized as IgG isotype (IgG subclasses 1 and 4) specifically binding to the following domains on the Heavy (HC) and Light Chain (LC): respectively A1, A2 and C1.

Discussion

In non-severe haemophilia A, immunization against FVIII is present in 5 to 13% of patients who have been treated with factor VIII concentrates [1,2]. It usually occurs before 150 EDs, but can also be observed at an early stage [3]. The antibody can be directed against both the exogenous non-mutated wild FVIII administered to the patient, and the endogenous mutated self FVIII, thus resulting in worsening the severity of haemophilia phenotype [4,5,6].

As in severe haemophilia A, *F8* gene mutations remain the deciding risk factor in immunization. *F8* missense mutations are more involved in the development of inhibitors in non-severe haemophilia A patients. The main epitopic regions targeted by the inhibitor are located in the A2, A3 and C2 domains [7]. More specifically, the *exon 23 missense mutation p.Arg2169His of the F8 gene (legacy numbering: Arg2150His or R2150H)* of the C1 domain has been described in the literature as associated with a 5% incidence rate of immunization in moderate to mild haemophilia A [3]. Due to its location on the C1-C2 junction [5], the developed inhibitor has the specificity of being directed against the C1 domain of the exogenous wild non-mutated FVIII, whilst sparing the endogenous mutated FVIII [6,8,9]. This selective inhibition of exogenous FVIII is also descripted, for example, in the presence of the Glu272Lys mutation of the A1 domain in mild haemophilia A [10].

In our patient, the *exon 23 missense mutation p.Arg2178Cys in the F8 gene (legacy numbering: Arg2159Cys or R2159C)* of the C1 domain was immunogenic, described in the literature as possibly corresponding to 3 different haplotypes, with an incidence rate of 1.9% [3]. It was not associated with haplotype 1 (despite being white European). Its characterization was very close to the well-known substitution associated with a high risk of inhibitor development, the *exon 23 missense mutation p.Arg2169His of the F8 gene (legacy numbering: Arg2150His or R2150H)* of the C1 domain, with a difference of only 9 amino acids. Both mutations are located in the C1 domain, on the light chain.

The inhibitor developed after 16 cumulative EDs to Octocog alfa (associated with Haplotype 2). It selectively inhibited the exogenous, non-mutated wild FVIII. In 60% of cases, the antibody disappears after approximately 9 months, with a non-excluded risk of an anamnestic response but rather a scarcer one [5], possibly explained by an immune tolerance induced by the endogenous FVIII despite an impaired function. In our case, the inhibitor is still present after 4 years and 9 months, at a low titre. No FVIII treatment was re-introduced.

Despite its specificity against domains A1, A2 (HC) and C1 (LC), this inhibitor's mechanism of action has not yet been reported: what we uncovered in this patient was a selective inhibition of wild type FVIII. These findings suggest a possible association of the *exon 23 missense* mutation *p.Arg2178Cys* with only the exogenous FVIII specific inhibitor.

This case emphasizes the need for close monitoring and regular testing for inhibitors, even in patients with mild haemophilia, particularly after acute treatment periods [11].

Conclusion

In mild haemophilia A patients, the development of inhibitors A also makes treatment management difficult, especially when desmopressin is ineffective, and increases the risk of bleeding. Genetic testing can be useful to evaluate the risk of developing an inhibitor, and should therefore be considered, regardless of the severity of the haemophilia. In our case, the *exon 23 missense* mutation *p.Arg2178Cys*, a close relative of the *exon 23 missense mutation p.Arg2169His*, played a rather important part in the immunization process, generating a high titre inhibitor, a strong anamnestic response, and the persistent of the inhibitor over years despite any exogenous factor VIII reintroduction. Finally, this case highlights the importance of genetic mutation identification and regular inbitor's monitoring in all patients with mild haemophilia.

Consent was obtained from the patient for the publication of this observation.

Author Statements

Addendum

P. Chamouni and M. Souissi wrote the manuscript, P. Billoir performed biological analyses, M. Fretigny, V. Barbay and V. Le Cam Duchez revised the manuscript.

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Conflict of Interest Disclosure

The authors have no competing interests.

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