



ORIGINAL ARTICLE

Allergic reaction in a cohort of haemophilia A patients using plasma-derived factor VIII (FVIII) concentrate is rare and not necessarily triggered by FVIII

S. A. L. MONTALVÃO, A. C. TUCUNDUVA, L. H. SIQUEIRA, A. L. A. SAMBO, S. S. MEDINA and M. C. OZELO

Hemophilia Unit 'Cláudio L. P. Correa', INCT do Sangue Hemocentro de Campinas, Centro de Hematologia e Hemoterapia da Unicamp, University of Campinas, Campinas, Brazil

Summary. In contrast to haemophilia B, allergic manifestations are rare complications in haemophilia A (HA) patients treated with factor VIII (FVIII) concentrates. Nevertheless, it can be serious and hamper replacement therapy in these cases. The aims of this study were to evaluate the frequency of allergic reaction in a cohort of HA patients treated only with plasma-derived FVIII (pdFVIII) concentrates, and assess the possible immune mechanisms involved. History of allergic reaction was retrospectively assessed. Patients with allergic manifestations were followed, and had plasma samples collected in different timepoints in relation to the allergic episode. These samples were analysed for the presence of inhibitor and anti-FVIII immunoglobulins subclasses. Three of 322 HA patients (0.9%) developed allergic reaction after exposure to pdFVIII products during the last 15 years in our centre. The first patient, with severe HA, without inhibitor, had anti-pdFVIII IgE

and IgG4, but no anti-recombinant FVIII (rFVIII) IgE. The second patient, with severe HA, and high-responding inhibitor, presented allergic manifestation with both, pdFVIII concentrate and activated prothrombin complex concentrate. Although anti-pdFVIII and anti-rFVIII IgG4 were detected, no anti-FVIII IgE was present. The third patient, with moderate HA without inhibitor, atopic, had no anti-FVIII immunoglobulin detected, and allergic symptoms disappeared after switching to rFVIII concentrate. This study corroborates the low incidence of allergic reactions in HA patients. In the three cases presented, the anti-FVIII immunoglobulin profile demonstrated that the allergic manifestation was triggered by other proteins contained in pdFVIII products, and not directed to FVIII.

Keywords: allergic reaction, factor VIII, haemophilia A, immunoglobulin E, Inhibitor

Introduction

Allergic manifestations, including anaphylactic reaction, are well-known complications in haemophilia B, and are implicated in the higher risk of inhibitor development [1]. Nevertheless, allergic reactions in haemophilia A (HA) were more commonly seen when treatment was based on cryoprecipitate and low-purity plasma-derived factor VIII (pdFVIII) concentrate [2,3].

Nowadays, with the development of recombinant products, allergic reactions are unlikely to occur, in spite of being more frequently notified [4,5].

Limited numbers of studies were able to demonstrate the mechanism involved in allergic reactions and this issue remains unclear. In rare cases, immunoglobulins (Ig) IgG4 and IgG2 were associated with allergic conditions in HA [6,7]. Indeed, previous report demonstrated that IgG4 is associated with inhibitory antibodies to FVIII [8,9]. Despite the occurrence of inhibitor in up to 30% of severe HA patients, allergic reaction in this population is rare.

The most common allergic reaction to drugs is type I hypersensitivity (or immediate hypersensitivity) reaction which is mediated by IgE isotype [10]. So far, there is just one report of anaphylactic reaction associated with the use of recombinant FVIII (rFVIII) concentrate that was proved to be IgE mediated [11].

Correspondence: Margareth C. Ozelo, MD, PhD, IHTC Hemophilia Unit Cláudio Luiz Pizzigatti Corrêa, INCT do Sangue Hemocentro Unicamp, University of Campinas, Rua Carlos Chagas 480, Cidade Universitária, Campinas, São Paulo 13.083.878, Brazil.

Tel.: +55 19 3521 8756; fax: +55 19 3521 8670;
e-mail: margaret@unicamp.br

Accepted after revision 12 January 2015

The objective of this study was to evaluate the occurrence of allergic reactions in a cohort of HA patients in a single reference Hemophilia Treatment Center during 15 years. Among the patients with allergic reaction to pdFVIII concentrates, we identified the immunoglobulins isotypes involved in the development of this complication.

Method

Patients

All HA patients followed at the Hemophilia Unit from Hemocentro Unicamp in Campinas, Sao Paulo, during the past 15 years had their medical history revised for the incidence of allergic reaction with the use of pdFVIII concentrates, which were the only type of FVIII-containing product available in our centre during the time of the study.

Patients presenting allergic symptoms after administration of pdFVIII concentrates were prospectively assessed for the occurrence of allergic reactions in subsequent infusions and evaluated for the presence of neutralizing antibodies against FVIII (inhibitors) and the anti-FVIII immunoglobulins subclasses.

The procedures followed were approved by the Institutional Review Board, and were in accordance with the ethical standards and with the Helsinki Declaration.

Detection of neutralizing antibodies against FVIII

Inhibitors were determined by Bethesda–Nijmegen modification assay, as previously described [12]. To improve the sensitivity, all plasma samples were treated with heat at 56°C for 30 min, before the assay [13].

ELISA for detection of binding antibodies

Anti-FVIII immunoglobulins were detected by ELISA method, according to standard procedures, using anti-human-Ig specific for subclasses IgG1, IgG4 and IgE (Southern Biotechnology Inc., Birmingham, AL USA). The plates were coated with a pdFVIII, Octavi SD Optimum® (Octapharma, Lingolsheim France), or a full-length rFVIII concentrate, Advate® (Baxter, Zürich Switzerland). Plasma samples were diluted 8-fold from 1:10 to 1:1280. Samples from 20 healthy individuals were used to validate the test.

Results

Three of 322 HA patients (0.9%) presented with allergic reaction after exposure to plasma-derived products [pdFVIII or activated prothrombin complex concentrate (aPCC)] during the past 15 years in our centre. Table 1 summarizes the clinical and laboratory findings of these three cases.

Patient 1

The first case was a 46-year-old man, with severe HA (FVIII < 1 IU dL⁻¹), without history of inhibitor, and no evidence of atopic diseases. As complications, he has positive serology for hepatitis C, and mild haemophilic arthropathy. During the previous 5 years, he presented sporadic allergic reactions with the use of several different pdFVIII concentrates. The symptoms included tremor, and pruritus with urticaria that were controlled with intravenous (i.v.) corticosteroids. Prophylactic i.v. administration of antihistamines was occasionally used to prevent these symptoms. In the investigation, no deficiency of IgA was detected, while elevated serum level of IgE was observed. During the

Table 1. Clinical and laboratory findings of three cases with allergic reaction to pdFVIII concentrates.

	Patient 1	Patient 2	Patient 3
Age at enrolment (year)	46	35	5
Race	Black	Caucasian	Black
Diagnosis	Severe HA	Severe HA	Moderate HA
FVIII genotype	ND	p.Q1659Stop	ND
Inhibitor history	No	High-responding	No
Comorbidities	HCV and chronic arthropathy	HCV and chronic arthropathy	None
Type of products related to allergic reactions	Several pdFVIII	Several pdFVIII and aPCC	Several pdFVIII
Atopic history	No	No	Yes
Family allergy history	No	No	Yes
Serum IgE level, IU mL ⁻¹	1430 (NR < 100 IU mL ⁻¹)	6430 (NR < 100 IU mL ⁻¹)	3560 (NR < 90 IU mL ⁻¹)
IgA deficiency	No	No	No
Anti-pdFVIII IgE	Positive	Negative	Negative
Anti-pdFVIII IgG4	Positive	Positive	Negative
Anti-pdFVIII IgG1	Positive	Negative	Positive
Anti-rFVIII IgE	Negative	Negative	Negative
Anti-rFVIII IgG4	Positive	Positive	Negative
Anti-rFVIII IgG1	Negative	Positive	Negative

HA, haemophilia A; ND, not determined; HCV, hepatitis C virus; pdFVIII, plasma-derived factor VIII concentrate; rFVIII, recombinant FVIII; aPCC, activated prothrombin complex concentrate; NR, normal range according to the age.

treatment of a joint bleeding episode with pdFVIII concentrate, (Octavi SD Optimum®; Octapharma) he developed a severe allergic reaction, with urticaria, laryngeal oedema and bronchospasm. The symptoms resolved with i.v. corticosteroids. Plasma samples were collected and analysed 3 h, 15 and 25 days after the allergic reaction, and were compared to samples collected 6 months before the event. Anti-pdFVIII IgG4 was detected in the samples collected at the same day of the allergic reaction and a peak titre was found after 15 days. Interestingly, anti-pdFVIII IgE was also detected in the same samples. However, no IgE was

observed when ELISA plates were coated with rFVIII, and low titre of IgG4 was detected in these analyses, although inhibitor presence remained negative during the whole period (Fig. 1a).

Patient 2

The second case was a 35-year-old man, with severe HA (FVIII < 1 IU dL⁻¹), and history of hepatitis C, and haemophilic arthropathy. Ten years before, he developed allergic reactions to pdFVIII concentrate, characterized by fever and chills. In that occasion,

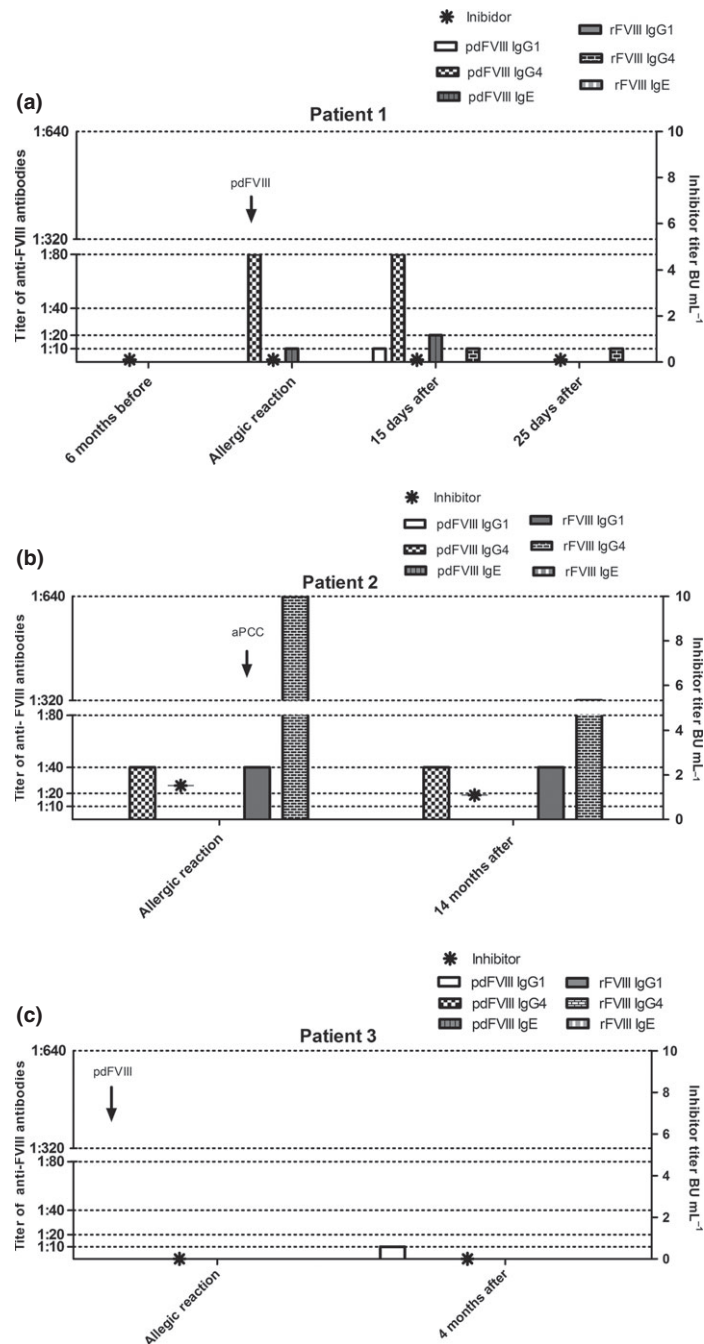


Fig. 1. Longitudinal assessment of antifactor VIII (FVIII)-specific antibodies and inhibitor titres of each patient. (a) Patient 1: plasma samples were collected 6 months before the allergic reaction, and 3 h, 15 and 25 days later. (b) Patient 2: plasma samples were collected the day of allergic symptoms, and 14 months later. (c) Patient 3: plasma samples were collected 48 h after a severe allergic reaction and 4 months later. Samples from all time points were tested for inhibitor and anti-plasma-derived FVIII and anti-recombinant FVIII IgG1, IgG4 and IgE.

inhibitor was detected for the first time, with peak titre of 160 BU mL⁻¹. He started to receive aPCC showing similar symptoms of allergic reaction, controlled with i.v. corticosteroids, and prevented with prophylactic i.v. administration of antihistamines. No evidence of IgA deficiency or clinical manifestations of atopic diseases were observed, despite the high serum level of IgE. Plasma samples were collected when he presented allergic symptoms after receiving aPCC for the treatment of a haemarthrosis, and were compared with plasma samples collected after 14 months, without recent exposure to plasma-derived concentrates. The analyses in those two occasions showed that anti-FVIII IgG1 and IgG4 were detected when the ELISA plates were coated with both pdFVIII and rFVIII (Fig. 1b). Anti-FVIII IgE was negative in these analyses.

Patient 3

The third one was a 5-year-old boy with moderate HA (FVIII 4.6 IU dL⁻¹), with history of atopy, manifested as allergic rhinitis and asthma, with high serum level of IgE. One year before, during the treatment of a mild haematoma with pdFVIII, he developed urticarial reaction associated with bronchospasm, fever and facial angioedema controlled with i.v. corticosteroids. Less intense allergic reactions had already been observed upon use of pdFVIII. These allergic manifestations disappeared after switching to rFVIII concentrate. Inhibitor was never detected and IgA deficiency was excluded. Plasma samples were collected 48 h after a severe allergic reaction with the use of plasma-derived product and 4 months later. No anti-FVIII immunoglobulin was detected in the samples first collected, and only low titre of anti-pdFVIII IgG1 was observed in the samples collected 4 months after the reaction (Fig. 1c).

Discussion

Allergic reactions with the use of FVIII-containing concentrates are not frequently observed [5]. In the past 15 years, we followed more than 300 HA patients, that had only received pdFVIII products during this period. In this cohort we observed the occurrence of allergic reactions in 0.9% of these patients.

The use of rFVIII concentrates can be an alternative for most of the cases in which the allergic manifestation is related to other proteins present in plasma-derived products. However, in the case of FVIII driven allergic reaction the use of rFVIII concentrates will not be the best choice, and in fact, this can intensify the reaction. Kadar *et al.* [11] described a case with

anaphylactic reaction after the administration of rFVIII, and proved for the first time that it was mediated by anti-FVIII IgE.

In this study, the patient 3, with personal and family history of atopy, had no longer allergic symptoms after switching to rFVIII. Indeed, the *in vitro* analyses showed no specificity to either pdFVIII or rFVIII.

The patient 2 with high-responding inhibitor history developed allergic reaction to both pdFVIII and aPCC. The ELISA results demonstrated the presence of anti-rFVIII IgG1 and IgG4, and anti-pdFVIII IgG4. No anti-FVIII IgE was detected. This immunoglobulin profile can be associated only with the presence of inhibitor and it does not necessarily explain the allergic manifestations. This patient was never exposed to rFVIII concentrate due to the presence of inhibitor and the shortage of this type of product at that time in our country. However, based on these analyses, we believe that the use of rFVIII concentrate will be an alternative for an immune tolerance induction treatment for him.

Only in patient 1 the type I hypersensitivity reaction mediated by IgE isotype directed to pdFVIII was observed. However, the IgE was negative when the ELISA was performed with rFVIII, proving that there was no specificity to FVIII, and the reaction was probably triggered by other proteins present in the pdFVIII concentrate.

In conclusion, in our cohort of HA patients, the incidence of allergic reaction to pdFVIII products was lower than 1%. The *in vitro* evaluation performed in samples from the three patients who developed these events, was not able to demonstrate that the allergic manifestation was related to FVIII. Thus, except for the patients with inhibitors, the use of rFVIII concentrate can be an option for the management of the patients with allergic symptoms to pdFVIII-containing products.

Author contributions

S.A.L.M. designed the study, performed the assays, collected and analysed the data and wrote the manuscript; A.C.T. and L.H.S. performed the assays, A.L.A.S. collected the data; S.S.M. analysed the data and reviewed the manuscript and M.C.O. designed the study, collected the data and critically revised and approved the final version of the manuscript.

Disclosures

The authors received funding support from the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). The authors stated that they had no interests that which be perceived as posing a conflict or bias.

References

- 1 Recht M, Pollmann H, Tagliaferri A, Musso R, Janco R, Neuman WR. A retrospective study to describe the incidence of moderate to severe allergic reactions to factor IX in subjects with haemophilia B. *Haemophilia* 2011; 17: 494–9.
- 2 Ahrons S, Glavind-Kristensen S, Drachmann O, Kissmeyer-Nielsen F. Severe reactions after cryoprecipitated human factor VIII. *Vox Sang* 1970; 18: 182–4.

- 3 Pernod G, Armari C, Barro C *et al.* Anaphylaxis following the use of a plasma-derived immunopurified monoclate-P, and the recombinant Recombinate and Kogenate factor VIII: a therapeutic challenge. *Haemophilia* 1999; 5: 143–4.
- 4 Shopnick RI, Kazemi M, Brettler DB *et al.* Anaphylaxis after treatment with recombinant factor VIII. *Transfusion* 1996; 36: 358–61.
- 5 Jadhav M, Warriar I. Anaphylaxis in patients with hemophilia. *Semin Thromb Hemost* 2000; 26: 205–8.
- 6 Tsuchiya H, Shima M, Yoshioka A. Anaphylactic response to factor VIII preparations in a haemophilic child with an inhibitor of high titre during the tolerance induction. *Eur J Pediatr* 1998; 157: 85.
- 7 Shakib F, Stanworth DR. xIgG4: a possible mediator of anaphylaxis in a haemophiliac patient. *Clin Allergy* 1979; 9: 597–603.
- 8 Whelan SF, Hofbauer CJ, Horling FM *et al.* Distinct characteristics of antibody responses against factor VIII in healthy individuals and in different cohorts of hemophilia A patients. *Blood* 2013; 121: 1039–48.
- 9 Lindgren A, Wadenvik H, Tengborn L. Characterization of inhibitors to FVIII with an ELISA in congenital and acquired haemophilia A. *Haemophilia* 2002; 8: 644–8.
- 10 Simons FE. Anaphylaxis. *J Allergy Clin Immunol* 2010; 125: S161–81.
- 11 Kadar JG, Schuster J, Hunzelmann N. IgE-mediated anaphylactic reaction to purified and recombinant factor VIII in a patient with severe haemophilia A. *Haemophilia* 2007; 13: 104–5.
- 12 Verbruggen B, Novakova I, Wessels H, Boezeman J, van den Berg M, Mauser-Bunschoten E. The Nijmegen modification of the Bethesda assay for factor VIII:C inhibitors: improved specificity and reliability. *Thromb Haemost* 1995; 73: 247–51.
- 13 Miller CH, Platt SJ, Rice AS, Kelly F, Soucie JM, Hemophilia Inhibitor Research Study I. Validation of Nijmegen–Bethesda assay modifications to allow inhibitor measurement during replacement therapy and facilitate inhibitor surveillance. *J Thromb Haemost* 2012; 10: 1055–61.