



Jornal Brasileiro de Patologia e Medicina Laboratorial

ISSN: 1676-2444

[jbpml@sbpc.org.br](mailto:jbpml@sbpc.org.br)

Sociedade Brasileira de Patologia  
Clínica/Medicina Laboratorial  
Brasil

Carvalho, Rosineide A.; da Silva, Paulo Henrique; Henneberg, Railson  
Incidence of factor VIII inhibitory antibodies in patients with hemophilia A seen at  
HEMOCE, Ceará, Brazil  
Jornal Brasileiro de Patologia e Medicina Laboratorial, vol. 52, núm. 6, diciembre, 2016,  
pp. 382-386  
Sociedade Brasileira de Patologia Clínica/Medicina Laboratorial  
Rio de Janeiro, Brasil

Available in: <http://www.redalyc.org/articulo.oa?id=393549337004>

- How to cite
- Complete issue
- More information about this article
- Journal's homepage in [redalyc.org](http://redalyc.org)

[redalyc.org](http://redalyc.org)

Scientific Information System

Network of Scientific Journals from Latin America, the Caribbean, Spain and Portugal

Non-profit academic project, developed under the open access initiative

# Incidence of factor VIII inhibitory antibodies in patients with hemophilia A seen at HEMOCE, Ceará, Brazil

## *Prevalência de inibidores do fator VIII em pacientes com hemofilia A atendidos no HEMOCE, Ceará, Brasil*

Rosineide A. Carvalho<sup>1</sup>; Paulo Henrique da Silva<sup>2</sup>; Railson Henneberg<sup>2</sup>

1. Centro de Hematologia e Hemoterapia do Ceará (HEMOCE), Ceará, Brazil. 2. Universidade Federal do Paraná (UFPR), Paraná, Brazil.

### ABSTRACT

**Introduction:** Hemophilia A is an inherited disease caused by a deficiency of factor VIII, which results from a genetic inheritance located on the X chromosome. During treatment of patients with this disorder, factor VIII inhibitors may be present, which are primarily antibodies type immunoglobulin G (IgG), and interfere with the activation of factor VIII. **Objectives:** The present study aims to investigate and quantify the presence of antibodies against factor VIII:C in patients with hemophilia A, treated at the Ceará Hematology Center (HEMOCE). **Material and methods:** Screening for the inhibitor against factor VIII was performed according to the original Bethesda method or the Nijmegen modified assay. **Results:** One hundred eighty-four patients with hemophilia A were evaluated, from November 2012 to February 2015. From the patients evaluated, 149 (80.98%) showed no inhibitor presence, while in 35 patients (19.02%) the presence of the inhibitor was detected. Among inhibitor carriers, most hemophilia patients had high titers of the inhibitor (57.2%). **Conclusion:** The high incidence of factor VIII inhibitor in the study population can be explained by the type of treatment used at HEMOCE, which is based on the factor VIII in its recombinant form. The results should be evaluated carefully, so that the treatment and monitoring of these patients are conducted in the safest way possible.

**Key words:** hemophilia A; factor VIII inhibitor.

### INTRODUCTION

Hemophilia A is a congenital factor VIII deficiency that affects one in 5000 men<sup>(1-3)</sup>, being a sex-linked genetic disorder. Although it is an inherited disease, in 30%-40% of the cases there is no family history of bleeding disorders<sup>(4)</sup>. Carriers of hemophilia A may present bleeding within any organs after a vascular lesion<sup>(3)</sup>. The most common bleeding manifestations are the hemarthrosis and the most affected joints are knees, elbows, ankles, shoulders, and wrist<sup>(5)</sup>. Patients with severe hemophilia show a history of bleedings since their childhood, with later events of bleeding into the joints, post-traumatic and muscular bruising<sup>(6)</sup>.

The diagnosis of hemophilia A is based on clinical background, physical examination and laboratory tests. The laboratory test is suggested from the activated partial

thromboplastin time (aPTT) alteration, which is altered when the average concentration of the factor VIII is lower than 30% of the normal concentration<sup>(4)</sup>.

A major problem in the treatment of patients with hemophilia A is the development of anti-factor VIII inhibitor antibody. The antibodies against clotting factors are classified as non-specific (lupus antibodies) or specific inhibitors (factor VIII inhibitors). Patients with factor VIII, in whom the aPTT does not correct to normal according to the mixing study, they must be submitted to the anti-factor VIII inhibitor antibody investigation. The aPTT show correction when the hemophilic plasma is mixed with equal volume of normal plasma. If the hemophilic plasma contains an anti-factor VIII inhibitor antibody the aPTT on a similar mixture is prolonged, but incubation of the mixture for 1 or 2 hours at 37°C is required to detect the prolongation<sup>(3)</sup>.

About 5%-30% of the patients with hemophilia A develop anti-factor VIII inhibitor antibody, which are antibodies type immunoglobulin G (IgG). The affected patients are mostly those who suffer from the severe form of the disease. The presence of inhibitors is noticed by the lack of response to the traditional treatment or by the increase in the frequency and/or severity of bleeding episodes<sup>(7)</sup>. The identification of anti-factor VIII inhibitor antibody is important to explain the need for higher or lower therapeutic schemes doses of factor VIII. It is recommended that all medical centers attending to hemophilia patients perform the test to detect these inhibitors once a year<sup>(8,9)</sup>.

This study aims to evaluate the presence of anti-factor VIII inhibitor antibody in patients admitted at Hematology and Hemotherapy Center of Ceará (HEMOCE) from November 2012 to February 2015. It will also analyze the age of the patients and determine the distribution of the severity levels of hemophilia A according to the presence of inhibitors.

## MATERIAL AND METHODS

One hundred and eighty-four hemophilia A carriers, all admitted at HEMOCE from November 2012 to February 2015, were evaluated. The date of diagnosis, patient age, severity level of hemophilia and presence of anti-factor VIII inhibitor antibody were considered for all patients.

The research of the anti-factor VIII inhibitor antibody was performed according to the original Bethesda method or the Nijmegen modified assay<sup>(10)</sup>. This research uses a pool of normal plasma incubated in equal proportions with the patient's plasma. In parallel, in another tube, the pool is incubated in equal proportions with an imidazole 0.1 M pH 7.4 buffered solutions. Then, the activity of factor VIII clotting (factor VIII:C) is measured in both mixtures of the pool (the one with the patient's plasma and the one with the imidazole buffer). The difference in FVIII:C activity between both mixtures is called residual activity of factor VIII:C, and is associated to the loss of factor VIII activation after 2 hours in 37°C incubation. The level of inhibitors will be referenced by the use of a standard inhibitor screen defined by the Bethesda method in accordance with the level of residual FVIII:C obtained in the test<sup>(11)</sup>. The methodology used the equipment CA1500 to perform the tests.

## RESULTS

Distribution of patients according to age is shown in **Table 1**.

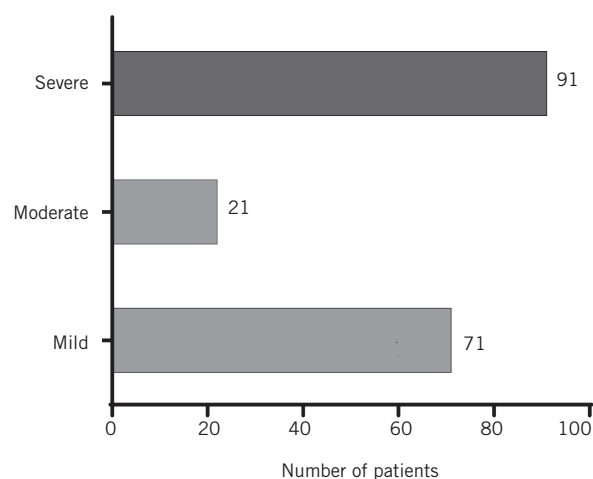
**TABLE 1** – Distribution of patients according to age

Individuals	Frequency	%
Children	75	40.76
Adults	109	59.24
Total	184	100

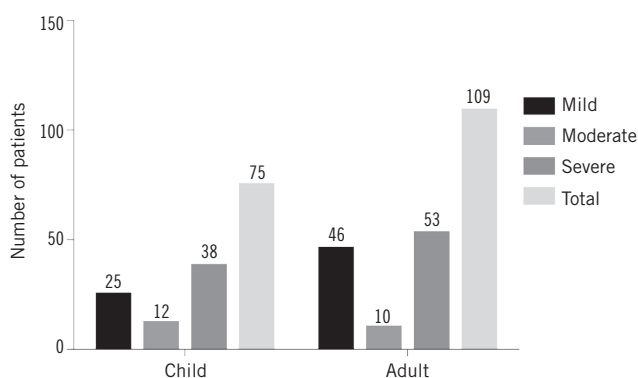
**Figure 1** shows the distribution of the three levels of hemophilia A, mildly, moderately and severely affected patients.

**Figure 2** shows the distribution of hemophilia A categories by age. In children, 33.3% showed mild hemophilia, while 16% showed moderate hemophilia and 50.7%, severe hemophilia. In adults, 42.2% showed mid, 9.2% showed moderate and 48.6%, severe hemophilia.

**Table 2** shows the incidence of the anti-factor VIII inhibitor antibody in patients. The presence of the inhibitor antibody was identified in 19% of patients.



**FIGURE 1** – Distribution of the three levels of severity of hemophilia A



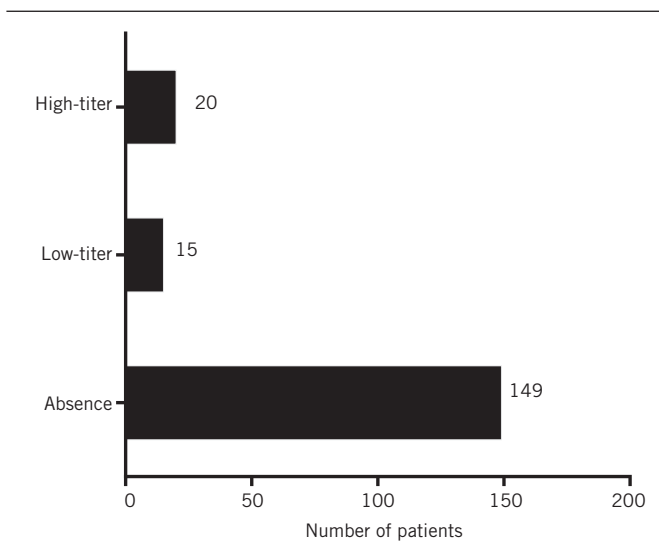
**FIGURE 2** – Distribution of the three levels of severity of hemophilia according to age group

**TABLE 2** – Incidence of anti-factor VIII inhibitor antibody in studied patients

Inhibitor antibody	Frequency	%
Negative	149	80.98
Positive	35	19.02
Total	184	100

The inhibitor antibody was only found in three patients (10.33%) with moderate hemophilia. The presence of the inhibitor antibody in 23 patients with severe hemophilia is in accordance with data found in the literature relating the severity of the disease to the presence of the inhibitor.

**Figure 3** shows the presence of the anti-factor VIII inhibitor antibody according to its titer, following the original Bethesda method or the Nijmegen modified assay<sup>(11)</sup>. According to the Italian Association of Hemophilia Centers (IAHC) the response is considered low (low titer) when the inhibitor titers are less than or equal to 5 UB/ml in children and adults. From the 35 hemophilia patients who carry an anti-factor VIII inhibitor antibody, 15 (42.8%) presented low titer and 20 (57.2%), high titer.

**FIGURE 3** – Presence of the of anti-factor VIII inhibitor antibody

## DISCUSSION

The only efficient therapy for hemophilia A is the compensation of factor VIII, which can be carried out by the administration of factor VIII, in its plasma-derived or recombinant form. The formation of inhibitors is the main complication in the treatment<sup>(1)</sup>.

Many risk factors for the development of anti-factor VIII inhibitor antibody have been described in patients with hemophilia A. The incidence of inhibitors depends on genetic and non-genetic factors.

Among the genetic factors, the severity of hemophilia, the mutation type, the ethnicity, the family history of inhibitors, and the human leukocyte antigen (HLA) genotype stand out. The intensity and duration of the treatment are the main non-genetic factors. The type of factor VIII concentration that is used might also influence the incidence of inhibitors. The frequency of inhibitors in patients treated only with factor VIII concentrated from plasma is lower in comparison with the use of recombinant factor VIII<sup>(12)</sup>.

The inhibitors are polyclonal populations of IgG that have better affinity to the antigenic sites in the A2, A3 and C2 factor VIII domains<sup>(1)</sup>. The inhibitors neutralize factor VIII and increase the morbidity and mortality of patients with hemophilia when compared to those who do not carry the inhibitors<sup>(13)</sup>.

For this study, 184 hemophilia A patients admitted at HEMOCE were investigated, including children and adults. After the determination of the inhibitor's presence, its quantification was performed. In the sample that was analyzed, 62 patients (33.7%) had mild hemophilia, 22 patients (11.96%), moderate hemophilia and 100 patients (49.46%) showed severe hemophilia. The risk of inhibitor development increases in the first 10-15 days after exposure to the treatment with factor VIII. For this reason, the studies that aim to explain the genetic and non-genetic factors for the development of inhibitors have sought non-treated hemophilia patients<sup>(13)</sup>. In this study, the period time of treatment initiation was not evaluated due to limitations to obtain that information.

The frequency of inhibitors in the studied population was of 19%, with predominance of the inhibitor associated to the severe form of the disease. There was no difference in the presence of inhibitors related to the age group. Such frequency (of 19%) is considerably higher to that found in other research projects. Wang *et al.* (2010)<sup>(1)</sup> studied 1,435 hemophilia patients and verified a low frequency (3.9%) of inhibitors, with predominance in patients with severe hemophilia A. Aznar *et al.* (2014)<sup>(13)</sup> found low incidence (9.3%) of inhibitors analyzing hemophilia patients who had been exposed to the treatment for an average of 958 days. In this study, the participants were submitted to changes in the therapeutic schemes regarding the source of factor VIII, which did not have influence in the incidence of inhibitors. Gown *et al.* (2015)<sup>(14)</sup> studied 366 hemophilia A patients and found 87 patients with inhibitors, 69 with high titer and 18 with low titer.

There are controversies regarding the incidence of factor VIII inhibitors in connection with the type of factor VIII used in the treatment (plasma-derived or recombinant), or if the commercial brand factor VIII might influence this sensitization<sup>(13)</sup>.

Goudemand *et al.* (2006)<sup>(12)</sup> researched the presence of factor VIII inhibitors in 148 hemophiliac patients before initiating the

treatment. Comparing the types of factor VIII that were used, 14 hemophilia patients developed inhibitors with the use of plasma-derived factor VIII while 49 developed inhibitors using recombinant factor VIII. In this study, all patients were treated with recombinant factor VIII. There is a higher risk (2.5 to 3 times) of inhibitor development when hemophilia patients are treated with recombinant factor VIII<sup>(12)</sup>.

The type of treatment is a fundamental factor to explain the incidence of inhibitors in hemophilia. The 19% index in this study might be explained by the fact that the patients were treated with recombinant factor VIII, which must be critically evaluated by the health professionals' team at the HEMOCE.

## CONCLUSION

The results of this study may be used as important tools for the HEMOCE to achieve a better understanding in the treatment of hemophilia patients. The presence of anti-factor VIII inhibitor antibody might represent changes in therapeutic schemes of hemophilia patients and explain possible clinical complications in such patients. The incidence in hemophilia A carriers attended at HEMOCE can be considered high when compared to other studies. The treatment with recombinant factor VIII must be evaluated for the implementation of measures that could avoid the development of factor VIII inhibitors in patients who have not initiated treatment yet.

## RESUMO

**Introdução:** A hemofilia A é uma doença hereditária causada pela deficiência do fator VIII, resultante de uma herança genética ligada ao cromossomo X. Durante o tratamento de pacientes com essa doença, pode ocorrer presença de inibidores do fator VIII, os quais são, em sua maioria, anticorpos do tipo imunoglobulina da classe G (IgG), que interferem na ativação do fator VIII. **Objetivos:** O presente estudo tem como objetivo pesquisar e quantificar a presença de anticorpos contra o fator VIII:C em pacientes com hemofilia A, atendidos no Hemocentro Ceará (HEMOCE). **Material e métodos:** A pesquisa para detecção do inibidor do fator VIII foi realizada de acordo com a modificação do método de Nijmegen Bethesda original. Foram avaliados 184 pacientes com hemofilia A entre novembro de 2012 e fevereiro de 2015. **Resultados:** Dos pacientes avaliados, 149 (80,98%) não revelaram presença do inibidor, enquanto em 35 (19,02%) essa presença foi detectada. Entre os portadores dos inibidores, a maioria dos pacientes hemofílicos apresentaram títulos elevados do inibidor (57,2%). **Conclusão:** A incidência elevada de inibidor do fator VIII na população em estudo pode ser explicada pelo tipo de tratamento utilizado no HEMOCE, o qual se baseia no fator VIII na sua forma recombinante. Os resultados devem ser avaliados com critério para que o tratamento e o acompanhamento desses pacientes sejam realizados da maneira mais segura possível.

**Unitermos:** hemofilia A; inibidor do fator VIII.

## REFERENCES

1. Wang XF, Zhao YQ, Yang RC, et al. The prevalence of factor VIII inhibitors and genetic aspects of inhibitors development in Chinese patients with haemophilia A. *Haemophilia*. 2010; 16(4): 632-9.
2. Bitchell TC. Distúrbios hereditários da coagulação. In: Lee GR, Bitchell TC, Foerster J, Athens JW, Lukens JN. *Wintrobe Hematologia Clínica*. vol II. 9 ed. São Paulo: Editora Manole; 1998. Cap. 56, p. 1562-1616.
3. Silva PH, Hashimoto Y. Coagulação visão laboratorial da hemostasia primária e secundária. Rio de Janeiro: Livraria e Editora Revinter; 2006. p. 136.
4. Green D, Dimichele DM. Hemophilia — factor VIII deficiency. In: Loscalzo J, Schafar AI. *Thrombosis and hemorrhage*. 2 ed. Williams & Wilkins; 1998. Cap. 35, p. 757-72.
5. Vilanção PR, Carneiro JDA, D'amico EA. Hemofilias. In: Zago MA, Falcão RP, Pasquini R. *Hematologia: fundamentos e prática*. São Paulo: Atheneu; 2001. Cap. 73, p. 803-18.
6. Kruse-Jarres R. Hemofilia A e inibidores: avanços na prevenção e otimização do tratamento da hemofilia. *ITI*. 2014; 20 suppl.6.1.
7. Chaves DG, Rodrigues CV. Desenvolvimento de inibidores do fator VIII na hemofilia A. *Rev Bras Hematol Hemoter*. 2009; 31(5).
8. Kasper CK, Aledort LM, Counts RB, et al. A more uniform measurement of factor VIII inhibitors. *Thromb Diath Haemorrh*. 1975; 34: 869-72.
9. Verbruggen B, Novakova I, Wessels H, Boezeman J, van der Berg M, Mauser-Bunschoten E. The Nijmegen modification of the Bethesda assay for factor VII:C inhibitors: improved specificity and reliability. *Thromb Haemost*. 1995; 73: 247-51.

10. Austen DE, Lechner K, Rizza CR, Rhymes IL. A comparison of the Bethesda and New Oxford methods of factor VIII antibody assay. *Thromb Haemost.* 1982; 47(1): 72-5.
11. Brasil. Ministério da Saúde. Manual de diagnóstico laboratorial das coagulopatias hereditárias e plaquetopatias. Brasília; 2012. p. 81-8.
12. Goudemand J, Rothschild C, Deminguel V, et al. Influence of the type of factor VIII concentrate on the incidence of factor VIII inhibitors in previously untreated patients with severe hemophilia A. *Blood.* 2006; 107(1).
13. Aznar JA, Moret A, Ibanez F, Vila C, Cabrera N, Mesa E, Bonanad S. Inhibitor development after switching of FVIII concentrate in multi-transfused patients with severe haemophilia A. *Haemophilia.* 2014; 20: 624-9.
14. Gouw S, van der Bom JG, Marijke van der Berg H. Treatment-related risk of inhibitors development in previously untreated patients with hemophilia A; the CANAL Cohort Study. *Blood.* 2015; 109(11): 4648-54.

---

**CORRESPONDING AUTHOR**

**Railson Henneberg**

Universidade Federal do Paraná, campus 3; Departamento de Análises Clínicas; Avenida Lothario Meissner, 632; Jardim Botânico; CEP: 80210-170; Curitiba-PR, Brasil, e-mail: railson@ufpr.br.