Journal of Blood Transfusions and Diseases
JBTD, 3(1): 126-129
www.scitcentral.com
ISSN: 2641-4023
Original Research Article: Open Access

FVIII Inhibitors during Primary Prophylaxis for Hemophilia A: A Pilot Study in Cameroon

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Received November 19, 2019; Accepted November 25, 2019; Published April 25, 2020

ABSTRACT

The occurrence of inhibitors of anti-hemophilic factors during treatment remains the main complication in the management of hemophilia. In Cameroon, there are over 160 people diagnosed with hemophilia, usually receiving "on-demand" treatment, with restricted access to primary prophylaxis. Thus, such prophylaxis is poorly assessed in Africa.

Objective: To determine the frequency of inhibitors in patients with hemophilia A during primary prophylaxis.

Method: A cohort of 5 patients undertaking primary prophylactic treatment of hemophilia A was followed from January to December 2018. The screening for inhibitors using the APTT to test 50:50 plasma patients mixed with normal plasma was performed every 5 cumulative exposure days. We performed the titration of inhibitors using the Bethesda Nijmegen method on all positive samples.

Results: Out of the five patients included in our cohort, four patients were tested positive at least once during treatment. Two patients had developed high responder inhibitors before prophylaxis and had a bleeding frequency of 2.5 to 3 per month of which 40 to 80% were hemarthrosis. The other two patients developed low responder inhibitors during treatment with a bleeding frequency of 1 per month, of which 19-20% had hemarthrosis. The only patient who did not develop inhibitors was never subjected to hemarthrosis.

Conclusion: The primary prophylactic treatment may be effective in improving the quality of life of patients and may be responsible for the appearance of low responder inhibitors with minimal influence on treatment in Cameroon.

Keywords: Hemophilia, Primary prophylaxis, Inhibitors of anti-hemophilic factors

INTRODUCTION

Hemophilia affects nearly 400,000 people worldwide and although the management of this pathology has improved significantly since the advent of anti-hemophilic factor concentrate substitution therapy, it is still a real challenge in Africa given the relatively high cost and low availability of replacement factors [1]. The primary prophylactic treatment that prevents the risk of bleeding and especially the occurrence of arthropathy is recommended by the World Federation of Hemophilia but has been very little experienced in Sub-Saharan Africa. Yet it would be an ideal tool for improving the quality of life of hemophiliac patients. Although it has been shown that the use of low doses of anti-hemophilic factor (AHF) in the management of this pathology gives satisfactory results [2]. The fact remains that the most frequent and most feared complication is the appearance of AHF inhibitors which neutralize the procoagulant activity of the injected factors [3]. It develops mainly during the treatment of hemophilia A, in 20 to 30% of the treated patients and generally appears during the first 50 cumulative exposure days (CED) [3]. As part of a pilot study at the Hemophilia Treatment Center at the Yaoundé University Teaching Hospital, concerning the primary prophylactic treatment of hemophilia A patients on long-acting factor concentrates, this study was carried out to examine the occurrence of AHF inhibitors. Furthermore, the study intended to describe the types of bleeding that occurred during primary prophylaxis and to identify the profile of patients who developed these inhibitors.

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Citation: Chendjou A, Ndoumba A, Kalla C, Tayou C & Mbanya D. (2020) FVIII Inhibitors during Primary Prophylaxis for Hemophilia A: A Pilot Study in Cameroon. J Blood Transfusions Dis, 3(1): 126-129.

MATERIALS AND METHODS

We conducted a prospective cohort study that included hemophilia patients on primary prophylaxis with long-acting AHF concentrates. The study took place at the Yaoundé University Teaching Hospital from January 2018 to December 2018. The participants were recruited through the information contained in their medical records (including telephone contacts) stored at the Yaounde Hemophilia Treatment Center. Children with hemophilia who were younger than 7 years old, with less than 2 documented histories of joint bleeding, not having clinical arthropathy were included in the study. Furthermore, they have to live in Yaoundé and with parental commitment to regularly bring the children to the hospital, at least once a week, for prophylaxis and follow-up. The parents of those who met the clinical criteria were contacted and following their consent. Patients who dropped out were automatically excluded. Prophylaxis consisted in injection of 10-20 UI/kg of AHF twice a week at regular interval at the Hemophilia Treatment Center.

For each patient included in the cohort study, screening and titration of AHF inhibitors was done from the 10th CED and every 5 CED until the 50th CED or until the appearance of the inhibitors. Blood samples were collected from all patients into tubes containing sodium citrate (0.109 M) and centrifuged at 1700 g for at least 10 min [4]. The Start 4 semi-automatic coagulometer and STA reagents (Diagnostica Stago, Paris, France) were used to perform the analysis on the patient plasmas. Inhibitor screening was done using an Activated Partial Thromboplastin Time (APTT) assay and the Rosner index was determined, based on the formula [5]:

$$100 \times [(\text{APTT mixture} - \text{control APTT})/\text{sick APTT}]$$

All patients with a Rosner score greater than or equal to 12% were considered positive for inhibitors.

Inhibition titration by the Bethesda Nijmegen method was performed in all patients who screened positive. The source of factor VIII was a plasma pool of 20 normal individuals with no history of hemostatic disorder. This plasma pool was buffered with 1 M Hydroxy Ethyl Piperazine Ethan Sulfonic: HEPES Buffer (SIGMA Aldrich, St. Louis USA) to improve the stability of Factor VIII (FVIII) during incubation [6,7]. The residual FVIII level of this mixture was then measured by comparing the value of its APTT with that of the standard sample. Based on the FVIII level obtained, the inhibitor titer of the sample was determined, as defined by a Bethesda Unit (BU) which is the amount of inhibitor that neutralizes 50 % of the activity of an International Unit (IU) of FVIII in two hours of incubation at 37°C [4,7]. The sensitivity threshold for this technique was 0.6 Bethesda units per milliliter (BU/mL). A patient was classified as a low responder when his inhibitor titer was less than 5 BU/mL and a high responder when the titer was greater or equal to 5 BU/mL [4,8]. The CS-Pro Version 7.00 and Microsoft Excel 2013 software were used to analyze the data obtained.

RESULTS

Our cohort consisted of 5 hemophiliac A patients, of which 2 had moderate hemophilia and 3 had severe disease. The age of the patients ranged between 1 and 7 years. Patients 2 and 4 were cousins **(Table 1)**. The age of discovery of the disorder ranged from one to 18 months and the circumstances of discovery of the pathology were gum bleeds or bleeding during circumcision. All the patients were already taking anti-hemophilic drugs prior to the implementation of the prophylaxis scheme, with a maximum of 22 CED. The two cousins included in our study previously had inhibitors at the time of first screening, although they had less than 10 CED when prophylaxis was initiated **(Table 1)**.

Table 1. Characteristics of a population of 5 hemophilia A patients during a primary prophylaxis treatment.

Patients	Type of hemophilia	FVIII rate (%)	Severity of the deficit	Age (year)	Circumstance of discovery	CED before prophylaxis	Relationship
Patient 1	A	<1%	Severe	01	Circumcision	01	No
Patient 2	A	<1%	Severe	03	Gum bleeding	04	Cousin of the patient 4
Patient 3	A	1%	Moderate	04	Hematoma, epistaxis	22	No
Patient 4	A	4.3%	Moderate	04	Gum bleeding	09	Cousin of the patient 2
Patient 5	A	<1%	Severe	07	Circumcision	01	No

Out of the 5 hemophiliac patients on prophylaxis, 4 were screened positive for AHF inhibitors during the study. Patients who were already developing inhibitors at the time of first screening were “high responders”. However, patients who developed inhibitors with a progressive onset were “low responders”. The “high responder” patients developed

the inhibitors throughout the study; the prophylaxis was interrupted at the 30th CED because of bleeding manifestations and an Immune Tolerance Induction (ITI) was initiated. In "low responder" patients, inhibitors appeared transiently throughout the study **(Table 2)**.

Table 2. Evolution of the AHF inhibitors titer during a primary prophylactic treatment.

CED	0	10	15	20	25	30	35	40	45	50
	Titer of AHF Inhibitors (BU/mL)									
Patient 1	-	-	-	-	-	-	-	-	-	-
Patient 2	-	>16	>16	>16	>16	>16	End of prophylaxis and switch to ITI			
Patient 3	-	-	-	-	-	-	-	-	0.70	-
Patient 4	-	>16	>16	>16	>16	>16	End of prophylaxis and switch to ITI			
Patient 5	-	-	-	-	-	1.34	-	-	-	0.75

Throughout the prophylaxis, high responders to AHF inhibitors had 2-3 times more bleeding (2.5 to 3 monthly bleeds) than the rest of our study population with relatively high joint bleeding frequencies (40 to 80% bleeding observed) compared to the others **(Table 3)**.

Table 3. Monthly types and frequencies of bleeding during primary prophylaxis treatment.

Patients	Type of inhibitors	Type de bleeding (%)			Frequency of bleeding/Month
		Hematoma	Hemarthrosis	Others	Median (interval)
Patient 1	-	86%	-	14%	1 (0-2)
Patient 2	High responder	60%	40%	-	2.5 (1-3)
Patient 3	Low responder	69%	19%	12%	1 (0-4)
Patient 4	High responder	20%	80%	-	3 (1-3)
Patient 5	Low responder	60%	20%	20%	1 (0-2)

DISCUSSION AND CONCLUSION

Our study revealed that four of the five patients in this study had a positive Bethesda Nijmegen test at least once during prophylaxis among which two were high responders.

This is the first study in Central Africa that reports appearance of inhibitors in hemophiliac patients during primary prophylaxis. Few studies have reported the frequency of inhibitors in hemophiliac patients under prophylaxis in Africa. Kraiem et al. [10] in Tunisia found only 5% of positive patients for inhibitors in a cohort of 32 patients in 2012. Similarly, Balogog et al. [11] in Cameroon had found a prevalence of inhibitors of 19% in a population of 42 hemophilia patients of whom 38 were diagnosed hemophilia A. These are different from the results we obtained probably because of the small size of our sample. In addition, according to data from a Swedish registry that included 460 pairs of hemophilia A or hemophilia B brothers, the existence of a family history significantly increases the risk of inhibitors [12]. It is also described that the risk of inhibitors is higher in black subjects and in families with antecedent of antibodies compared to the others [12]. This could explain the consanguinity found between the two high responding patients to AHF inhibitors in this study. In contrast to high responders, inhibitors in low responders appeared relatively late in prophylaxis and resolved spontaneously. This better reflects the characteristics of transient inhibitors, which are defined as "low-titer inhibitors, sometimes exceeding 5 BU and "spontaneously disappearing" after a certain time, without modification of the therapeutic regimen" [3,7].

The higher frequency of bleeding in high responders compared to others shows the impact of AHF inhibitors on the health and well-being of patients by rendering the treatment in place completely ineffective. Unfortunately, in our context, we do not have by-pass products whose effectiveness has however been demonstrated in such cases [7,13,14]. Low responder patients had a predominance of hematomas compared with joint bleeds during prophylaxis. Thus showing the action, although reduced but presents, of low responder inhibitors on the primary prophylactic treatment put in place. This is especially true since the only

patient who had never developed inhibitors throughout the study, was not subject of joint bleeding.

The study population consisted exclusively of moderate and severe hemophilia A patients, who met the set eligibility criteria. Hemophilia A occurs more frequently worldwide, and would explain why they were predominant in this study. A relationship was found between two patients included in our cohort, coincidentally and despite the small size of our sample. This is not surprising given the hereditary nature of the pathology because in about two-thirds of cases, patients have a family history of hemorrhagic disease [3].

Among the secondary findings, we noted that the age of discovery of the pathology was relatively low in our study population (less than 18 months). This may be due to the rapid management of patients during the first year of their life, which will have made it possible to prevent bleeding episodes and thus preserve their joint function. This is also the reason why all had already received AHF. Circumcision and gum bleeding were the most common circumstances of discovery of pathology in our cohort. This is close to the results of the 2001 French cohort showing that the hemorrhagic event represents the first diagnostic circumstance for hemophilia, accounting for 59.9% of cases [9]. The two cousins included in our study already had inhibitors despite having less than 10 CED. This makes us think that genetic factors including the abnormality of the FVIII gene would have predisposed these patients to a rapid development of inhibitors. In-depth genetic testing would provide more insight for our 2 patients. The results of the AFSSaPS report (Agence Française de Sécurité Sanitaire des Produits de Santé) on the development of inhibitors and the management of hemophilia patients supports this hypothesis. According to this report, the type of abnormality affecting the FVIII gene significantly modifies the risk of inhibitors up to 90% of the most deleterious abnormality cases [8]. In addition, the early establishment of prophylaxis in children at the first exposure could have an effect comparable to that of the Immune Tolerance Induction (ITI) treatment and induce the disappearance of early-onset AHF inhibitors.

We recognize that the main limit of this study is the sample size. This is a pilot study that can be considered as the first description of inhibitors appearance in western African hemophiliac patients. However, it indicates that exposition to regular AHF during primary prophylaxis may increase the frequency of inhibitors. Primary prophylaxis in Africa needs to be better assessed to find the best strategy that will balanced the benefit in terms of reduction in frequency of bleeding with the risk of inhibitor appearance.

ACKNOWLEDGEMENT

The authors of this article declared that they had no interests which might be perceived as posing a conflict or bias. A special thanks to the staff at the Hematology and Blood Transfusion unit of the Yaoundé University Teaching Hospital. Our deepest gratitude to all the patients and parents who participated in this study. Also acknowledge the WFH who provided the clotting factors.

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