Scholars Journal of Medical Case Reports
Abbreviated Key Title: Sch J Med Case Rep
ISSN 2347-9507 (Print) | ISSN 2347-6559 (Online)
Journal homepage: https://saspublishers.com

OPEN ACCESS

Medicine

Monitoring of Hemophilia A in Morocco

Amina Chraibi[1,2*], Hassane Mamad[1,2], Khalid Edderdouri[1,2], Adjimon Ganhoutode[1,2], Souad Benkirane[1,2], Azlarab Masrar[1,2]

[1]Hematology Laboratory, Ibn Sina University Hospital - Rabat
[2]Faculty of Medicine and Pharmacy - Mohamed V University of Rabat

DOI: 10.36347/sjmcr.2024.v12i04.025 | **Received:** 01.03.2024 | **Accepted:** 08.04.2024 | **Published:** 18.04.2024

***Corresponding author:** Amina Chraibi
Hematology Laboratory, Ibn Sina University Hospital - Rabat

Abstract **Original Research Article**

Hemophilia A is a hemorrhagic disorder caused by a deficiency in factor VIII coagulation. Its incidence is 1 to 2 per 10,000 male births. Hemophilia A is characterized by intra-articular, mucosal, or cutaneous bleeding, and its management relies on the substitution of factor VIII with plasma derived or recombinant products. However, the major complication of treatment is the development of anti-factor VIII inhibitors, rendering substitution therapy ineffective. The aim of this study is to investigate the prevalence of anti-factor VIII inhibitors, the severity of hemophilia, and the follow-up of hemophilia patients developing anti-FVIII inhibitors in a hemophiliac population by presenting their epidemiological and biological characteristics. We conducted a retrospective descriptive cross-sectional study at the Central Hematology Laboratory of the Ibn Sina Hospital Center in Rabat (Morocco) over a period of 37 months, from December 1, 2020, to December 31, 2023. The study included 172 hemophilia A patient, with a mean age of 25 years. 55 patients developed anti-FVIII inhibitors (32%). The majority of positive inhibitor hemophiliacs were severe hemophiliacs (87%), including 23 cases of low responders (42%) and 32 cases of high responders (58%). The results obtained partly agree with most national studies (H. Mamad 2021, Y. El Aissaoui 2016, and F. Zizi in 2011), and international ones. However, the development of inhibitors varies from one individual to another according to several criteria (genetic and non-genetic).

Keywords: Hemophilia A, Factor VIII, Anti-Factor VIII Inhibitor, Severe Hemophilia, High Responders, Low Responders.

INTRODUCTION

Hemophilia A is a bleeding disorder caused by a deficiency in factor VIII of coagulation. It is a genetic disease with recessive X-linked transmission. It manifests in males and is transmitted by carrier females.

It is a relatively rare condition, but it remains the most common congenital coagulopathy [1]. Its incidence is 1 to 2 per 10,000 male births, while female hemophilia is exceptional [2].

Clinically, hemophilia A is characterized by intra-articular, mucosal, or cutaneous bleeding. However, deep bleeds, though less common, are severe and require urgent management.

Depending on the blood level of factor VIII, hemophilia A can be mild, moderate, or severe. Diagnosis is based on screening in families at risk, or on hemorrhagic signs in sporadic cases.

Management of hemophilia A is essentially based on the substitution of antihemophilic factor by plasma-derived or recombinant substitutes, for curative and preventive purposes [3]. However, a major complication of treatment lies in the development of inhibitors directed against factor VIII, rendering substitute treatment ineffective.

Through this study, we report the experience of the Central Hematology Laboratory of the Ibn Sina Hospital (CHIS), Rabat (Morocco), in examining the occurrence and prevalence of anti-factor VIII inhibitors, the severity of hemophilia A, and the follow-up of hemophilia patients developing anti-FVIII inhibitors in an exposed hemophilia population, outlining their epidemiological and biological characteristics.

MATERIALS

This is a retrospective, descriptive cross-sectional study involving patients with hemophilia A,

conducted at the Central Hematology Laboratory of Ibn Sina Hospital Center in Rabat, between December 1, 2020, and December 31, 2023, spanning a 37-month period.

Inclusion Criteria:

Hemophilia A patients with factor VIII levels ≤ 40%, and those with anti-FVIII inhibitor titers ≥ 0.6 UB/ml.

Exclusion Criteria:

Non-hemophilia A patient with factor VIII levels > 30%, and hemophilia A patients classified as inhibitor-negative with inhibitor titers below 0.6 BU/ml.

For each patient, we collected epidemiological information (name, first name, gender, age), and biological data (degree of severity of hemophilia, presence or absence of inhibitors, kinetics of low and high inhibitor responders). All our patients underwent the following hematological tests and assays: Activated partial thromboplastin time (APTT), Prothrombin time (PT), Fibrinogen assay (Fg), Factor VIII activity assay, and screening and titration of FVIII inhibitors.

METHODS

Pre-Analytical Phase

Our patients underwent venous sampling performed by direct puncture, with a loosely applied tourniquet for less than a minute, then promptly transported to the laboratory at a temperature of 20°C +/- 2°C.

Sampling was done using 2 citrate tubes (5 ml of venous blood), of which the filling volume must be respected (1V citrate/9V blood), followed by double thermostat-controlled centrifugation (15 to 20°C), at 2500g for 15 minutes, or frozen at -80°C if the analysis was delayed.

Analytical Phase

The conducted tests were divided into two categories: tests to assess hemophilic involvement and its progression, and tests to detect the presence or absence of inhibitors, measure their inhibition levels, and perform their titration. Hemostasis tests were conducted using the optical method on the ACLTOP 750 Werfen analyzer.

In hemophilia, the activated partial thromboplastin time (APTT) is prolonged, while the prothrombin time (PT) and platelet count are normal. Specific factor assays determine the type and severity of hemophilia. Furthermore, the biological diagnosis of an anti-FVIII inhibitor is generally based on demonstrating its inhibitory effect on the procoagulant functions of FVIII.

a. **Prothrombin Time (PT):** This measures the coagulation time at 37°C of platelet-poor, decalcified plasma in the presence of excess calcium thromboplastin. It is expressed in seconds and compared to a control, then converted to prothrombin percentage (PT%). Reference values in adults: PT: 70-100%

b. **Activated partial Thromboplastin Time (APTT**): This measures the coagulation time of platelet-poor plasma at 37°C in the presence of phospholipids (cephalin), a contact phase activator (ellagic acid, micronized silica, kaolin, celite), and calcium chloride. It is typically the first test conducted for hemophilia diagnosis, and a prolonged APTT is characteristic. A normal APTT is indicated by the ratio of patient APTT to control APTT, with a value equal to or less than 1.2 in adults.

c. **Functional Fibrinogen Assay:** Conducted using the Von Clauss method. Reference value in adults: 2 to 4 g/L.

d. **Mixing Test (Inhibitor Detection):** This test is conducted to investigate the cause of APTT prolongation. It is essential to calculate the Rosner index, defined as

[(APTT (P+T) – APTT (T)) / APTT (P)] * 100, with times expressed in seconds.

P: Patient Plasma, C: Control Plasma.

A Rosner index below 12% indicates no inhibitory effect, while a value above 15% suggests the presence of an inhibitor. If the index falls between 12% and 15%, the result is considered equivocal.

In hemophilia patients, failure to correct should prompt further investigation for the presence of an inhibitor.

e. **Determination of FVIII Activity (Baseline Level):** Currently, there are two techniques for measuring FVIII activity. The first method is the chronometric method, and the second is the chromogenic method (involving the addition of a chromogenic substrate).

f. **Screening and Titration of Anti-FVIII Inhibitors:** Screening and titration of anti-FVIII inhibitors: The detection and titration of inhibitors are conducted using the Nijmegen method, which is a modification of the Bethesda method designed to enhance sensitivity and specificity. This method is currently regarded as the reference standard by the FVIII/FIX Scientific Subcommittee of the International Society on Thrombosis and Haemostasis. Inhibitor titration is expressed in Bethesda units (BU/ml), representing the quantity of antibodies capable of inhibiting 50% of FVIII in 1

ml of plasma. This titration is determined using a theoretical curve, factoring in pre-dilution. The currently accepted positivity threshold is 0.6 BU/ml.

Data were initially collected in an Excel spreadsheet and then analyzed using SPSS software.

RESULTS

Our study included 172 patients with hemophilia A, all of whom were male, with an age range from 1 to 78 years old (the average age range was between 21 and 25 years).

Table I: Distribution of different age groups of patients with hemophilia A

Age	Number of Patients (N)	Percentage (%)
< 6 months	0	0
6 months - 5 years	23	6,39
6 years - 10 years	28	13,37
11 years - 15 years	21	16,27
16 years - 20 years	21	12,2
21 years - 25 years	24	13,95
26 years - 30 years	18	10,46
> 30 years	47	27,32
Total	172	100.0

However, individuals with hemophilia who tested positive for inhibitors were of various ages, ranging from 2 to 70 years old. There was a predominance of individuals aged between 21 and 25 years, accounting for 20%.

Table II: Distribution of different age groups of positive inhibitor patients

Age	Number of patients (N)	Percentage (%)
< 6 months	0	0
6 months- 5 years	5	9,09
6 years - 10 years	10	18,18
11 years - 15 years	9	16,36
16 years - 20 years	5	9,09
21 years - 25 years	11	20
26 years - 30 years	6	10,9
> 30 years	9	16,36
Total	55	100

Among the 172 patients collected at the hematology laboratory, 55 patients tested positive for inhibitors, with a prevalence of 32%, while 123 did not develop anti-FVIII inhibitors (inhibitor-negative). (Figure 1)

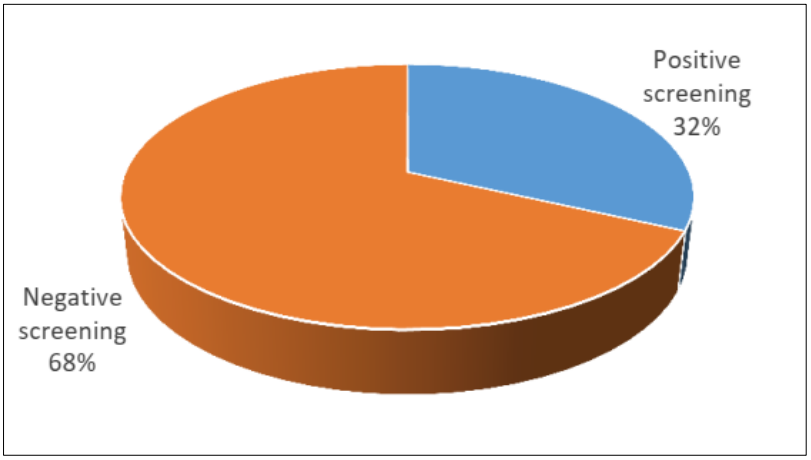

Figure 1: Screening results of inhibitors in hemophilia a patients

Our patients were categorized according to the severity of hemophilia into 97 severe hemophiliacs, 41 moderate hemophiliacs, and 34 mild hemophiliacs. (Figure 2)

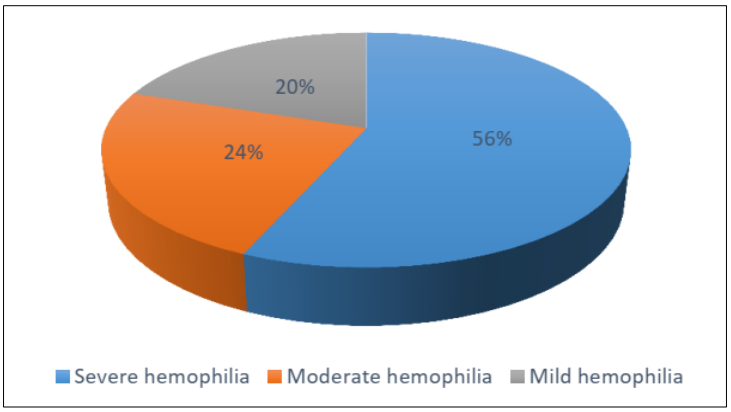

Figure 2: Representation of all hemophilia A patients according to their severity

Among positive inhibitors, we identified 48 severe hemophilia A patients and 7 moderate hemophilia A patient. (Figure 3)

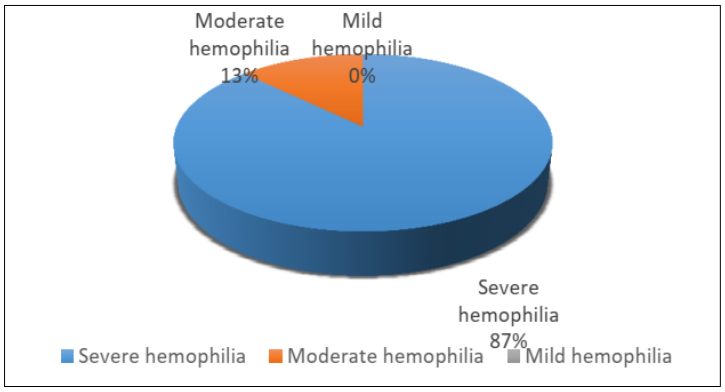

Figure 3: Representation of positive inhibitors according to their severity

The inhibitor levels range from 26% to 100%. However, the titer of anti-FVIII inhibitors ranges from 0.72 to 138.88 BU/ml, with an average value of 27.94 BU/ml.

Table III: Statistical representations of inhibition levels and inhibitor titers

	N	Maximum	Minimum	Mean	Standard deviation
Inhibition level (%)	55	100	26,2	83,09	21,8
Inhibitor titer UB/ml	55	138,88	0,72	27,94	37,77

We classified patients who developed anti-FVIII inhibitors based on their anti-FVIII titers into two categories: low responders (inhibitor titer ≤ 5 BU/ml) and high responders (inhibitor titer > 5 BU/ml). In this study, we identified 23 cases of low responder inhibitors, accounting for a frequency of 42%, and 32 cases of high responder inhibitors, accounting for 58%. (Figure 4)

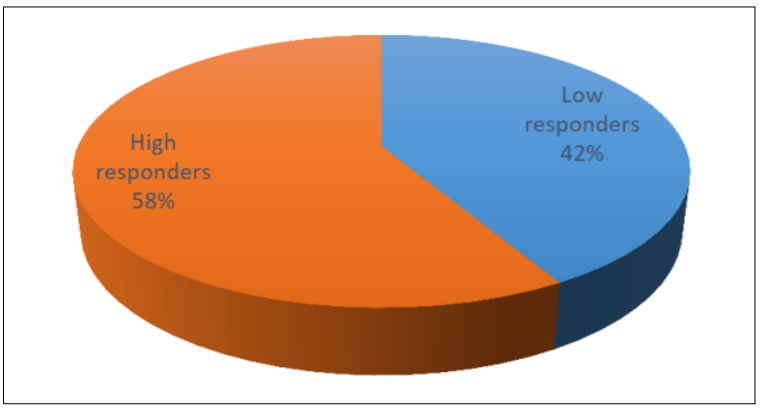

Figure 4: Distribution of high and low responder inhibitors

Among the high responders, the inhibitor titer ranged from 5.64 to 138.88 BU/ml, with 29 cases identified as severe hemophilia A patient, representing a frequency of 90.62%, and 3 cases identified as moderate hemophilia A patient, representing 9.37%.

As for the low responders, the inhibitor titer ranged from 0.72 to 4.72 BU/ml, with 19 cases identified as severe hemophilia A patient, accounting for 82.6%, and 4 cases identified as moderate hemophilia A patient, accounting for 17.39%

Table IV: Distribution of low and high responder inhibitors according to severity level

Severity level	Inhibitors	
	Low responders	High responders
Severe hemophilia	19 (82,6%)	29 (90,62%)
Moderate hemophilia	4 (17,39%)	3 (9,37%)
Mild hemophilia	0 (0%)	0 (0%)
Total	23 (100%)	32 (100%)

In order to adapt the replacement therapy and due to the chronic nature of the disease and the ongoing screening for inhibitors, we observed that out of 55 positive inhibitor patients, 24 underwent multiple Factor VIII assays and inhibitor titrations.

Among these 24 patients, we observed four distinct evolution kinetics based on their inhibitor titers:

- 9 highly responsive patients with inhibitor titers consistently above 5 BU/ml.
- 4 low responder patients with inhibitor titers consistently below 5 BU/ml.
- 3 patients transitioned from being high responders to low responders in terms of inhibitor titration.
- 8 patients transitioned from being low responders to high responders in terms of inhibitor titration.

DISCUSSION

Hemostasis is a physiological process involving a series of phenomena triggered by vascular injury, aimed at plugging the bleeding and involving a complex interplay of intracellular biochemical mechanisms and plasma coagulation factors.

The balance between these mechanisms, pro-coagulant factors, and anticoagulant mechanisms is crucial, as disruption of this balance can lead to either hemorrhagic or thrombotic processes [4].

Hemophilia A is an inherited hemorrhagic disorder linked to a deficiency in factor VIII. Since factor VIII is a crucial plasma glycoprotein in the coagulation process, its deficiency leads to decreased and delayed thrombin generation, with abnormalities in fibrin clot formation resulting in the hemorrhagic syndrome [5].

It is a recessive X-linked genetic disorder affecting 1 in 5000 male newborns. Furthermore, the FVIII level in heterozygous females is, on average, half of that found in the general population.

There are three forms of hemophilia A based on the measured factor VIII level: severe (<1%), moderate (1-5%), and mild (5-40%) [6].

The diagnosis is usually made before the walking age. Additionally, in the neonatal period, severe or moderate hemophilia should be suspected in cases of intracerebral bleeding, intra-abdominal organ bleeding, or unusual hematomas during the first vaccinations.

Muscle hematomas are a common hemorrhagic manifestation of hemophilia, while the central nervous system, neck, and oral floor are particularly dangerous locations, involving a life-threatening prognosis or the risk of permanent functional sequelae.

Joint bleeds are both a clinical feature of hemophilia and a key element in functional prognosis, most commonly occurring in the knees, ankles, and elbows. Repeated bleeding into a joint can lead to complications such as hemophilic arthropathy [5].

The cornerstone of medical management of hemophilia A is the infusion of factor VIII concentrate. Several products are available, including plasma-derived and genetically engineered recombinant products. Non-substitutive therapies such as Emicizumab, Concizumab, and Fitusiran are also available. Desmopressin, indicated in mild hemophilia, mobilizes the reserves of factor VIII and von Willebrand factor contained in endothelial cells, thereby increasing plasma levels [3-5].

Two modalities are possible: "on-demand" treatment, mainly indicated in moderate or mild hemophiliacs, and often in adults with severe hemophilia. This treatment involves early injection of factor VIII as soon as bleeding is diagnosed or suspected. "Prophylactic" treatment, indicated in severe hemophilic children, involves systematic injection of factor VIII two to three times a week to prevent hemorrhages [3].

The main complication of substitute treatments remains the risk of developing inhibitors (anti-FVIII antibodies) in over 30% of individuals with hemophilia a [6].

Given that these inhibitors are most often polyclonal IgG, they interfere very rapidly with injected FVIII, rendering substitute treatment completely ineffective.

Anti-FVIII inhibitors are essentially characterized by their titer, which corresponds to the patient's plasma capacity to neutralize FVIII in normal plasma. It is expressed in Bethesda units (BU) [5]. We distinguish between high responder inhibitors with a titer greater than 5 BU, characterized by an anamnestic response. This means antibody production is stimulated again upon re-exposure to the FVIII antigen without any chance of spontaneous acquisition of immune tolerance, leading to greater therapeutic difficulties. Low responder inhibitors have a titer less than or equal to 5 BU, which often have little significant impact on clinical or therapeutic management [7].

In this present study conducted at the central hematology laboratory of IBN SINA Hospital in Rabat, we retrospectively reviewed data from hemophilia A patients over a 37-month period to study the occurrence and prevalence of anti-factor VIII inhibitors, as well as the severity of hemophilia in a national population of hemophilia, by presenting their epidemiological and biological characteristics. In this regard, we gathered a sample of 172 hemophilia A patients, of whom 55 patients developed anti-FVIII inhibitors, representing a prevalence of 32%. This rate is comparable to what was previously reported by a national study conducted by H. Mamad in 2021 (25%) and Y. El Aissaoui in 2018 (38%) [8, 9]. However, it is far from what was reported by F. Zizi in 2010 (11%) [10]. This may indicate an increase in the rate of hemophilia patients developing anti-FVIII inhibitors, which could be due to improvements in hemophilia diagnostic techniques.

Regarding international studies, our results are consistent with the study by Calvez *et al*., who detected a prevalence of inhibitor development of 37.6% among 113 positive inhibitor patients in a population of 303 hemophilia A patients [11].

The patient's age at the time of initial treatment was among the risk factors for inhibitor development according to the study by Lorenzo *et al*., [12].

In our series, the mean age of patients was 25 years, with 27.32% of them belonging to an adult age group beyond 30 years. The mean age of patients reported by other studies was lower, between 6 months and 10 years for H. Mamad's study [8], between 11 and 15 years for Y. El aissaoui's study [9], and 12 years for F. Zizi's study [10]. However, it was consistent with the series of Laissouf *et al*., (24 years) [13], and Kang *et al*., (25 years) [14].

For hemophiliacs who developed inhibitors, they were of different ages between 2 and 70 years, with a predominance of the age group between 21 and 25 years, at 20%. In contrast, Y. El aissaoui's study reported an inhibition peak at 39.28% for the age groups between 11-15 years and 16-20 years [9].

In our study, hemophilia patients were mainly severe hemophiliacs (56%), the same finding was reported by H. Mamad (68%), Y. El aissaoui (63%), F. Zizi (52.59%), and Kang *et al*., (56.5%) [8-14].

Among all patients developing inhibitors, 87% are severe hemophiliacs (48/55), and 13% are moderate hemophiliacs (7/55). Our results align with those described by H. Mamad (94% severe hemophiliacs), Y. El Aissaoui (96% severe hemophiliacs), and F. Zizi (77.78% severe hemophiliacs) [8-10]. This indicates that the majority of hemophiliacs developing inhibitors are severe hemophiliacs. Additionally, a large retrospective study conducted by Darby *et al*., in the United Kingdom confirmed that this complication is four times more common in severe forms compared to non-severe forms [15].

We identified 23 cases of low responder inhibitors, accounting for 42%, compared to a rate of 44.44% found in F. Zizi's series, and 32 cases of high responder inhibitors, accounting for 58%, against a percentage of 55.56% reported by F. Zizi [10]. Collins *et al*., also reported a frequency of 50.84% (60/118) in high responder inhibitors [16]. Our result is thus consistent with the literature.

Among the high responders, 29 cases are severe hemophilia A, representing a percentage of 90.62%, and 3 patients are moderate hemophilia A (9.37%). However, the study by Y. El Aissaoui revealed a rate of 100% severe hemophilia A [9].

As for the low responders, 19 cases are severe hemophilia A, representing 82.6%, and 4 cases are moderate hemophilia A, representing 17.39%. Y. El Aissaoui reported 77.77% severe hemophilia A, 11.11% moderate hemophilia A, and 11.11% minor hemophilia A [9].

The increase in the incidence of inhibitors in more recent series compared to historical series may be related to the greater sensitivity of biological tests and intensified screening analyses optimizing the diagnosis of low-titre inhibitors or to transformations altering the immunogenicity of FVIII concentrates [7].

In this series, we also studied the kinetics of inhibitors in 24/55 patients who underwent multiple FVIII assays and inhibitor titrations. Consequently, we identified 13 patients who consistently maintained their inhibitor response type, with 9 of them being high responders and 4 being low responders. Additionally, 3 patients changed from high responder inhibitor titration

to low responder, and 8 patients changed from low responder inhibitor titration to high responder.

It can be concluded that inhibitor titers are inconstant. In high responders, the inhibitor titre rises rapidly after FVIII administration, and in the absence of new stimulation, this titre gradually decreases, then antibody production is stimulated again upon re-exposure to FVIII (anamnestic response), with no chance of spontaneous acquisition of immune tolerance. However, in low responders, the inhibitor titre remains largely unaffected by new administrations of FVIII, as it may be saturated by the antigen, thus conventional substitutive treatment with FVIII concentrates usually retains some effectiveness. These inhibitors may spontaneously disappear within weeks or months. They are termed transient and have no impact on long-term treatment, but they may subsequently increase significantly, thus transforming the patient from a low responder to a high responder.

There is also another category of antibodies called non-neutralizing antibodies. They neither inhibit the coagulant activity of FVIII nor alter the therapeutic effectiveness of conventional substitutive treatment. The frequency of these antibodies is less studied, and they are widely underestimated, so their potential impact on the subsequent appearance of an inhibitor is poorly understood.

Better understanding of all risk factors and their consideration when initiating treatment in young children with severe hemophilia A could reduce the incidence of inhibitors, thereby preserving patients' health status and quality of life as well as for medical-economic reasons. Thus, several categories of inhibitor risk factors have been identified. These include genetic factors intrinsic to the patient, which, although unmodifiable, leave little room for potential prevention measures during treatment. Environmental factors related to treatment implementation conditions and those directly related to FVIII concentrate. It is through intervention on these non-genetic factors that the risk of inhibitor can be modulated, thus affecting the frequency, severity, and/or evolution of this complication [7].

CONCLUSION

The development of inhibitors represents the major complication of substitutive treatment, especially for young children with severe hemophilia A. This complication should be targeted for eradication based on immune tolerance induction (ITI), involving frequent and repeated injections of Factor VIII concentrates, often for several months or even years.

Hemophilia is a multidisciplinary disease, requiring complex management involving specialized medical and paramedical teams, physiotherapists, and potentially other specialties depending on the complications. These healthcare professionals must be regularly trained and informed to effectively manage hemophiliacs. Additionally, emphasis should be placed on the essential role of parents and the hemophiliac individual in their own care, as they are at the forefront of monitoring and administering treatments. This necessitates professional therapeutic education.

Furthermore, the future sees two types of treatments on the horizon: gene therapy and long-acting antihemophilic factors.

Conflicts of Interest: The authors declare no conflicts of interest.

REFERENCES

1. Boumahni, B., Jacquemont, M. L., & Stoven, C. (2017). Découverte d'une hémophilie A sévère lors du test de dépistage néonatal. *Archives de P diatrie*, *5*(24), 515-516. doi: 10.1016/j.arcped.2017.02.025.
2. Hedner, U., Ginsburg, D., Lusher, J. M., & High, K. A. (2000). Congenital hemorrhagic disorders: new insights into the pathophysiology and treatment of hemophilia. *ASH Education Program Book, 2000*(1), 241-265. doi: 10.1182/asheducation-2000.1.241.
3. Théron, A., & Schved, J. F. (2018). Hémophilie. EM-Hématologie, doi: 10.1016/S1634-6939(18)74032-5
4. Nizamaldin, Y., Abi Najm, S., El Hage, M., & Samson, J. (2012). Hémostase locale en chirurgie orale. 1ère partie: physiologie de l'hémostase. *Médecine Buccale Chirurgie Buccale*, *18*(2), 119-127. doi: 10.1051/mbcb/2012005.
5. Gillet, B., & Trossaërt, M. (2017). Hémophilies, EM-Hématologie, doi: 10.1016/S1155-1984(17)74213-6
6. Peters, P., & Gothot, A. (2020). Hémophilie: une maladie en marche. *Revue Médicale de Liège*, *75*(5-6). https://orbi.uliege.be/bitstream/2268/256420/1/Pubmed%202020%20Vol%2075%20%285-6%29%20322.pdf
7. Chambost, H., Guillaume, Y., Falaise, C., Roux, C., & Monpoux, F. (2015). Update on the risk of inhibitor occurrence when initiating treatment with factor VIII concentrate in severe hemophilia A. *Journal of Pediatric Hematology Oncology*, *3* (2), 78-87. doi: 10.1016/j.oncohp.2015.03.002.
8. Mamad, H. (2021). Monitoring de l'hémophilie A au Maroc, mémoire N° : 44, Faculté de Médecine et de Pharmacie- Rabat.
9. El Aissaoui, Y. (2018), « Titrage des inhibiteurs anti facteur VIII dans l'hémophilie A : A propos de 28 cas, Thèse N° : 85 ; Faculté de Médecine et de Pharmacie - Rabat ». https://123dok.net/document/yjd2k0py-titrage-inhibiteurs-anti-facteur-viii-dans-hemophilie-propos.html

10. Zizi, F. Z. (2010). Dépistage des inhibiteurs dans l'hémophilie: étude rétrospective à propos de 121 cas. https://toubkal.imist.ma/bitstream/handle/123456789/13395/P0772010.pdf?sequence=1.
11. Calvez, T., Chambost, H., Claeyssens-Donadel, S., d'Oiron, R., Goulet, V., Guillet, B., ... & Goudemand, J. (2014). Recombinant factor VIII products and inhibitor development in previously untreated boys with severe hemophilia A. *Blood, The Journal of the American Society of Hematology*, *124*(23), 3398-3408. doi: 10.1182/blood-2014-07-586347.
12. Lorenzo, J. I., López, A., Altisent, C., & Aznar, J. A. (2001). Incidence of factor VIII inhibitors in severe haemophilia: the importance of patient age. *British journal of haematology*, *113*(3), 600-603. doi: 10.1046/j.1365-2141.2001.02828. x.
13. Aissouf, S., & Merah, F. (2013). Etude épidémiologique de l'hémophilie au CHU Tlemcen. Thesis. http://dspace1.univ-tlemcen.dz//handle/112/3385
14. Kang, P., Fang, Y., Cheng, Y., Zhang, X., Wang, J., Li, H., & Zhang, X. (2019). Analysis of haemophilia case information of Shandong Province in China: data from 2010 to 2017. *Haemophilia*, *25*(3), e209-e211. doi: 10.1111/hae.13724.
15. Darby, S. C., Keeling, D. M., Spooner, R. J., Kan, S. W., Giangrande, P. L., Collins, P. W., ... & UK Haemophilia Centre Doctors' Organisation. (2004). The incidence of factor VIII and factor IX inhibitors in the hemophilia population of the UK and their effect on subsequent mortality, 1977–99. *Journal of Thrombosis and Haemostasis*, *2*(7), 1047-1054. doi: 10.1046/j.1538-7836.2004.00710. x.
16. Collins, P. W., Palmer, B. P., Chalmers, E. A., Hart, D. P., Liesner, R., Rangarajan, S., ... & Hay, C. R. (2014). Factor VIII brand and the incidence of factor VIII inhibitors in previously untreated UK children with severe hemophilia A, 2000-2011. *Blood, The Journal of the American Society of Hematology*, *124*(23), 3389-3397. doi: 10.1182/blood-2014-07-580498.