Inhibitor development in nonsevere hemophilia A
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Chapter 5

Inhibitor incidence after intensive FVIII replacement for surgery in mild and moderate hemophilia A: a prospective national study in the Netherlands

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ABSTRACT

Inhibitor development is currently the most severe complication in mild/moderate hemophilia A patients, causing increased bleeding tendency, hospitalisation and mortality. It has been suggested that receiving high doses of factor VIII concentrates for surgical procedures is an important risk factor for inhibitor development in these patients. The aim of the current multicenter study was to determine prospectively the incidence of inhibitor development after intensive factor VIII replacement therapy for surgical procedures in patients with mild/moderate hemophilia A. All consecutive patients with mild/moderate hemophilia A were included when they required at least 10,000 IU of factor VIII concentrates (or 250 IU kg\(^{-1}\)) during five or more days for a surgical procedure. Potential clinical risk factors for inhibitor development and results of inhibitor tests were collected. Forty-six patients with a median age of 54 years (Inter Quartile Range [IQR], 40-59) were included in the study. Factor VIII genotyping revealed 20 different missense mutations. Patients received either recombinant (65%) or plasma derived factor VIII concentrates (35%), by intermittent bolus injections (41%) or continuous infusion (57%). Two patients developed a low titer inhibitor post-operatively. The incidence of inhibitor development following intensive treatment for surgery in this unselected prospective cohort of mild/moderate hemophilia A patients was 4% (95% confidence interval [CI], 0.5-14.8).
INTRODUCTION

Hemophilia A is an X-linked bleeding disorder caused by a mutation in the factor VIII gene (F8) resulting in a deficiency of plasma factor VIII. Classically, hemophilia A is divided into three forms of severity, based on the level of plasma factor VIII (FVIII:C): severe (FVIII:C, <2 International Units per deciliter [IU dL-1]), moderate (FVIII:C, 2-5 IU dL-1) and mild (FVIII:C, 6-40 IU dL-1). In mild hemophilia A spontaneous bleeding rarely occurs and bleeding is mostly provoked by (minor) trauma or surgery. Bleeding episodes are preferably treated or prevented by desmopressin (DDAVP) in responders, while major bleeds or invasive procedures requiring prolonged increased plasma levels of factor VIII are treated with factor VIII concentrates.

The development of inhibiting antibodies towards factor VIII is the most severe complication of the treatment with factor VIII concentrates. Inhibitor formation represents a major challenge in the management of hemophilia A as it renders the administered factor VIII concentrates ineffective, leading to increased complications and mortality. In patients with mild/moderate hemophilia inhibitors can also cross-react with endogenous factor VIII resulting in a more severe phenotype with spontaneous bleeding. Bleeding symptoms are often severe and life-threatening, forcing these patients to change their lifestyle completely.

Inhibitor eradication therapy by repeated high dose of intravenous factor VIII administration (Immune Tolerance Induction) is only successful in 51-76% of the severe hemophilia A patients with a high titer inhibitor and is very expensive. Moreover, in mild/moderate hemophilia A no standardized therapies for inhibitor eradication are available.

Inhibitors occur more frequently in patients with severe hemophilia (incidence, 25-30%) as compared to patients with mild/moderate hemophilia (incidence, 3-13%). However, inhibitor development in patients with mild/moderate hemophilia A seems to be rising; in the Netherlands more than one third of the newly diagnosed inhibitors were shown to occur in mild/moderate hemophilia. Since more than 50% of all hemophilia patients have a mild/moderate form, the clinical impact of the problem is substantial.

Previous studies have demonstrated that inhibitor development in mild/moderate hemophilia A is multi-factorial involving both genetic and environmental risk factors. The genetic predisposition for inhibitor development is associated with the underlying F8 mutation and a positive family history of inhibitor development. Mild/moderate hemophilia A is generally caused by a missense mutation in the F8 gene. Previous research showed that specific mutations that alter the three-dimensional structure of the factor VIII protein are associated with a higher incidence of inhibitor formation (i.e. Arg593Cys and Arg2150His).

Especially in mild/moderate hemophilia A, concerns have been raised about the association between inhibitor development and intensive treatment with factor VIII.
concentrates for surgical procedures, particularly in patients carrying high risk F8 mutations and in older patients. This could be explained immunologically by the combination of cytokine release through tissue damage resulting from surgery, combined with excessive amounts of exogenous factor VIII antigen. Both may render the immune system more susceptible to develop inhibitors. The influence of other possible contributing factors such as the type of administered factor VIII concentrate, administration by continuous infusion instead of intermittent bolus injections and the occurrence of concomitant infection or inflammation needs further investigation.

It is important to know the incidence of inhibitor development in patients with mild/moderate hemophilia A patients undergoing surgery, because this risk has to be considered when an elective surgical procedure is planned. Yet, prospective studies on this topic are lacking. Previous studies were all retrospective, performed in small populations or in selected groups of patients with a high proportion of high risk mutations. Although mild/moderate hemophilia A patients are not treated frequently, they will usually have received several doses of factor VIII concentrate at the moment that they need surgery. To generate data that are relevant for clinical practice, we designed a prospective multicenter study to determine the incidence of inhibitor development in a consecutive group of previously treated mild/moderate hemophilia A patients receiving intensive factor VIII replacement therapy for surgical procedures.

PATIENTS AND METHODS

Patient selection
All mild (FVIII:C, 6-40 IU dL\(^{-1}\)) and moderate (FVIII:C, 2-5 IU dL\(^{-1}\)) hemophilia A patients, at least 12 years of age and requiring intensive treatment with factor VIII concentrates for elective surgery were eligible for study entry. Intensive treatment of factor VIII was defined as the cumulative use of at least 10,000 International Units (IU) or 250 IU per kilogram bodyweight (IU kg\(^{-1}\)) during five or more consecutive days. Patients were excluded if they had any other hemostatic disorder or a positive history of inhibitors.

Patients were recruited consecutively at seven hemophilia treatment centers in the Netherlands during a prospective period of four years. The source population from which eligible patients were recruited existed of all patients with mild/moderate hemophilia A cared for by these seven centers (approximately 750 mild/moderate hemophilia A patients). The study protocol was conducted in accordance with the Declaration of Helsinki and ethical approval was obtained in each participating center. Informed consent was obtained in writing from all subjects before study entry.
Data collection
After inclusion, clinical data were collected from hospital databases and patient files by the patient’s physician at least one week before surgery and included: date of birth, ethnicity, cumulative number of exposure days to factor VIII concentrates, previous intensive treatments for bleeding or surgery, family history of hemophilia A and inhibitors, co-morbidity and medication.

During surgery, patients were treated according to national guidelines under supervision of their treating hemophilia specialist. The surgery took place in one of the participating hemophilia treatment centers. Data recorded peri-operatively included: type of surgery and indication, type of factor VIII concentrate, mode of factor VIII administration (intermittent bolus injections or continuous infusion), number of exposure days, cumulative amount of factor VIII concentrate administered, peri-operative medication and complications during the first week after surgery. Patients were seen at a follow-up visit at four to eight weeks after the surgical procedure. During this visit all post-operative complications were recorded and a blood sample was obtained.

Laboratory assessment
EDTA anti-coagulated blood for factor VIII genotyping was sent to the Academic Medical Center genomics laboratory, Amsterdam. F8 mutation was determined by sequencing of the F8 gene. Laboratory assessments that were performed at each local laboratory included: FVIII:C by one-stage clotting assay, von Willebrand factor (vWF) activity, vWF antigen, anti-factor VIII, antibodies against hepatitis A virus, hepatitis B virus, hepatitis C virus and Human Immunodeficiency Virus (HIV).

Inhibitors were locally tested by the Nijmegen modification of the Bethesda assay. Patients were tested for inhibitors before surgery and at the follow-up visit after surgery or earlier in case of a clinical indication (i.e. no response to factor VIII treatment or increased bleeding tendency). A titer of 1-4 Bethesda Unit per milliliter (BU mL\(^{-1}\)) was defined as a low inhibitor titer and a titer of at least 5 BU mL\(^{-1}\) was defined as a high inhibitor titer. If the result of the Bethesda assay was between 0.6 and 1.0 BU mL\(^{-1}\), the patient was classified as an inhibitor patient if the patient presented with spontaneous bleeding symptoms, or if the FVIII:C ratio (FVIII:C during inhibitor/ FVIII:C before inhibitor) was 0.5 or less.

Statistics
All data was collected prospectively. In the description of patient characteristics continuous data are presented as medians and Inter Quartile Ranges (IQR).
RESPECT

Patient characteristics and genotype
During the enrolment period, 54 patients were admitted to one of the participating hemophilia treatment centers for a surgical procedure; 48 (89%) complied with the inclusion criteria. Of the six excluded patients, one patient was a female carrier of hemophilia A, three patients turned out to have von Willebrand disease type 2N and two patients had a positive inhibitor history. Of the 48 included patients, two patients were excluded during study follow-up because they received less factor VIII concentrates than initially estimated, both patients did not develop an inhibitor.

In total 46 hemophilia A patients, 43 mild and three moderate, with a median FVIII:C of 16 IU dL⁻¹ (IQR, 8-25) and a median age at inclusion of 54 years (IQR, 40-59) underwent intensive factor VIII treatment for a surgical procedure and were included in the study. Baseline characteristics of the patients are listed in Table 1. Forty-three patients (93%) were genotyped, revealing 20 different F8 missense mutations, including four novel mutations not previously reported in the HaMSTERS database (Table 2).

Medical history of patients
More than half of the patients (n = 26; 57%) had one or more co-morbidities besides hemophilia A. Eight patients (17%) were suffering from cardiovascular disease; five of them were taking anticoagulants (thrombocyte aggregation inhibitor [n = 4] or heparin [n = 1]). Fifteen patients (32%) were infected with hepatitis C, none were infected with HIV. Other co-morbidities were malignancy (n = 3), epilepsy (n = 1), non-insulin dependent diabetes mellitus (n = 1), hypertension (n = 2) and hypercholesterolemia (n = 1).

There was a broad range in previous exposure days (Table 1). Seventy percent (n = 33) of the patients had undergone one or more surgical procedures before inclusion; eleven of them had received peri-operative treatment for a period of more than three consecutive exposure days in the past. Fourteen patients (38%) were intensively treated for one or more bleeding episodes prior to study participation.

Surgical procedures
A variety of surgical procedures was performed (Table 3). Thirty patients (65%) were treated with a recombinant factor VIII product to cover the surgical procedure. The other 16 patients (35%) received plasmaderived factor VIII products. Patients were exposed to factor VIII concentrates for a median of nine cumulative exposure days (IQR, 7-13) following surgery and received a median cumulative factor VIII of 453 IU kg⁻¹ (IQR, 251-610). In 26 patients (59%) administration of factor VIII concentrates started by continuous infusion
Inhibitors after intensive treatment for surgery

and was followed after several days by intermittent bolus injections for a median of nine exposure days (IQR, 7-12). The cumulative use of factor VIII in patients who received factor VIII concentrates exclusively by bolus injections was slightly lower as compared to continuous infusion (median, 430 IU kg\(^{-1}\); IQR, 217-633; versus median, 472, IQR, 337-571).

### Inhibitor development

Two patients (4%; 95% CI, 0.5-14.8) developed an inhibitor post-operatively. Case 1 is a 35-year-old male with mild hemophilia A (FVIII:C, 9 IU dL\(^{-1}\)) of Syrian origin. At the age of 25 years he had a spinal intramedullary hemorrhage following a traffic accident and he has been paraplegic since then. Multiple surgical procedures have been covered by factor...
Chapter 5

Table 2. Characteristics of the 20 F8 gene missense mutations identified in 43 mild/moderate hemophilia A patients

<table>
<thead>
<tr>
<th>Aminoacid substitution</th>
<th>No. of patients (%)</th>
<th>FVIII:C (IU dL⁻¹)</th>
<th>Reported previously (Y/N)†</th>
<th>Inhibitors reported† (Y/N/U)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Val140Ala</td>
<td>1 (2)</td>
<td>22</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>Asp167Gly</td>
<td>2 (5)</td>
<td>13</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>Asp459Glu</td>
<td>1 (2)</td>
<td>12</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Arg527Trp</td>
<td>1 (2)</td>
<td>11</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>Arg531Cys</td>
<td>3 (6)</td>
<td>16</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Ala544Gly</td>
<td>1 (2)</td>
<td>22</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>Cys554Tyr</td>
<td>1 (2)</td>
<td>9</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Arg593Cys</td>
<td>9 (20)</td>
<td>16</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Asn618Ser</td>
<td>3 (6)</td>
<td>26</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Thr657Ser</td>
<td>5 (11)</td>
<td>25</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Arg698Trp</td>
<td>1 (2)</td>
<td>21</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>Glu720Lys</td>
<td>1 (2)</td>
<td>49</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>Arg1689Cys</td>
<td>3 (7)</td>
<td>9</td>
<td>Y</td>
<td>N</td>
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<tr>
<td>Arg1749Cys</td>
<td>1 (2)</td>
<td>46</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>Arg1781His</td>
<td>1 (2)</td>
<td>3</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Gly2057Gly</td>
<td>1 (2)</td>
<td>9</td>
<td>N</td>
<td>N</td>
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<tr>
<td>Arg2150His</td>
<td>5 (11)</td>
<td>10</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Arg2159Cys</td>
<td>1 (2)</td>
<td>16</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>Gln2246Arg</td>
<td>1 (2)</td>
<td>6</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>Trp2271Arg</td>
<td>1 (2)</td>
<td>5</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

Y, yes; N, No; No., number.

† One of the patients with the Arg531Cys and one of the patients with the Arg593Cys missense mutation developed an inhibitor.

† According to the HaMSTERS database.²¹

VIII concentrates previously in another hemophilia treatment center; his previous exposure status was not exactly known but estimated to be at least over 50 exposure days. This time he underwent plastic surgery for decubitus ulcers as a result of his paraplegia in combination with the excision of an infected femur head. Surgery was covered by continuous infusion of recombinant factor VIII concentrate for 14 days; he received a total cumulative amount of 100,000 units factor VIII. In the post-operative period factor VIII dosing had to be increased daily to keep the FVIII:C at an adequate level. Hemostasis was optimal and no bleeding complications occurred. Two days after continuous infusion was stopped, he tested positive for an inhibitor (2.5 BU mL⁻¹, also peak titer). His plasma FVIII:C level was similar to prior levels (FVIII:C, 7 IU dL⁻¹). Immune tolerance induction was started on the same day with a regimen of 25 IU kg⁻¹ day⁻¹ three times per week. After three months the inhibitor disappeared. F8 gene
mutation analysis identified a missense mutation (Arg531Cys). His two brothers who both have hemophilia A did not develop an inhibitor; no further information was available about the number of exposure days that these brothers had received.

Case two is a 58 year-old Caucasian male with mild hemophilia A (FVIII:C, 9 IU dL⁻¹). He developed a low-titre inhibitor (1 BU mL⁻¹) after vascular surgery. Prior to this procedure his lifetime cumulative number of exposure days was below ten exposure days and he had
not been treated intensively with factor VIII concentrates or for surgery before. Besides hemophilia A he suffered from vascular disease, hypercholesterolemia and hypertension. His surgery was managed by continuous infusion for five days followed by daily bolus injections for four days (a total of nine consecutive exposure days); a total amount of 73,000 units recombinant factor VIII concentrate was administered. Hemostasis was optimal and no bleeding or other complications occurred. An inhibitor (0.8 BU mL⁻¹) was detected 40 days after surgery. His baseline FVIII:C level remained stable (FVIII:C, 8 IU dL⁻¹). There were no bleeding complications and the inhibitor was followed up by regular testing. Peak inhibitor was 1.0 BU mL⁻¹ three months after surgery and the inhibitor spontaneously disappeared within several months. F8 gene mutation analysis indentified a missense mutation (Arg593Cys).

DISCUSSION

This is the first prospective nationwide study measuring the cumulative incidence of inhibitor development after intensive factor VIII replacement therapy for surgery in mild/moderate hemophilia A. Two out of 46 patients (4%; 95% CI, 0.5-14.8) developed an inhibitor post-operatively. The frequency of inhibitor development after surgery that we found in this relative large cohort of consecutive patients was lower than the frequency observed in the smaller study by Sharatkumar and colleagues (25%; 95% CI, 7.3-52.4)¹⁷ and the previous retrospective cohort study at one of the participating hemophilia treatment centers (17%; 95% CI, 7.2-32.1).¹⁵ The higher inhibitor incidence in those studies may be explained by the selection of high risk patients for inclusion in these studies. In the study of Sharatkumar and colleagues, four patients out of 16 who where treated intensively with factor VIII concentrates developed an inhibitor. Three of these patients were previously untreated patients (PUPs) and one had only one previous exposure day to factor VIII concentrate. In the cohort study that found an inhibitor incidence of 17% after surgery (7/41), a large proportion (38%) of the included patients carried the Arg593Cys mutation which is associated with an increased risk of inhibitor development.

In the present study the majority of the surgical patients that were consecutively included (70%) had been challenged before in one or more periods of (intensive) factor VIII treatment without developing inhibitors. Remarkably one of the patients that developed an inhibitor did so after more than 50 exposure days while he had faced many prior challenges – including surgery, inflammation and intensive treatment – without developing an inhibitor. An unexpectedly late occurrence of inhibitors in mild/moderate patients has also been observed in a case-control study by Kempton and colleagues.¹⁶ In this study 42% of the case subjects developed an inhibitor after more than 50 cumulative exposure days, illustrating that previously treated (>50 exposure days) mild/moderate hemophilia A patients are still at
risk for inhibitor development. This may also be the case for severe patients, as demonstrated recently by Hay and colleagues in a national study in the United Kingdom comprising 2,528 severe hemophilia A patients who were followed up for a median of 12 years.\textsuperscript{22} Although the incidence of inhibitors was highest among the patients below five years of age (64/1,000 treatment years) there was a second peak of significantly increased inhibitor incidence among the age group of > 60 years of age (11/1,000 treatment-years).

As life expectancy increases, the rising incidence of inhibitor development in older patients with hemophilia A will become an important clinical challenge.\textsuperscript{23} Moreover, age-related health problems such as cancer and arthrosis will increase the need for intensive treatment with factor VIII concentrates for surgery in these patients.\textsuperscript{24} In case 1, intensive treatment for surgery was performed while infection was present (infected femur head). Environmental circumstances such as high factor VIII antigen load, tissue damage and inflammation may trigger the immune system to form inhibitor antibodies, irrespective of previous cumulative exposure days. Further research is required to explore the pathophysiology of late-onset inhibitors and to elucidate the role of other potentially synergistic risk factors for inhibitor development at advanced age to enable preventive measures.

The genotype may be an important contributing factor to inhibitor development in the two cases of the present study. Both cases were carrying high risk $F_8$ missense mutations, caused by a cysteine replacement. The formation of disulphide bridges by cysteine replacement may alter the folding of the factor VIII protein and thereby induce recognition of wild-type factor VIII by the immune system.\textsuperscript{25,26} Among the patients with missense mutations which are associated with inhibitor development according to the HaMSTERS database (i.e. Arg531Cys, Arg593Cys, Asn618Ser, Arg1781His and Arg2150His) the incidence of inhibitors in our cohort is 10% (2/21).\textsuperscript{21}

The incidence of inhibitor development that we found in this observational study may be influenced by multiple factors including age, previous (intensive) factor VIII exposures and the presence of high risk $F_8$ genotypes. The absence of a reference group in this study restricted the analysis to calculating the incidence of inhibitor development after surgery.

Nevertheless, the results emphasize the importance of being aware of inhibitor risk in these patients irrespective of age and cumulative exposures to factor VIII concentrates. Caution should be taken when treating mild/moderate hemophilia A patients intensively with factor VIII concentrates for surgery and alternative or additional use of desmopressin should be considered whenever possible. Routine inhibitor testing should be performed post-operatively to detect inhibitors at an early stage. Further study is needed to confirm these findings in mild/moderate patients with other characteristics (e.g. previously untreated patients) and to investigate potential risk factors that may play a role in post-operative inhibitor development. This will ultimately help to optimize inhibitor prevention in future treatment.
REFERENCES


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