Inhibitor development in nonsevere hemophilia A

Eckhardt, C.L.

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Intensive peri-operative use of factor VIII and the Arg593→Cys mutation are risk factors for inhibitor development in mild/moderate hemophilia A


*Both authors contributed equally to this study

ABSTRACT

Background
A severe and challenging complication in the treatment of hemophilia A is the development of inhibiting antibodies (inhibitors) directed towards factor VIII. Inhibitors aggravate bleeding complications, disabilities and costs. The etiology of inhibitor development is incompletely understood.

Objectives
In a large cohort study in patients with mild/moderate hemophilia A we evaluated the role of genotype and intensive factor VIII exposure in inhibitor development.

Patients/methods
Longitudinal clinical data from 138 mild/moderate hemophilia A patients were retrospectively collected from 1-1-1980 to 1-1-2008 and analyzed by multivariate analysis using Poisson regression.

Results
Genotyping demonstrated the Arg593Cys missense mutation in 52 (38%) patients, the remaining 86 patients had 26 other missense mutations. Sixty-three (46%) patients received intensive factor VIII concentrate administration, 41 of them for surgery. Ten patients (7%) developed inhibitors, eight of them carrying the Arg593Cys mutation. Compared to the other patients, those with the Arg593Cys mutation had a tenfold increased risk of developing inhibitors (Relative Risk [RR], 10; 95% confidence interval [CI], 0.9-119). The other two inhibitor patients had the newly detected mutations Pro1761Gln and Glu2228Asp. In both these patients and in five patients with genotype Arg593Cys, inhibitors developed after intensive peri-operative use of factor VIII concentrate (RR, 186; CI, 25-1403). In five of the ten inhibitor patients factor VIII was administered by continuous infusion during surgery (RR, 13; CI, 1.9-86).

Conclusion
The Arg593Cys genotype and intensive peri-operative use of factor VIII, especially when administered by continuous infusion, are associated with an increased risk for inhibitor development in mild/moderate hemophilia A.
INTRODUCTION

A severe and challenging complication in the treatment of hemophilia is the development of factor VIII inhibiting antibodies (inhibitors). Inhibitors compromise the management of bleeding symptoms, thereby aggravating complications, disabilities and costs.\textsuperscript{1,2} Inhibitors arise more frequently in severely affected patients than in those who are mildly or moderately affected. The cumulative incidence of inhibitors is estimated to be 23\% in the former group and 8\% in the latter.\textsuperscript{3} The lower incidence of inhibitor development in mild and moderate hemophilia may be explained by the circulating factor VIII protein in these patients. Mild and moderate hemophilia A is caused by missense mutations in the factor VIII gene and patients have a residual level of circulating endogenous factor VIII activity.\textsuperscript{4} This endogenous factor VIII presumably induces tolerance towards infused factor VIII. In contrast, severely affected hemophilia A patients have deletions or inversions of the factor VIII gene and no measurable factor VIII in their plasma.

However, when inhibitors arise in patients with mild or moderate hemophilia, the effects may be severe. These inhibitors may not only neutralize the effect of factor VIII replacement therapy, but may also cross-react with endogenous factor VIII, causing severe spontaneous bleeding.\textsuperscript{5-7} From a case series of 26 patients with mild or moderate hemophilia who developed inhibitors, two patients died from uncontrollable haemorrhage.\textsuperscript{5} Since more than 50\% of all hemophilia patients have a mild or moderate form,\textsuperscript{9} the clinical impact of the problem is substantial. A national study comprising a 22 year observation period demonstrated that almost one third of the newly diagnosed inhibitors occurred in mild or moderate haemophiliacs.\textsuperscript{10}

Most research on the etiology of inhibitor development has been performed in patients with severe hemophilia A. Previous studies in severe hemophilia A demonstrated that both genetic and environmental factors play a role in the etiology of inhibitor development.\textsuperscript{11} Genetic factors associated with inhibitor development are located within the factor VIII gene, i.e. specific missense mutations in regions encoding the A2 or C2 domain of the factor VIII protein or outside the factor VIII gene, e.g. African or Asian ethnic origin and genetic polymorphisms in the IL 10 or TNFA gene.\textsuperscript{6,12-16} Environmental factors that have been reported as potential risk factors for inhibitor development are: early intensive treatment, mode of factor VIII administration (bolus injections or continuous infusion), type of administered factor VIII concentrate, and change of factor VIII products.\textsuperscript{6,17-23} It has also been suggested that infection, trauma and surgery may render the immune system more susceptible to inhibitor formation.\textsuperscript{19,20,24,25}

So far, reports on the possible association of certain risk factors and inhibitor development in mild and moderate forms of hemophilia only consist of case reports and case series, while larger, comparative studies are absent. In order to evaluate the potential
role of genotype and intensive use of factor VIII in the development of inhibitors in mild or moderate hemophilia A, we performed a longitudinal observational study in a well-defined group of consecutive patients.

**PATIENTS AND METHODS**

**Patient selection and genotyping**
All mild (factor VIII clotting activity [FVIII:C], 6-40 International Units per milliliter [IU mL⁻¹]) and moderate (FVIII:C, 2-5 IU mL⁻¹) hemophilia A patients, who visited the hemophilia treatment center of the Academic Medical Center on at least three occasions during the study’s observation period were eligible for this observational cohort study. The observation period started January 1st, 1980, when intermediate purity factor VIII concentrates were introduced, and lasted until January 1st, 2008. Patients who used no factor VIII concentrate during the study period were excluded. Patients were followed until inhibitor development, death, or the end of the observation period. Mutation analysis was performed, aided by pedigree analysis for deceased patients of whom no DNA was available.

**Data collection**
Clinical data were collected from hospital databases and patient files by two investigators (CLE, LAM), and discussed with two experienced hemophilia consultants (KF, MP) whenever clarification was necessary. The number of exposure days and the cumulative amount of factor VIII concentrates administered during the study period were recorded. A period of intensive use of factor VIII concentrate was defined as the cumulative use of at least 250 International Units per kilogram bodyweight (IU kg⁻¹) within five consecutive days, or at least 30 IU kg⁻¹day⁻¹ during more than five consecutive days. The reason for intensive use of factor VIII was classified as being surgery or a bleeding episode. Data collected on other potential risk factors for inhibitor development included: ethnic origin, type of factor VIII product, product change and mode of factor VIII administration (bolus injections or continuous infusion).

**Inhibitor development**
FVIII:C was assessed by a one-stage clotting assay. Inhibitors were identified by the Bethesda assay as described by Kasper et al. From 1996 onward, the Nijmegen modification of the Bethesda assay was used. Patients were tested for inhibitors when factor VIII response declined after a period of intensive use of factor VIII, when bleeding tendency increased or once a year if factor VIII concentrate had been used in the past 12 months. A titer of at least 1 Bethesda Unit per milliliter (BU mL⁻¹) was defined as a low inhibitor titer, 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 and 1 BU mL\(^{-1}\), the patient was classified as an inhibitor patient if the patient presented with spontaneous bleeding symptoms, or if the factor VIII ratio (FVIII:C during inhibitor/ FVIII:C before inhibitor) was 0.5 or less.

**Statistics**

In the description of patient characteristics continuous data are presented as medians and Inter Quartile Ranges (IQR). In order to evaluate the role of genotype, product change, intensive use of factor VIII, continuous infusion and surgical procedures in the development of inhibitors, a Poisson regression model was fitted. Inhibitor risk of individuals from the same family was assumed to be correlated by including an unobserved “frailty” or random effect for the event which was assumed to have a normal distribution on the linear scale of the predictors. Potential risk factors with values changing over time (intensive factor VIII administration, continuous infusion and product change) were entered as time-dependent covariates. The effect of these covariates was assumed to last for three months after the exposure. Results were presented as relative risks (RR). Poisson regression analyses were performed in the R statistical program.

**RESULTS**

**Patient characteristics, genotype and clinical risk factors**

The study population consisted of 128 mild and 10 moderate hemophilia A patients and comprised 6,525 exposure days during an observation period of 1,536 patient years. The median period of observation was 10 years (IQR, 5-17). All but two patients were of Caucasian origin. Genotyping demonstrated the Arg593Cys missense mutation in 52 patients (38%). The ancestors of the 17 families to whom they belonged all originated from the close vicinity of Amsterdam. In the remaining 86 patients (62%), 26 other missense mutations were found in 47 independent families. The characteristics of all patients are depicted in Table 1.

During the study period, 63 patients (46%) received at least one period of intensive factor VIII concentrate administration. In 41 patients a surgical operation was the reason for the first intensive factor VIII administration. Twenty-two patients received their first intensive factor VIII concentrate administration for a bleeding episode (Figure 1). After this first intensive exposure, another 61 periods of intensive factor VIII concentrate administration were observed, of which 34 for surgery and 27 for a bleeding episode.
Chapter 4

Inhibitor development

Inhibitor tests were performed in 131 patients (95%) with a median of six inhibitor tests per patient (IQR, 3-8). The median period between inhibitor testing was 13 months (IQR, 11-19).

In the other seven patients no inhibitor test was performed during the study period. These patients had a low number of exposure days to factor VIII concentrates (median, 2; IQR, 2-5) and did not have an immediate indication for testing such as intensive exposure or increased bleeding tendency.

Ten patients (7%) developed inhibitors (Table 2). Four were low titer and five were high titer inhibitors, the peak titer was unknown in one patient. In six patients severe spontaneous bleeding occurred, leading to death in one of them. This patient died from uncontrollable hemorrhage and sepsis after immunosuppressive treatment. Three other inhibitor patients died from causes unrelated to their inhibitor.

All inhibitor patients were of Caucasian origin and the median age at inhibitor development was 37 years (IQR, 18-64). Inhibitors arose after a median of 27 exposure days (IQR, 12-34) during which a median of 107,200 IU of factor VIII concentrate had been administered (IQR, 39,200-187,300). Six of the ten inhibitor patients had been treated with recombinant factor VIII concentrates prior to inhibitor development and the other four had been treated with plasma derived factor VIII concentrates.

The Arg593Cys mutation was present in eight inhibitor patients, in the other two the newly detected missense mutations Pro1761Gln and Glu2228Asp were found. The incidence rate of inhibitor development was 13/1,000 patient years in the Arg593Cys group, compared

Table 1. Characteristics of the 138 mild and moderate hemophilia patients at the end of the observation period

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N (%) or median (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total length of observation, years</td>
<td>1,536</td>
</tr>
<tr>
<td>Total no. exposure days</td>
<td>6,525</td>
</tr>
<tr>
<td>Age, years</td>
<td>41 (18-61)</td>
</tr>
<tr>
<td>Baseline FVIII:C, IU dL⁻¹</td>
<td>14 (8-20)</td>
</tr>
<tr>
<td>Total no. of patients with the Arg593Cys mutation</td>
<td>52 (38)</td>
</tr>
<tr>
<td>Cumulative amount of factor VIII administered, IU</td>
<td>57,800 (8,800-81,900)</td>
</tr>
<tr>
<td>Cumulative number exposure days</td>
<td>10 (6-29)</td>
</tr>
<tr>
<td>Type of FVIII product</td>
<td></td>
</tr>
<tr>
<td>Plasma derived</td>
<td>47 (34)</td>
</tr>
<tr>
<td>Recombinant</td>
<td>91 (66)</td>
</tr>
<tr>
<td>No. of product changes</td>
<td>1 (0-2)</td>
</tr>
<tr>
<td>No. inhibitor tests during the study period</td>
<td>6 (3-8)</td>
</tr>
<tr>
<td>No. months between inhibitor tests</td>
<td>13 (10-19)</td>
</tr>
</tbody>
</table>

FVIII, factor VIII, No, number
Surgery and the arg593cys mutation are risk factors to 2/1,000 patient years in the other mutation group (crude RR, 6.5; 95% confidence interval [CI], 3.5-18.5) (Table 3). The number of exposure days at the end of the study was comparable for the Arg593Cys group and the other mutation group, median 10 exposure days (IQR, 6-27) and median 10 exposure days (IQR, 5-31) respectively, limiting the chance that the results are confounded by the number of exposure days.

In seven patients (including five from the Arg593Cys mutation group), inhibitors arose after intensive peri-operative use of factor VIII. Surgical procedures were: percutaneous coronary intervention for coronary heart disease, total thyroidectomy for thyroid carcinoma, total mesorectal excision for rectal cancer, bilateral orchidectomy for testicular torsion, inguinal hernia repair, tonsillectomy and nose correction. Desmopressin response had been tested in five of these seven patients and FVIII:C response infusion varied from 38 to 93 IU dL⁻¹.

All post-surgical inhibitors arose after the first surgical procedure. Consequently, only the first surgical procedure per patient was taken into account in the multivariate analysis.

Figure 1. Flow chart of patients
Flow chart of all included patients (n = 138), classified into (B) those with and without the Arg593Cys mutation, further classified into (C) those who underwent intensive factor VIII exposure for surgery or for bleeding and those who did not. Finally, patients were distributed in (D) those who received intensive factor VIII exposure by continuous infusion (n = 10) or bolus injections. The number of inhibitors for each sub classification is shown in row (E). Arg593Cys, Arg593Cys mutation in factor VIII gene; Surgery, intensive factor VIII exposure for surgery; Bleeding, intensive factor VIII exposure for bleeding without surgery; No intensive treatment, no intensive factor VIII exposure during the observation period; CI, continuous infusion of factor VIII concentrates; Inhibitors, inhibitor development.
The median number of days between the start of the first peri-operative use of factor VIII concentrate administration and the detection of inhibitors was 47 (IQR, 26-70). Intensive peri-operative use of factor VIII concentrate was identified as a risk factor for inhibitor development (RR, 186; CI, 25-1403) (Table 4). Intensive treatment with factor VIII concentrates was not only given for surgery but also for bleeding, this was the case in 22 patients. None of the 22 patients that received intensive factor VIII concentrates for bleeding episodes developed an inhibitor (Figure 1).

When the risk for inhibitor development in the Arg593Cys mutation group was adjusted for intensive peri-operative use of factor VIII, continuous infusion, product change and family structure, the RR was 10 (CI, 0.9-119) (Table 4).

### Table 2. Characteristics of the patients who developed an inhibitor

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Age</th>
<th>Mutation</th>
<th>Preceding event</th>
<th>Type of F VIII used</th>
<th>Inhibitor peak titer (BU mL⁻¹)</th>
<th>FVIII:C before inhibitor (IU dL⁻¹)</th>
<th>FVIII:C during inhibitor (IU dL⁻¹)</th>
<th>FVIII:C ratio</th>
<th>Spontaneous bleeding</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>63</td>
<td>Arg593Cys</td>
<td>Surgery</td>
<td>MP</td>
<td>&gt;400</td>
<td>22</td>
<td>&lt;2</td>
<td>&lt;0.5</td>
<td>Y</td>
<td>O</td>
</tr>
<tr>
<td>2</td>
<td>51</td>
<td>Arg593Cys</td>
<td>Surgery</td>
<td>RP</td>
<td>1.2</td>
<td>19</td>
<td>8</td>
<td>&lt;0.5</td>
<td>N</td>
<td>U</td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>Arg593Cys</td>
<td>Surgery</td>
<td>RP</td>
<td>0.2</td>
<td>27</td>
<td>5</td>
<td>&lt;0.5</td>
<td>N</td>
<td>U</td>
</tr>
<tr>
<td>4</td>
<td>18*</td>
<td>Arg593Cys</td>
<td>Surgery</td>
<td>IP</td>
<td>22</td>
<td>20</td>
<td>&lt;2</td>
<td>&lt;0.5</td>
<td>Y</td>
<td>S</td>
</tr>
<tr>
<td>5</td>
<td>68</td>
<td>Arg593Cys</td>
<td>Surgery</td>
<td>RP</td>
<td>94</td>
<td>12</td>
<td>&lt;2</td>
<td>&lt;0.5</td>
<td>Y</td>
<td>D</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>Arg593Cys</td>
<td>2 days of F VIII exposure for a joint bleeding</td>
<td>RP</td>
<td>1.7</td>
<td>15</td>
<td>5</td>
<td>&lt;0.5</td>
<td>Y</td>
<td>S</td>
</tr>
<tr>
<td>7</td>
<td>23</td>
<td>Arg593Cys</td>
<td>Infection antibiotics</td>
<td>RP</td>
<td>0.5**</td>
<td>17</td>
<td>4</td>
<td>&lt;0.5</td>
<td>Y</td>
<td>S</td>
</tr>
<tr>
<td>8</td>
<td>18*</td>
<td>Arg593Cys</td>
<td>None</td>
<td>IP</td>
<td>9</td>
<td>20</td>
<td>3</td>
<td>&lt;0.5</td>
<td>Y</td>
<td>O</td>
</tr>
<tr>
<td>9</td>
<td>67</td>
<td>Pro1761Gln</td>
<td>Surgery</td>
<td>RP</td>
<td>9</td>
<td>6</td>
<td>&lt;2</td>
<td>&lt;0.5</td>
<td>N</td>
<td>O</td>
</tr>
<tr>
<td>10</td>
<td>16</td>
<td>Glu2228Asp</td>
<td>Surgery</td>
<td>MP</td>
<td>1.6</td>
<td>17</td>
<td>18</td>
<td>1</td>
<td>N</td>
<td>U</td>
</tr>
</tbody>
</table>

* Patients are HLA-identical twin brothers.  
† Type F VIII concentrate used at time of inhibitor development. MP = monoclonal purified plasma derived concentrate, IP= intermediate purity plasma derived, concentrate, RP= recombinant product.  
‡ FVIII:C ratio = FVIII:C during inhibitor/ FVIII:C before inhibitor.  
§ Y = yes, N = no.  
¶ Outcome = clinical manifestation of inhibitor, D = patient died, death was related to inhibitor, O = patient died, death was not related to inhibitor. S = severe spontaneous bleeding, U = unchanged bleeding pattern.  
** Inhibitor peak titer unknown due to a long term stay abroad.
Surgery and the arg593cys mutation are risk factors

There was no significant difference between the surgical patients and the patient group treated intensively for bleedings with respect to the number of exposure days preceding intensive treatment (median, 2; IQR, 0-8; versus median 2; IQR, 0-11, respectively) or the number of exposure days during intensive treatment (median, 8; IQR 7-10; versus median, 7; IQR,5-11, respectively) or the total dose of factor VIII concentrates (median, 26,500; IQR, 18,000-41,500; versus median,21,500; IQR, 11,000-33,100) during the intensive exposure. The seven patients who developed an inhibitor after intensive peri-operative use of factor VIII concentrates for surgery had a preceding factor VIII exposure status of 12 exposure days (IQR, 8-24), which is higher than the median of two exposure days in the total group of patients receiving intensive factor VIII treatment for surgery. Hence, it seems unlikely that the observed association between intensive peri-operative use of factor VIII concentrates and inhibitor development was confounded by a low number of exposure days prior to surgery.

In five (50%) of the inhibitor patients factor VIII was administrated by continuous infusion (Figure 1). Continuous infusion was used in ten patients of this study, exclusively in surgical patients and not for treatment of bleeding episodes. The high incidence (5/10) of inhibitors after continuous infusion suggested an association between continuous infusion of factor VIII concentrates and inhibitor development. The adjusted relative risk of continuous infusion for inhibitor development was 13 (CI, 1.9-86) (Table 4).

In the remaining three inhibitor patients, all Arg593Cys genotype, inhibitor development was not preceded by a period of intensive factor VIII exposure. The first patient had been treated with factor VIII concentrate (50 IU kg\(^{-1}\) day\(^{-1}\)) for a joint bleeding in his ankle for two days prior to inhibitor detection. The second patient was suffering from recurrent infections combined with the use of antibiotics short before inhibitor development. For the third patient, no remarkable event had taken place in the six months prior to inhibitor detection. He had

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Table 3. Crude (i.e. not corrected for family structure) incidence rates of putative risk factors and inhibitor development by univariate analysis

<table>
<thead>
<tr>
<th>Putative risk factor</th>
<th>Exposed to risk factor</th>
<th>Unexposed to risk factor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. inhibitors*</td>
<td>PYAR</td>
</tr>
<tr>
<td>Arg593Cys mutation</td>
<td>8</td>
<td>621(^\dagger)</td>
</tr>
<tr>
<td>First intensive FVIII treatment for surgery</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Continuous infusion of FVIII concentrates</td>
<td>5</td>
<td>1.9</td>
</tr>
<tr>
<td>FVIII product change</td>
<td>1</td>
<td>61</td>
</tr>
</tbody>
</table>

\(^*\) Total number of patients who developed an inhibitor during the 28-years observation period.

\(^\dagger\) PYAR = person-years at risk.

\(^\ddagger\) Person-years at risk = person-years observed (PYO), because mutation is a time-independent covariate.
Table 4. Multivariate survival analysis of putative risk factors of inhibitor development, adjusted for family structure

<table>
<thead>
<tr>
<th>No. exposures to risk factor</th>
<th>No. inhibitors</th>
<th>Adjusted RR (CI)*</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg593Cys mutation</td>
<td>52</td>
<td>8</td>
<td>10  (0.9-119)</td>
</tr>
<tr>
<td>First intensive FVIII treatment for surgery‡§</td>
<td>41</td>
<td>7</td>
<td>186 (25-1403)</td>
</tr>
<tr>
<td>Continuous infusion of FVIII concentrates§</td>
<td>10</td>
<td>5</td>
<td>13 (1.9-86)</td>
</tr>
<tr>
<td>FVIII product change†</td>
<td>220</td>
<td>1</td>
<td>1.1 (0.1-11.7)</td>
</tr>
</tbody>
</table>

CI, 95% confidence interval; FVIII, factor VIII; No., number; RR, relative risk.

* The significance level was set at P<0.05.
† Each variable was adjusted for all the others and for family structure, to adjust for confounding and correlated data.
‡ In a period of 3 months prior to inhibitor development.
§ For calculating the relative risk of intensive use of factor VIII and continuous infusion of factor VIII concentrates only the first surgical procedures were taken into account, as all post-surgical inhibitors arose after the first surgical procedure.

received factor VIII replacement therapy on about five occasions before he developed an inhibitor.

Half of the patients (n = 70) switched factor VIII product for a total of 220 times. In seven patients this product change took place at the moment of surgery. Only one patient developed an inhibitor within three months after changing factor VIII product, he switched from a recombinant product to another recombinant product on the day he was operated and developed an inhibitor 64 days later. (Table 2, patient number 3) In the whole study population there was no significant relation between factor VIII product change and inhibitor development (RR, 1.1; CI, 0.1-11.7) (Table 3-4).

DISCUSSION

This first cohort study on the etiology of inhibitors in patients with mild or moderate hemophilia strongly suggests that intensive peri-operative use of factor VIII concentrate, especially when delivered by continuous infusion, and to a lesser extent the Arg593Cys mutation are risk factors for the development of inhibitors in this group. No inhibitors occurred after intensive use of factor VIII concentrate for bleeding. The number of inhibitor patients was too small to investigate the possible interaction of the Arg593Cys mutation and intensive peri-operative factor VIII exposure. Two new missense mutations were observed in the patients who developed inhibitors after intensive peri-operative use of factor VIII: Pro1761Gln and Glu2228Asp.
Previously, case reports have been published about inhibitor development in patients with Arg593Cys missense mutation.\textsuperscript{6,30-35} These reports concern case reports of three patients from the current study (Table 2, patient number 1, 4 and 8), one patient from Canada,\textsuperscript{33} and two patients from Sweden.\textsuperscript{34} No previous study has addressed the causative role of the mutation in a cohort study or case control study. The mutation leads to replacement of the amino acid arginine by cysteine at position 593 in the A2 domain of the factor VIII protein. Because cysteine is involved in the formation of disulphide bridges, its presence may result in a conformational change of the factor VIII protein. Therefore, it may alter the immunogenic characteristics of the endogenous factor VIII. Upon the administration of wild-type factor VIII, this might predispose for inhibitor development.\textsuperscript{31,35}

The pedigrees of the Arg593Cys patients in this study suggested a founder effect. Since other genetic factors than the mutation in the factor VIII gene can contribute to the risk of inhibitor development, it can not be excluded that shared immunological genes other than the Arg593Cys mutation might play a role in the observed risk for inhibitor development in these patients.\textsuperscript{14,15} Therefore we adjusted for family structure in the multivariate analysis. However, except for two HLA identical twin brothers, the patients in whom inhibitors developed were not closer than 4th to 11th degree family members. Since all but two patients were of Caucasian origin, we could not investigate whether ethnicity is a risk factor for inhibitor development in mild or moderate hemophilia.

Both intensive peri-operative use of factor VIII and continuous infusion have been mentioned as possible risk factors for inhibitor development in patients with mild or moderate hemophilia.\textsuperscript{17,18} The risk of intensive peri-operative use of factor VIII was demonstrated in severe hemophilia A patients in the CANAL cohort study,\textsuperscript{23} but it has never been studied in mild or moderate hemophilia A before. As no inhibitors developed after intensive treatment with factor VIII for bleeding, tissue damage that results from surgery may play an important pathophysiological role by triggering the immune system in such a way that it is more prone to develop inhibitors.\textsuperscript{24,36,37} When interpreting the increased risk found for continuous infusion on inhibitor development the low number of patients that received factor VIII by continuous infusion should be taken into account. Nevertheless, our results strongly suggest an attributive risk of continuous infusion during surgery for inhibitor development. The antibody response against exogenous factor VIII in these patients may be explained by the theoretical release of danger signals elicited by subcutaneous leakage of factor VIII concentrate during continuous infusion. Other factors possibly contributing to this increased risk are the modification of factor VIII protein during storage in infusion pumps, or concomitant thrombophlebitis at the infusion site.\textsuperscript{17-19} However, since two patients who developed inhibitors post surgically had exclusively received bolus injections, the risk of post-surgical inhibitors could not be attributed solely to the use of continuous infusion.
In our cohort, all post-surgical inhibitors occurred after the first surgical procedure, suggesting that especially this first exposure to surgery combined with intensive use of factor VIII selects those patients who are more susceptible to develop inhibitors. As all post-surgical inhibitors were detected within three months after surgery, this period was used as time window for this covariate in the Poisson regression model. The extremely high relative risk found for intensive peri-operative use of factor VIII on inhibitor development is partially due to this narrow time window during which the variable is assumed to be effective, nevertheless the association of intensive peri-operative use of factor VIII on inhibitor development seems strong.

Although inhibitor development after changing factor VIII product has been suggested in mild hemophilia A, until now there is no evidence that a change of product is associated with an increased inhibitor risk. In our cohort only one patient developed an inhibitor after product change. As this product change was combined with intensive peri-operative factor VIII exposure on the same day, the risk attributable to product change could not be discerned.

Several studies have demonstrated that age at first factor VIII exposure is inversely associated with inhibitor risk in severe hemophilia A. Because of the advanced age of our cohort, many patients had been treated with cryoprecipitate or other blood products before factor VIII concentrates became available. Since reliable data could not be retraced, we did not analyze factor VIII concentrate that was administered before 1980 or prescribed outside the study center and the influence of age at first factor VIII exposure on inhibitor development could not be analyzed. The reported cumulative number of exposure days and cumulative amount of factor VIII concentrates may underestimate the total lifetime exposure, although we expect that this occurred randomly and that the observed relations are not confounded.

To summarize, the intensive use of factor VIII concentrates in surgical procedures and the Arg593Cys mutation are both associated with a high risk of inhibitor development in mild or moderate hemophilia A. In order to prevent the severe complication of developing inhibitors, we propose that this risk should be taken into account when treating patients with mild and moderate hemophilia A intensively with factor VIII concentrates for surgical procedures, particularly in those carrying high risk mutations. Hence, surgery should only be performed for clear indications. Our results imply that limiting intensive factor VIII exposure or alternative therapeutic options, such as the additional use of DDAVP in mild hemophilia A, should be considered whenever possible.
REFERENCES


Surgery and the arg593cys mutation are risk factors


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