Inhibitor development in nonsevere hemophilia A
Eckhardt, C.L.

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Chapter 6

Surgery and inhibitor development in hemophilia A: a systematic review

C.L. Eckhardt, J.G. van der Bom, M. van der Naald, M. Peters, P.W. Kamphuisen, and K. Fijnvandraat

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ABSTRACT

Background
Although the association between intensive treatment and the formation of inhibiting antibodies towards factor VIII in hemophilia A has been demonstrated, the attributing effect of surgery is presently unclear. The release of immunological danger signals resulting from tissue damage in surgery in the presence of a high factor VIII antigen load may elicit the formation of factor VIII antibodies. The aim of this systematic review was to investigate the role of surgery on the inhibitor risk associated with intensive treatment as compared to treatment for bleeding and prophylactic administration of factor VIII.

Methods
A comprehensive literature search was performed that identified four cohort studies and three case control studies, comprising 342 inhibitor patients among a total of 957 hemophilia A patients.

Results
Intensive treatment increased the inhibitor risk, most pronounced with intensive treatment of ≥ 5 exposure days compared to < 3 exposure days (Odds Ratio [OR], 4.1; 95% confidence interval [CI], 2.6-6.5). Pooled odds ratio for inhibitor development in severe hemophilia patients that received intensive treatment for surgery at first exposure was 4.1 (CI, 2.0-8.4) compared to treatment for bleeding or prophylaxis. Information on continuous infusion, previously treated patients and non-severe hemophilia A was insufficient for valid meta-analyses.

Conclusions
Intensive factor VIII treatment for surgery at first exposure leads to a higher inhibitor risk in hemophilia A patients as compared to intensive treatment for bleeding.
INTRODUCTION

The development of factor VIII inhibiting antibodies (inhibitors) is the most severe complication of the treatment with clotting factor concentrates for hemophilia A. Inhibitors compromise the ability to manage hemorrhage in affected patients, resulting in a considerable increase in complications, disability and costs.\(^1,2\) About one in four severe, and one in 15 nonsevere hemophilia A patients develop inhibitors during their treatment.\(^3\) The reason why these patients develop inhibitors is not completely elucidated, and this hampers developing an effective strategy to reduce the risk.

The etiology of inhibitor development is a complex process in which multiple genetic and environmental factors interact dynamically.\(^4\) Patients who develop inhibitors are likely to have high risk genotypes. Inhibitor development is triggered by certain environmental factors during their treatment; such as intensive treatment with clotting factor concentrates, inflammation and infection.\(^5,6\) Inflammation may provoke antibody formation by the concurrent presence of cytokine release arising from injured tissues, so called “danger signals”.\(^7\)

Although the association between intensive treatment and the formation of inhibiting antibodies towards factor VIII in hemophilia A has been demonstrated, it is presently unclear through which mechanism this severe complication is elicited.\(^8\) Moreover, the influence of reason for treatment – surgery versus bleeding – is indistinct. Not only may tissue damage and injury elicit immunological danger signals during surgery, several other factors such as anaesthetic drugs and inflammation may contribute to antibody formation. In addition, administration of factor VIII concentrates by continuous infusion has been suggested to contribute to a higher incidence of inhibitors peri-operatively. Several conditions related to continuous infusion such as subcutaneous leakage of factor VIII concentrate, factor VIII protein modification during storage in infusion pumps, or concomitant thrombophlebitis at the infusion site may lead to danger signal release and thereby activation of the immune system.\(^9\)

Peri-operative factor VIII replacement regimens are targeted to prevent bleeding and do not take the potential inhibitor risk into account. Over the last few decades this has resulted in a tendency to aim for higher factor VIII levels, leading to the use of higher doses of factor concentrates in surgical procedures.\(^10\) If inhibitor risk of intensive treatment for surgical interventions could be predicted in individual patients, clinicians would be able to adapt their management of these patients to reduce the risk.

Although numerous reports on intensive treatment with factor VIII concentrates in combination with surgery as a risk factor for inhibitor development in hemophilia A have been published, no systematic review of the literature is available. By combining data of individual studies, the inclusion of a high patient number will lead to the critical mass that is needed to quantify more accurately the effect of this risk factor.
The purpose of this systematic review was to address the contribution of surgery to inhibitor formation in patients with hemophilia A receiving intensive treatment with factor VIII concentrates.

Therefore we formulated three research questions, based on contrasting the effect of high and low doses of factor VIII (a) and by contrasting the effect of the presence of immunological danger signals with their absence (b), as illustrated in Figure 1:

1. Is there an association between intensive treatment with factor VIII concentrates for surgery and inhibitor development as compared to prophylaxis?
   (a) High versus low load of factor VIII antigen, and
   (b) Presence versus absence of danger signals.

2. Is there an association between intensive treatment with factor VIII concentrates for surgery and inhibitor development as compared to intensive treatment for a bleeding episode?
   (a) High versus intermediate load of factor VIII antigen, and
   (b) Higher versus lower presence of danger signals.

3. Is continuous administration of factor VIII concentrates during intensive treatment for surgery associated with a higher risk for inhibitor development as compared to administration by bolus infusions?
   (a) Both high load of factor VIII antigen, and
   (b) Higher versus lower presence of danger signals.

Figure 1. Research questions
METHODS

We conducted a systematic review and meta-analysis according to the Meta-analysis of Observational Studies in Epidemiology (referred to as “MOOSE”) Group checklist. This MOOSE consensus statement was developed to provide uniform guidance for the conduct of meta-analysis of observational studies, in order to increase their quality.

Study selection
We comprehensively searched the literature of CENTRAL, MEDLINE and EMBASE (January 1966 - December 2010) using terms for hemophilia (e.g., Haemophilia*, Hemophilia*, Haemophiliac* or Hemophiliac*) factor VIII (e.g., factor VIII*, FVIII*, or factor 8*), inhibitor (e.g., inhibit* or antibody*) and epidemiology (Epidemiolog*, epidemiologic factors). The full list of terms is available in supplemental table 1. Abstracts of empirical studies that appeared potentially relevant were identified independently by two of the authors (CE and KF) by checking the records retrieved by the electronic search. For those studies that were potentially relevant, full papers were obtained and studies were selected for inclusion. Additional articles were identified by reviewing reference lists. Searches were not restricted to language. The manufacturers of factor VIII concentrates (Bayer Healthcare, Baxter, CSL Behring and Wyeth Pharmaceuticals) were contacted for additional data and information about unpublished data of factor VIII treatment for surgery.

Randomised controlled trials, cohort studies and case control studies were included if the participants were hemophilia A patients (irrespective to age, severity, or treatment history), if treatment for surgery, bleeding and/or prophylaxis was reported, and if the outcome was inhibitor development. Studies were included when either all participants were previously untreated patients at the beginning of the observation, or if previously treated participants had never been tested positive for inhibitors and the number of previous exposure days to factor VIII concentrates were reported. Surgery was defined as an invasive medical procedure involving an incision of skin or mucosa with instruments, or closure of a previously sustained wound. Any surgical procedure was considered, irrespective of urgency, type of procedure, body system involved, degree of invasiveness, and kind of instrumentation. Other procedures were considered as surgery (e.g., angioplasty or endoscopy) if they involved “common” surgical procedure or settings, such as use of a sterile environment, anaesthesia, antisepctic conditions, typical surgical instruments, and suturing or stapling. Intensive treatment with factor VIII concentrates was defined as the use of factor VIII concentrates during at least 3 days. We defined inhibitor development as at least one positive inhibitor assay (≥0.6 BU mL⁻¹) during the study observation period.

Studies were excluded if there was no contrast of study populations within the study, i.e., determinant series (all patients underwent surgery) or case-series (all patients developed an
inhibitor) or case-reports. Also studies including only patients with acquired hemophilia A or female carriers of hemophilia A were excluded.

**Data extraction**

Two authors (CE and MN) independently extracted data from full articles of the included studies. If needed, the authors of the primary studies were contacted for clarification of data and additional information. The assessments were done without any masking. A standardized data extraction form was used to retrieve data of interest, including study characteristics, participant characteristics, treatment prior and during study period, continuous/bolus infusion, measure of inhibitor development, follow-up, and potential confounders.

**Assessment of methodological quality**

Five authors (CE, JB, PK, MP and KF) assessed the methodological quality of the studies by a modified quality assessment scale, each study being independently reviewed by two individuals. The modified quality assessment scale was based on earlier described checklists for observational studies according to Evidence Based Medicine Criteria. The assessment of methodological quality concerned the following items: comparability of groups, selection bias, follow-up, outcome, blinding and confounders. Confounders considered in each study were: age, preceding number of exposure days, previous intensive treatment and/or surgery, product type and product switch, ethnicity, positive family history of inhibitors, factor VIII genotype and severity of disease. The methodological quality assessment criteria for observational studies are described in Table 1. For the methodological quality assessment of case-control studies, the criteria were slightly adapted with regard to the selection of cases and controls. Cases and controls had to be selected based on comparable patient characteristics (i.e. age, number of prior exposure days to factor VIII concentrates and factor VIII mutation). Discrepancies between reviewers regarding choice of articles meriting inclusion, data extraction and quality assessment were resolved by discussion.

**Statistical analyses**

We used Review Manager (Review Manager version 5.0.15, Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2008) for data analyses. Because of the use of observational data and the risk of bias, only studies that specifically aimed to study the risk of surgery on inhibitor development were included in the meta-analysis. Incidence of inhibitor development in patients that received intensive treatment with factor VIII concentrates for surgery was compared to the incidence of inhibitor development in others who were treated with factor VIII concentrates for prophylaxis or bleeding. Raw dichotomous data were calculated as odds ratios (OR) or Relative Risks (RR) with 95% confidence intervals.
### Table 1. Methodological quality assessment criteria for observational studies on surgery and inhibitor development

<table>
<thead>
<tr>
<th>Comparability of groups</th>
<th>Selection Bias</th>
<th>Follow-up</th>
<th>Outcome</th>
<th>Confounders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete</td>
<td>Cohort study: if the group consisted of consecutive or obviously representative series of hemophilia A patients (&gt;90% of the original cohort of hemophilia A patients). Or if it was an appropriate random sample with respect to the previous exposure to FVIII concentrates was taken.</td>
<td>If there was follow up of at least three months after intensive treatment with factor VIII concentrates for surgery or bleeding or after prophylactic treatment in all patients. And if inhibitor tests (a minimum of 2 tests) were performed up to the end of the follow-up period. If the outcome was assessed at the end date of the study for more than 90% of the study group of interest.</td>
<td>If the outcome definition was objective and precise, i.e., if all patients underwent inhibitor tests during the study period. And if there was a statement of no history of inhibitors for all patients. And if inhibitor levels have been measured within three months after intensive treatment for surgery/bleeding or prophylactic treatment.</td>
<td>If other important risk factors (age, number of preceding exposure days, previous intensive treatment and/or surgery, product type and product switch as genetic risk factors: ethnicity, pos. family history of inhibitors, FVIII genotype and severity of disease) have been identified and association with inhibitor development have been analyzed for these factors in univariate analysis. And if important prognostic factors (age, number exposure days and previous intensive treatments/surgeries) and follow-up were taken adequately into account.</td>
</tr>
</tbody>
</table>

- If type and extent of surgery was clear, together with description of exposure to FVIII concentrates before (cumulative ED before surgery), during and following surgery for all patients who underwent surgery during the study period. And if mode of administration of factor VIII concentrates (bolus or continuous infusion) during surgery is clear. And if choice and type of control group is clear (treatment for bleeding or prophylactic treatment with FVIII concentrates), together with description of exposure to FVIII concentrates.

- Cohort study: if type and extent of surgery was clear, together with description of exposure to FVIII concentrates before (cumulative ED before surgery), during and following surgery for all patients who underwent surgery during the study period. And if mode of administration of factor VIII concentrates (bolus or continuous infusion) during surgery is clear. And if choice and type of control group is clear (treatment for bleeding or prophylactic treatment with FVIII concentrates), together with description of exposure to FVIII concentrates.

- Case-control study: For the risk of bias assessment of case-control studies, the criteria are adapted with regard to the selection of cases and controls. Cases and controls have to be selected from the same source population (preferably stratified on number of previous exposure days).
Table 1. Continued

<table>
<thead>
<tr>
<th>Comparability of groups</th>
<th>Selection Bias</th>
<th>Follow-up</th>
<th>Outcome</th>
<th>Confounders</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Source population</strong></td>
<td>Source population is defined as follows: 'If the controls would have become a case, they would have been selected in the current study as a case-patient.'</td>
<td>If time of follow up is explicitly stated in the method section on the study design. And if time of follow up is adequate considering the specific aim of the study. And if the outcome was assessed at the end date of the study for 80% of the study group of interest. Or if description was provided of those lost and if subjects lost to follow up were unlikely to introduce bias.</td>
<td>If description was provided of those who were not tested and that there was no clinical likelihood of inhibitor presence (i.e., no spontaneous bleeding symptoms and no decreased response to FVIII concentrates).</td>
<td>And if relative risk, odds ratio, attributable risk, linear or logistic regression model, or mean difference, was calculated for more than 90% of the population.</td>
</tr>
<tr>
<td><strong>Adequate</strong></td>
<td>If surgery status is described for all patients And exposure before and during the peri-operative period is described</td>
<td>Cohort study: If eligible HA patients over a defined period of time, in a defined catchment area, or in a defined hospital or clinic, group of hospitals or health maintenance organization were included. Case-control study: If all patients were drawn from the same community or source independent of factor exposure history.</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>And</strong></td>
<td></td>
<td></td>
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</table>

And if cumulative number of preceding exposure days, severity of disease and FVIII mutation are mentioned for all patients. And if a multivariate analysis has been conducted, but not with all above mentioned prognostic factors and/or follow-up.
intervals (CI) and were pooled using the Mantel Haenzel method. A random effects model was employed because heterogeneity was assumed to exist, based on the observational character of the included studies.

RESULTS

Our initial search yielded 2494 literature citations. After exclusion of 382 duplicates, 2,112 unique references remained (Figure 2). Of these, 1,961 were excluded after scanning titles or reading the abstracts. Of the 151 unique articles retrieved, 128 were excluded after full article review. In 60 studies there was no data on surgery or no surgery was performed during the observation period. 24 studies were case reports or case series in which all patients underwent surgery (23) or all had an inhibitor (1), in five studies inhibitors pre-existed before study-entry and in 21 studies information on inhibitor outcome was not reported, four were reviews, eight were comments and/or contained no original data, two were experimental reports, four studies were published in a non-English language (Spanish, Japanese, Chinese and Hungarian) and the full article could not be obtained.

Twenty-three studies were considered for inclusion in the systematic review.14-36 An attempt was made to contact all corresponding authors for lacking data; three authors and one pharmaceutical company provided additional data before January 1st, 2011.24;28;33 After thorough examination of the 23 articles, 16 were excluded from the systematic review, because they were not aimed to study the association between surgery and inhibitor development in hemophilia A. Consequently chronological data about prophylaxis, bleeding and surgery for each patient was lacking in these 16 studies, hampering the analysis of these determinants in cases and controls. The remaining seven studies,14;15;19;24;25;33;36 comprising 957 hemophilia A patients, were included in the systematic review. Risk of bias (study quality) was assessed for all seven studies and was adequate to complete for most of the studies. The overview of the results of the quality assessment results is listed in Table 2.

Study characteristics

Table 3 presents the characteristics of the seven included studies. Four of the seven studies were cohort studies14;19;24;25 and three case control studies15;33;36 Four studies (2 cohort, 2 case-control) included only patients with a severe form of hemophilia A (FVIII:C, ≤ 1%),15;24;25;33 and the other three (2 cohort, 1 case-control) included only nonsevere hemophilia A patients (FVIII:C, 2-40%).14;15;19;36

Type of surgical procedure varied greatly among the inhibitor patients, most performed procedures were dental extractions, orthopaedic procedures and catheter implantations.
The studies did not specify type of surgical procedures in non-inhibitor patients. Only one study mentioned cumulative factor VIII dose and number of exposure days peri-operatively.\textsuperscript{19} A total of 342 patients developed an inhibitor during the study observation period. The cut-off for a positive inhibitor assay ranged from $> 0.5$ BU mL\textsuperscript{-1},\textsuperscript{33} $\geq 0.6$ BU mL\textsuperscript{-1}\textsuperscript{14,24,36} to $\geq 1$ BU mL\textsuperscript{-1}\textsuperscript{15,19} and was not stated in one study.\textsuperscript{14} The frequency of inhibitor testing was defined as $\geq 4$/year,\textsuperscript{15,24,33} or $\geq 4/50$ exposure days\textsuperscript{24} or 1/year or after intensive treatment,\textsuperscript{19} and was not stated in two studies.\textsuperscript{14,36}

**Inhibitor risk of surgery at first exposure**
Data on treatment at first exposure was available in the four studies on patients with severe hemophilia A, two cohort studies and two case-control studies.\textsuperscript{15,24,25,33} In the multicenter
cohort of 366 severe hemophilia A patients by Gouw and colleagues.\textsuperscript{25} 65% (11/17) of the patients that were first treated for surgical procedures developed an inhibitor compared to 22% (65/286) of the patients who were treated for bleeding and 23% (8/37) of the patients who received prophylaxis at first treatment. For the other 26 patients the reason for first exposure to factor VIII and inhibitor development related to this first exposure was not stated in the article. Adjusted RR of surgical procedure at first treatment for inhibitor development was 2.6 (CI, 1.3-5.1).

The other study by Gouw and colleagues describes a cohort of 236 severe previously untreated patients derived from databases of four factor VIII product registration studies.\textsuperscript{24} Data on treatment at first exposure was available for 234 (99%) patients. Half of the patients (2/4, 50%) that received their first factor VIII concentrates for surgery developed an inhibitor, compared to 25% (45/180) of the patients that were treated for bleeding and 44% (7/16) who received prophylaxis at first treatment (data obtained from authors). For the other 34 patients the reason for first treatment was recovery (8 patients) or other, not further specified (26 patients), 13 of them developed an inhibitor (38%). Based on these data, we did not find a statistically significant association between surgery as reason for first exposure and inhibitor development (OR, 2.7; CI, 0.4-20.2).

The case-control study by Santagostino and colleagues included 60 inhibitor patients and 48 controls with moderate or severe hemophilia A.\textsuperscript{33} Surgery was the reason for starting factor VIII treatment in seven cases (12%), all with high-responding inhibitors and two (4%) controls. There was no statistically significant association between surgery compared to bleeding at first exposure and inhibitor development (crude OR, 3.0; CI, 0.6-15.4).

The other case-control study by Maclean and colleagues included 78 inhibitor cases and 78 age-matched controls.\textsuperscript{15} Four of the cases (5%) and two of the controls (3%) had surgery as reason for their first exposure. Compared to minor bleeds, there was no significant

### Table 2. Risk of bias

<table>
<thead>
<tr>
<th>Article</th>
<th>Good comparability of groups</th>
<th>No selection bias</th>
<th>Follow-up</th>
<th>Adequate outcome measurement</th>
<th>Adequate dealing with confounders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eckhardt et al.\textsuperscript{20}</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>-</td>
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<tr>
<td>Gouw et al.\textsuperscript{26}</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
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<tr>
<td>Gouw et al.\textsuperscript{25}</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Kempton et al.\textsuperscript{37}</td>
<td>-</td>
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<tr>
<td>Maclean et al.\textsuperscript{16}</td>
<td>+</td>
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<tr>
<td>Santagostino et al.\textsuperscript{34}</td>
<td>+</td>
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<td>++</td>
<td>+</td>
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<tr>
<td>Sharathkumar et al.\textsuperscript{15}</td>
<td>+</td>
<td>++</td>
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</table>

++ indicates complete; + indicates adequate; and – indicates inadequate, according to the methodological quality assessment criteria (Table 1).
association between surgery and inhibitor development (crude OR, 2.2; CI, 0.4-12). In both case-control studies the chronological order of events was not analyzed nor was surgery included in multivariate analysis.\textsuperscript{15,33}

Based on these 4 studies,\textsuperscript{15,24,25,33} crude pooled OR for inhibitor development of surgery compared to non-surgery (i.e. bleeding or prophylaxis) at first exposure was 4.1 (CI, 2.0-8.4) (Figure 3). This association remained when surgery was compared to bleeding alone (crude OR, 4.1; CI, 2.0-8.5). In only two studies,\textsuperscript{24,25} prophylactic treatment was given as reason for first exposure. Surgery was associated with a twofold increased risk for inhibitor development compared to prophylaxis (crude pooled RR, 2.1; CI, 0.8-5.2).

**Inhibitor risk of surgery in previously treated patients**

The four studies in patients with severe hemophilia A also reported data on surgery and inhibitor development in (minimally) previously treated patients.\textsuperscript{15,24,25,33} In the cohort study by Gouw and colleagues 25% (80/366) patients underwent 84 major surgical procedures, defined as surgery for which replacement therapy was given on at least 3 consecutive days.\textsuperscript{25} Major surgical procedures at any exposure day (including surgery at first exposure) were associated with a slightly increased risk for developing inhibitors (adjusted RR, 1.4; CI, 0.8-2.5) as compared to the period before surgery. Relative risk for inhibitor development after portacath implantations (adjusted RR, 1.4; CI, 0.7-2.7) was comparable to the relative risk of other surgical procedures (adjusted RR, 1.3; CI, 0.5-3.0).

In the other study by Gouw and colleagues major surgical procedures during the first 50 exposure days were associated with a higher risk (RR, 2.4; CI, 1.2-4.8) of developing clinically relevant inhibitors as compared to the period prior to surgery.\textsuperscript{24}

In both case-control studies no association between surgery at any exposure day and inhibitor development was present in univariate analysis.\textsuperscript{15,33} Because of the potential influence of time-varying confounding determinants (e.g. number cumulative exposure days, number preceding surgeries or treatment intensity) on the association of surgery at any exposure day and inhibitor development, we decided that pooling of individual studies was not justified.

**Inhibitor risk and treatment intensity**

In three studies in severe patients treatment intensity was analyzed as a potential risk factor irrespective of reason for treatment.\textsuperscript{15,24,25} In the two cohort studies two different classifications of treatment intensity were used according to the moment in time that intensive treatment took place: at first exposure or at any exposure.\textsuperscript{24,25} The intensity of treatment at first exposure was classified into three categories: < 3 consecutive exposure days, 3-4 consecutive exposure days and ≥ 5 exposure days (defined as intensive treatment). For treatment intensity at any exposure day two categories of treatment intensity were defined: peak treatment moments
defined as treatment on ≥ 3 exposure days, and major peak treatment moments defined as treatment on ≥ 5 exposure days.

In the first cohort study intensive treatment at 1st exposure was associated with a more than threefold increased risk (adjusted RR, 3.3; CI, 2.1-5.3). This association remained for peak treatment moments and major peak treatment moments at any exposure day but less pronounced (peak treatment: adjusted RR, 1.5; CI, 0.9-2.5; major peak treatment: adjusted RR, 1.6; CI, 1.0-2.6).

In the other cohort study the association between intensive treatment at 1st exposure and inhibitors was confirmed (adjusted RR 2.7; CI, 1.2-5.8). Again, in this study the associations of peak treatment and major peak treatment with inhibitor development were less pronounced (respectively adjusted RR, 1.6; CI, 1.0-2.6 and adjusted RR, 1.6; CI, 0.9-2.8).

Maclean and colleagues classified treatment intensity into four categories: < 3 exposure days and three cumulative categories: ≥ 3 exposure days (including the two following categories), ≥ 5 exposure days (including the following category) and ≥ 10 exposure days. Compared to non-intensive treatment (< 3 exposure days), the association of treatment intensity of at least 3, 5 or 10 exposure days and inhibitor development was increasingly pronounced with unadjusted ORs of respectively 1.7 (CI, 0.8-3.5), 2.4 (CI, 0.9-6.3) and 5.0 (CI, 1.03-24).

Pooled results of these three studies showed that intensive treatment of at least 3 consecutive exposure days at first exposure is associated with inhibitor development (crude OR, 2.1; CI, 1.2-3.7) compared to less than 3 exposure days. This association was more pronounced when intensive treatment of ≥ 5 exposure days was compared to < 3 exposure days (crude OR, 4.1; CI, 2.6-6.5) (Figure 4). None of the three studies reported reasons for treatment intensity nor adjusted for reasons of intensive treatment (i.e. bleeding or surgery), when analysing the association between treatment intensity and inhibitor development.
Inhibitor risk of surgery in patients with mild and moderate hemophilia A

We identified three studies on surgery and inhibitor development in nonsevere patients; two cohort studies,\textsuperscript{14,19} and one case-control study.\textsuperscript{36} In a cohort of 29 mild hemophilia patients described by Sharatkumar and colleagues, \textsuperscript{14}14% (4/29) of the patients developed inhibitors.\textsuperscript{14} All inhibitors occurred after intensive treatment with factor VIII concentrate, defined as more than six consecutive exposure days. In two patients inhibitor development was preceded by intensive treatment for surgery. The total number of surgical procedures in the other 25 non-inhibitor patients was not described; therefore the association between surgery or intensive treatment and inhibitor development could not be calculated.

In the other cohort study ten of the 138 patients (7%) developed an inhibitor.\textsuperscript{19} Of these ten patients, seven (70%) were treated intensively with factor VIII concentrate for surgery prior to inhibitor development. Intensive exposure was defined as at least five consecutive exposure days with a cumulative factor VIII use of at least 250 units per kilogram bodyweight or 30 IU kg\textsuperscript{-1} day\textsuperscript{-1}. If the reason for the first intensive exposure was a surgical procedure this was associated with a 186-fold increased risk for inhibitor development (CI, 25-1403) in the three months after the surgical procedure as compared to the period in which no intensive treatment of factor VIII was given. After the first intensive exposure period, 36 patients received one or more subsequent intensive treatments for surgery or bleeding. None of these patients developed an inhibitor.

In the case control study 36 inhibitor cases and 62 controls were included.\textsuperscript{36} Half of the inhibitor cases (18/36, 50%) and 18% of the controls (11/62) received intensive factor VIII treatment during the prior year, defined as six or more consecutive days of factor VIII replacement. Intensive treatment in the prior year was strongly associated with inhibitor development (OR, 4.6; CI, 2.8-11.7). Surgery was the indication for intensive treatment in 78% (14/18) of the cases compared to 36% (4/11) of controls. The association between surgery and inhibitor development was not further analyzed.

In these three studies in mild and moderate hemophilia A patients information on the chronological order of events was not presented.\textsuperscript{14,19,36} Because the contribution of these unknown factors to inhibitor development cannot be quantified, no pooled analysis of the association between surgery and inhibitor development in nonsevere patients could be made.

Continuous infusion versus bolus injections

Mode of administration during surgery was reported in the three studies including nonsevere hemophilia A patients.\textsuperscript{14,19,36} In the cohort study by Sharatkumar and colleagues seven of the 16 patients (44%) that underwent intensive treatment with factor VIII concentrates received continuous infusions.\textsuperscript{14} All four inhibitors occurred after continuous infusion (4/7 = 57% of all patients receiving continuous infusion). In the other cohort study continuous infusion was
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used in ten of the 41 patients (25%) during intensive treatment for their first major surgery.\textsuperscript{19} Five of these patients (50% of all patients receiving continuous infusion) developed an inhibitor afterwards. After adjustment for surgery, intensive treatment, product change, factor VIII genotype and family structure, the RR of continuous infusion for inhibitor development was 13 (CI, 1.9-86) in the three months following surgery covered by continuous infusion as compared to the period in which no intensive treatment of factor VIII was given.

In the case-control study in nonsevere patients by Kempton and colleagues continuous infusion of factor VIII was used during the period of intensive factor VIII treatment in seven of the 18 cases (39%) and in two of the 11 controls (18%).\textsuperscript{36} No significant association was found between continuous infusion and inhibitor development as compared to bolus injections ($p = 0.41$; OR and CI not mentioned). Because of heterogeneity of the studies and missing data we were not able to pool data of the studies to perform a meta-analysis.

### DISCUSSION

Our study shows that surgery in combination with factor VIII treatment is strongly associated with inhibitor development in hemophilia A as compared to intensive treatment for bleeding or prophylaxis alone. This association is especially pronounced if surgery is the reason for first treatment and seems present both in severe and nonsevere hemophilia A. Information on continuous infusion was scarce.

We comprehensively searched the literature for relevant articles following systematic review methods. The use of robust methodological procedures to estimate the risk of bias of potentially relevant articles strengthens our conclusions. The quality of retrieved studies was not always optimal, as information on prior exposure status of the patients, type of surgical procedure and chronological association between treatment and inhibitor development was frequently lacking. To reduce potential risk of bias, we restricted the meta-
analysis to studies specifically focussing on risk factors for inhibitor development. As these studies consisted of representative series of hemophilia A patients covering a population heterogeneous in origin, age and severity of disease, we assume that this inclusion criterium has not introduced severe selection bias.

Although our meta-analysis was limited to the use of crude risk estimates, the possibility of confounding seems to be limited, as adjustment for time-varying determinants in the two studies by Gouw and colleagues did not lead to major changes in risk assessments (major surgical procedures at any exposure crude RR, 2.4; vs. adjusted RR, 2.7; and surgical procedures crude RR, 3.7; vs. adjusted RR, 2.6). The association between intensive treatment > 5 exposure days and inhibitor development was previously acknowledged in the CANAL study. However, treatment for surgical procedures is in general more intensive than factor VIII treatment for bleeding episodes. It is therefore extremely difficult to study surgery and intensive treatment independent of each other. None of the included studies provided detailed information on the reason for intensive treatment. Another limitation was that actual levels of factor VIII were not reported in the studies that we reviewed and could not be included in the meta-analysis.

Previous studies showed that the risk of developing inhibitors is strongly associated with the number of previous exposures to factor VIII. In severe patients half of the inhibitors occur before the 15th exposure day and the other half occur with a sharply decreasing incidence rate relatively early afterward. After 50 exposure days the risk of developing new inhibitors is less than 1%. This review showed that especially intensive treatment for surgery at first treatment is associated with inhibitor development (OR, 4.1). Surgery at any exposure within the first 50 exposure days was also associated with inhibitor development but less pronounced (RR, 1.4-2.4). Thus, a higher number of exposure days prior to surgery may reduce the risk for inhibitor development.

Prophylaxis is already widely used as standard treatment regimen in severe hemophilia, based on favourable joint outcome resulting from decreased bleeding frequency. However, evidence is lacking on the optimal timing of initiation of primary prophylaxis. From an immunological point of view, the opportunity should be availed to obtain tolerance in a “danger-free” environment and therefore prophylaxis should be initiated before exposure related to surgery or a major bleed. Treatment was initially started as prophylaxis in a small proportion of patients (7-11%) in the two studies that were included in the meta-analysis. This may be due to the fact that most treatment regimens in severe patients start prophylaxis after the first bleeding episode (joint bleed or cerebral bleeding). Based on these two studies the pooled OR for inhibitor development following treatment for surgery in comparison to prophylaxis was remarkably less pronounced (OR, 2.1) than when surgery was compared to bleeding (OR, 4.1). As we used non-randomized data for unadjusted meta-analysis, this may be explained by the clinical selection of patients for initiation of prophylaxis.
before any intensive treatment period. Further research is needed to evaluate whether the protective effect of prophylaxis can be confirmed.

In nonsevere hemophilia A patients prophylactic treatment is rarely given because these patients have a less severe bleeding pattern. The presence of low amounts of circulating factor VIII protects these patients from spontaneous bleeding and therefore factor VIII concentrate is mostly administered for surgery or major bleed. Minor bleeds or interventions are preferably covered by desmopressin (DDAVP) in mild patients with a good response. Factor VIII is predominantly administered when immunological danger signals are present, when surgery is performed or major bleeds occur. This may well explain the relatively high incidence of inhibitors at a more advanced age in nonsevere patients after intensive treatment periods (Table 3).

In the article of Kempton and colleagues 42% of the nonsevere hemophilia A patients developed inhibitors after more than 50 exposure days, suggesting that nonsevere patients are longer at risk of inhibitor development than severe patients. Absence of information on previous intensive exposures, including previous surgical interventions, precluded estimation of the effect of surgery on inhibitor development in nonsevere patients. Certain missense mutations (e.g. Arg593Cys, Arg2150Cys) in mild hemophilia patients are associated with a higher risk of inhibitor formation. Well-designed further studies are needed in nonsevere hemophilia A patients to estimate the risk of surgical interventions on inhibitor development, taking the influence of other genetic and environmental risk factors into account. This will ultimately enable implementation of individual treatment strategies in nonsevere patients who are at high risk of inhibitor development.

**Clinical implications**

Considering the consistent finding of increased inhibitor incidence associated with surgical intervention across a number of heterogeneous patient groups, we believe that caution must be taken when treating patients intensively with factor VIII concentrates for surgery, especially in previously untreated patients. We recommend that intensive treatment with factor VIII concentrates should be avoided early in treatment whenever possible. Starting low dose prophylactic treatment at an early stage may provide an opportunity to induce tolerance in a “danger free” environment.

Because of the increased life-expectancy of hemophilia patients that will increase the incidence of age-related health problems such as cancer and arthrosis, a continued rise in the need for surgery in hemophilia A patients is to be expected. Therefore, risk estimation in previously treated patients and mild/moderate hemophilia A patients – who might face their first intensive exposure to factor VIII at an advanced age – should get more attention. As intensity of treatment is associated with the risk of inhibitor development, ways to lower the amount or duration of factor VIII replacement should be investigated. In mild hemophilia
Table 3. Characteristics of studies included in the systematic review

<table>
<thead>
<tr>
<th>Study</th>
<th>Study design</th>
<th>Total follow-up in ED</th>
<th>Severity of disease</th>
<th>Age Median (IQR)</th>
<th>Positive family history No (%)</th>
<th>Type of F VIII concentrates</th>
<th>FVIII exposure at study entry</th>
<th>Total N/ Inhibitors*</th>
<th>Surgery at 1st ED/ Inhibitors*</th>
<th>Total surgeries/ Inhibitors*</th>
<th>Adjustments for confounders for the association between surgery &amp; inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eckhardt et al. 20</td>
<td>Cohort</td>
<td>Median 10 ED (IQR 6-29)</td>
<td>128 mild 10 moderate</td>
<td>41 (18-61) yrs</td>
<td>NS</td>
<td>47 (34%) plasma 91 (66%) rec</td>
<td>138 PUP</td>
<td>138/10</td>
<td>NS/NS</td>
<td>75/7</td>
<td>Factor VIII product change, continuous infusion, family structure, Arg593Cys mutation, intensive treatment for bleeding</td>
</tr>
<tr>
<td>Kempton et al. 37 Case-control</td>
<td>48 pat &lt; 50 ED</td>
<td>48 Mild 31 yrs</td>
<td>Controls: 31 yrs</td>
<td>11 (11)</td>
<td>NS</td>
<td>62/36</td>
<td>NS/NS</td>
<td>4/14</td>
<td>NS</td>
<td>Baseline FVIII activity level, ethnicity, family history of inhibitors, age at first exposure and prophylaxis.</td>
<td></td>
</tr>
<tr>
<td>Maclean et al. 16 Case-control</td>
<td>&gt;50 ED</td>
<td>156 Severe Cases: 284 days</td>
<td>Controls: 289 days</td>
<td>45 (29%) plasma 111 (71%) rec</td>
<td>156 PUP</td>
<td>78/78</td>
<td>2/4</td>
<td>22/17</td>
<td>NS</td>
<td>Baseline FVIII activity level, ethnicity, family history of inhibitors, age at first exposure and prophylaxis.</td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Study design</td>
<td>Total follow-up in ED</td>
<td>Severity of disease</td>
<td>Age Median (IQR)</td>
<td>Positive family history No (%)</td>
<td>Type of F VIII concentrates</td>
<td>FVIII exposure at study entry</td>
<td>Total N* Inhibitors*</td>
<td>Surgery at 1st ED/Inhibitors*</td>
<td>Total surgeries/Inhibitors*</td>
<td>Adjustments for confounders for the association between surgery &amp; inhibitors</td>
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</tr>
<tr>
<td>Santagostino et al. 34</td>
<td>Case-control</td>
<td>&gt; 50 ED</td>
<td>108 severe</td>
<td>Cases: 11 (range 2 days-64) mo Controls: 13 (1 day -67)mo</td>
<td>13 (12) NS</td>
<td>108 PUP 48/60*</td>
<td>2/7*</td>
<td>11/15* NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sharathkumar et al. 15</td>
<td>Cohort</td>
<td>Mean 16 ED (Range 1-46)</td>
<td>54 Mild</td>
<td>5.4 yrs (range 5 days to 16.3 yrs)§§</td>
<td>NS</td>
<td>4 (14%) plasma 25 (86%)††</td>
<td>54 PUP†† 29/4</td>
<td>NS/NS NS/NS NS/NS NS</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ED, Exposure Day(s); IQR, Inter Quartile Range; NS, not stated; mo, month(s); yrs, year(s); pat, patients; PUP, Previous Untreated Patient; PTP, Previously Treated Patients; rec, recombinant factor VIII concentrate; plasma, plasma derived FVII concentrate.

* Total number of patients who developed de novo inhibitors during the study observation period.
† Total follow-up in years or cumulative ED in control patients, follow-up in inhibitor cases until inhibitor development.
‡ When analysing the association between surgical procedure as reason for first factor VIII treatment and inhibitor development.
§ When analysing the association between major surgical procedures at any exposure and inhibitor development.
¶ These data concerns a case-control study, numbers indicate “controls/cases”.
** Total surgeries in prior year before inhibitor development or study inclusion.
†† Only 29 participants were exposed to FVIII concentrates during the study observation period, the other 15 patients were PUP at the end of the observation period.
‡‡ At the end of study follow-up.
§§ At first FVIII infusion.
A patient's alternative or additional use of desmopressin should be considered to reduce intensive treatment.

Two studies in mild and moderate patients show a clear association between continuous infusion and inhibitor development. Storage conditions, phlebitis at the infusion site or other concomitant factors during continuous infusion may provoke the immune system to develop inhibitors. However, the number of patients that received continuous infusion in these studies was small.¹⁴,¹⁹ Therefore, recommendations concerning the use of continuous infusion to cover surgical procedures or bleeding episodes should await further investigations.

CONCLUSION

This systematic review shows that surgery in combination with intensive factor VIII treatment is strongly associated with inhibitor development in hemophilia A patients as compared to treatment for bleeding or prophylaxis. This review highlights the need for robust well-designed future studies on the association between surgery and inhibitor development in nonsevere hemophilia A patients and previously treated patients, in which potential confounding factors are taken into account. Also the role of continuous infusion should be further elucidated. This knowledge will ultimately help us to understand the pathophysiology of surgery causing inhibitor development and may enable further research on preventive strategies, such as prophylaxis or immunosuppressive therapy.
REFERENCES


**Supplemental Table 1. Search strategy**

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<td>9 9001-27-8.rn. (12355)</td>
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<td>28 from 27 keep 1-29 (29)</td>
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<td>29 exp clinical trial/ (563268)</td>
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EMBASE search strategy:

1. (haemophil* a or hemophil* a or haemophiliac* or hemophiliac*).tw. (5483)
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3. 1 or 2 (6964)
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5. fviii.tw. (2037)
6. rviii*.tw. (11)
7. tfviii*.tw. (142)
8. factor 8.tw. (416)
10. factor viii.rw. (0)
11. or/4-10 (14609)
12. blood coagulation factor inhibitors.mp. or exp Blood Coagulation Factor Inhibitors/ (34774)
13. antibodies.mp. or exp Antibodies/ (507763)
14. antibody formation.mp. or exp Antibody Formation/ (23869)
15. (ai or iml).fs. or inhibit*.tw. or antibod*.tw. or anti bod*.tw. or autoantibod*.tw. or anti porcine.tw. or alloantibod*.tw. or (circulat* adj4 anticoagul*).tw. (1488724)
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18. Epidemiologic Methods/ (69561)
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22. exp epidemiologic factors/ (826700)
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25. or/17-24 (1158373)
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27. exp Clinical Trial/ (547777)
28. letter/ (425115)
29. 27 or 28 (955423)
30. 3 and 11 and 16 and 29 (321)
31. 30 or 26 (526)
32. limit 31 to yr="2008 - 2009" (65)
33. from 32 keep 1-65 (65)

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