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Chapter 10

Dissecting intensive treatment as risk factor for inhibitor development in hemophilia

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Submitted for publication
ABSTRACT

The treatment of hemophilia A can be severely complicated by the development of neutralizing antibodies towards factor VIII (inhibitors). Intensive treatment with factor VIII concentrates has been previously indicated as an important risk factor for inhibitor development. As intensive treatment is administered for major bleeding or surgery, the association between intensive treatment and inhibitor development may originate from two different triggers of the immune system: a.) a high dose of factor VIII administered within a short time span and b.) immunological “danger signals” arising from tissue damage and inflammation. Before changing our treatment strategies in order to lower the inhibitor risk, the contribution of these factors has to be dissected in order to establish their causal role. In the present paper we discuss the methodological issues that are required for observational studies in order to distinguish the separate effects of high dose of factor VIII administration and immunological danger signs on inhibitor development.
INTRODUCTION

The development of inhibiting antibodies (inhibitors) against factor VIII is currently the most severe complication of the treatment with factor VIII concentrates in hemophilia A. Inhibitors complicate the management of hemorrhage in affected individuals, resulting in a greater rate of complications, costs and disability.1,2 If inhibitor risk in individual patients could be predicted, clinicians would be able to adopt their treatment regimens and reduce this risk.

Inhibitor risk is determined by the genetic background of the patient and environmental factors such as treatment characteristics.3,4 Genetic risk depends on the type of factor VIII gene mutation, family history of inhibitors, ethnicity and genetic variations in immunoregulatory genes.5-13 Treatment-related factors that affect the inhibitor risk include factor VIII product type, reason for treatment, dose, frequency, and mode of therapeutic factor VIII administration.14-22

As it seems biologically plausible that the exposure to high doses of a foreign antigen administered within a short time span is a stronger trigger for the immune system than low doses administered infrequently, the association between intensity of treatment and inhibitor development has been studied extensively in recent years. Several observational studies observed an association between intensive treatment and inhibitor development both in severe,15-18 and nonsevere hemophilia A patients.20-23 Intensity of treatment is a risk factor that could potentially be modified by strategies that reduce the factor VIII load within a certain time span. This makes it an attractive candidate for future preventive strategies. However, before changing our treatment strategies, we should first identify the culprit and dissect whether it is indeed the high factor VIII load within a short time span that confers the inhibitor risk.

In the context of intensive treatment, usually administered for major bleeding episodes or surgery, immunological factors may play a role as well, since both severe bleeding and surgery are associated with tissue damage. Thus, the association between intensive treatment and inhibitor development may originate from two different triggers of the immune system: a.) a high dose of factor VIII administered within a short time span and b.) immunological "danger signals" arising from tissue damage and inflammation, eliciting cytokine release and co-stimulatory signaling (Figure 1).24 The contribution of these factors has to be dissected in order to establish whether modified treatment strategies might effectively reduce inhibitor risks.

The current definition of intensive treatment, based on factor VIII dose, interval and duration, only addresses the first factor: high factor VIII antigen load (Table 1, Figure 2). It is important to account for the effect of the other determinant as well: immunological danger signals, as lowering the dose of factor VIII in an attempt to avert inhibitor formation will not confer protection against immunological danger signals.
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Intensive treatment with factor VIII concentrates

Figure 1. Clinical conditions in hemophilia A associated with intensive treatment with factor VIII concentrates have two characteristics in common: a.) a high factor VIII antigen load within a short time span and b.) immunological danger signals elicited by tissue damage and inflammation.

Table 1. Definition of intensive treatment in the studies that assessed the risk of intensive treatment on inhibitor development

<table>
<thead>
<tr>
<th>Definition of intensive treatment/peak treatment moment</th>
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<tbody>
<tr>
<td>Severe hemophilia</td>
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<tr>
<td>Gouw et al. Blood 2007(^{16})</td>
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<tr>
<td>Gouw et al. JTH 2007(^{15})</td>
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<tr>
<td>Gouw et al. Blood 2013(^{17})</td>
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<tr>
<td>Maclean et al. 2012(^{18})</td>
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<tr>
<td>Nonsevere hemophilia</td>
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<tr>
<td>Eckhardt et al. JTH 2009(^{20})</td>
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<tr>
<td>Kempton et al. JTH 2010(^{21})</td>
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<tr>
<td>Eckhardt et al. BHJ 2011(^{33})</td>
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<tr>
<td>Mauser Bunschoten et al. Haemophilia 2011(^{22})</td>
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In the present paper we will further discuss methodological issues that need to be addressed in studies that aim to distinguish the separate effects of lowering the dose of factor VIII administration and of the presence or absence of immunological danger signals on inhibitor development.

HEMOPHILIA SEVERITY AND INTENSITY OF FACTOR VIII EXPOSURE

The intensity of treatment with factor VIII concentrates is determined by hemophilia severity and the severity of bleeding (e.g. trauma, surgery). The bleeding phenotype is associated with the plasma level of factor VIII (FVIII:C) and ranges from frequent spontaneous bleeds in joints and muscles in patients with severe hemophilia A (FVIII:C, <1 IU dL⁻¹) to occasional bleeds after major trauma or surgery in mild hemophilia A patients (FVIII:C, 5-40 IU dL⁻¹). Factor VIII concentrates are generally administrated for three types of indications: a.) to treat bleeds that occur spontaneously or after trauma; b.) to treat or prevent bleeds after surgery or; c.) prophylactic treatment to prevent spontaneous bleeds and associated joint damage in the long-term. In severe hemophilia A patients prophylactic regimens are initiated at a young age and consist of a low dose of factor VIII concentrates (15-25 IU kg⁻¹) 1-3 times a week. Nonsevere hemophilia A patients are preferably treated with desmopressin for minor bleeds. They only require treatment with factor VIII concentrates in case of major bleeding.
or surgery, that is covered by high doses of factor VIII concentrates for several days to obtain prolonged adequate plasma levels of factor VIII.

Inhibitor development is provoked by exposure to factor VIII concentrates. Importantly, the risk to develop inhibitors is not equal for each exposure day and depends on the cumulative number of exposure days to factor VIII concentrates. In severe hemophilia A patients, the risk is highest during the first ten exposure days after which the risk gradually decreases towards below 1% at 50 exposure days.\textsuperscript{17,28} When assessing the inhibitor risk of time-dependent (treatment related) risk factors (such as factor VIII dose), this gradual decrease in risk should be taken into account.

\textbf{IMMUNOLOGICAL DANGER SIGNALS AND INHIBITOR DEVELOPMENT}

Following the traditional immunological principle that the immune system functions by discriminating between self and non-self,\textsuperscript{29} one might expect that all severe hemophilia A patients would recognize the infused factor VIII protein as non-self and therefore mount an immune response. However, this is not the case as approximately 30\% of the patients with severe hemophilia A develop an inhibitor and the other patients become tolerant towards the infused factor VIII.\textsuperscript{28} Why do not all patients develop inhibitors against foreign factor VIII? This may be explained by the “danger theory”. The danger theory proposes that the immune system’s primary driving force is the need to detect and protect against danger.\textsuperscript{24} The danger model, as described by Matzinger, assumes that immune responses are initiated by danger signals from injured tissues.\textsuperscript{24} Distressed or injured tissue cells release danger signals that activate antigen presenting cells that subsequently present antigens in an activated state to T lymphocytes (Figure 3). Activated T lymphocytes will then stimulate B lymphocytes to synthesize factor VIII specific antibodies.\textsuperscript{30}

\textbf{A THOUGHT EXPERIMENT: DESIGN OF A RANDOMISED CONTROLLED TRIAL TO STUDY HIGH FACTOR VIII ANTIGEN LOAD OR DANGER SIGNALS SEPARATELY}

In order to assess a causal claim for an independent determinant it is essential to compare two groups of patients that differ only for the determinant under investigation and are similar with respect to all other clinical features that may contribute to the risk (Figure 4). To assess the inhibitor risk of the two individual determinants (high factor VIII antigen load and presence of danger signals) they should be investigated separately. A randomised controlled trial (RCT) is a study design that allows manipulation of one single factor in an
Dissecting intensive treatment as risk factor

Figure 3. Danger theory: stimulation of CD4+ T cell by exposure to immunological danger signals released by distressed cells caused by tissue damage at time of exposure to foreign factor VIII (based on Matzinger).31

Figure 4. Model of risk assessment of immunological danger signals

intervention group that is comparable to a control group in all other aspects. An observed difference in outcome between the two groups can be ascribed to the single factor under study.31
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As a thought experiment we avail this opportunity to examine our two hypothesis: 1.) the presence of danger signals and 2.) high dose of factor VIII in a short time span increase inhibitor risk. In the first virtual trial previously untreated hemophilia A patients are randomised to receive either danger signals (intervention group) or no danger signals (placebo group) (Figure 5A). For this RCT design we elected a surgical procedure to be the intervention inducing danger signals. Both groups receive the same high doses of factor VIII concentrates (50 IU kg\(^{-1}\) day\(^{-1}\) for 5 consecutive days). During this episode of factor VIII exposure the intervention group will undergo a surgical procedure whereas the control group will not. The outcome ‘inhibitor development’ will be established within six months after the intensive treatment episode.

In the second virtual trial, previously untreated hemophilia A patients are randomised to receive either high doses of factor VIII (intervention group) or low doses of factor VIII (placebo group) (Figure 5B). Both groups will receive either the low or high factor VIII doses with the same frequency and in the same timeframe, as frequency and duration of factor VIII administration may also influence the inhibitor risk. Inhibitor development will be established within six months after factor VIII exposure.

Figure 5. Virtual design of a randomised controlled trial on the effect of a.) danger signals on inhibitor risk and b.) high factor VIII dose on inhibitor risk.
It is clear that these trials could never be performed for ethical reasons. Other options to obtain evidence about the role of high factor VIII dose or danger signals in a randomised setting are difficult to imagine. Although randomisation between prophylaxis (absence of danger signals) and on demand treatment (presence of danger signals) might provide an opportunity, this design is limited by the difference in treatment frequency and antigen load between the two treatment arms that will confound the association between the presence of danger signals and inhibitor development. Thus, as there is no possibility of conducting an RCT that will help us to evaluate the causal effect of danger signals and factor VIII load on inhibitor development, we have to rely on the results of observational studies.

**Figure 6.** Assessment of the causal effect of a.) danger signals and b.) factor VIII dose on inhibitor development in an observational setting.

**How to Study the Effect of High Factor VIII Load and Danger Signals in an Observational Setting?**

Patients with nonsevere hemophilia A patients differ substantially in treatment regimen from severe hemophilia A patients. Therefore, we will discuss the assessment of the causal effect of factor VIII load and danger signals on inhibitor development for these specific subgroups separately.
Severe hemophilia A
To assess the independent effect of high factor VIII load and danger signals on inhibitor development in an observational setting, clinical conditions should be compared with: a.) different "doses" of danger signal but an equal amount of factor VIII administration; or b.) different doses of factor VIII but an equal amount of danger signals (Figure 6). In situations requiring high doses of factor VIII there is variation in the strength of danger signals, depending on the reason for treatment (bleed or surgery) and the presence of other co-morbidities (e.g. infection, malignancy, major bleed). Assuming that surgery produces more extensive tissue damage and stronger danger signals than major bleeding episodes, this contrast was used to study the effect of danger signals in a systematic review comparing treatment for bleeds to prophylaxis. Pooling the results of four studies indicated an increased risk of inhibitor development for surgery (Odds Ratio [OR], 4.1; 95% Confidence Interval [CI], 2.0-8.5) as compared to treatment for bleeding. In the more recent RODIN study by Gouw and colleagues, the association between surgery and inhibitor development was less pronounced. Major surgery at first treatment requiring at least 3 consecutive treatment days, seemed to be associated with an adjusted relative risk of 2.1 (CI, 0.77-5.8) as compared to other treatment indications. As patients have the highest risk of developing inhibitors during the first ten exposure days, the highest risk of peak treatments is to be expected in this period. Major surgery at any time during the first 75 exposures was not clearly associated with inhibitor development (relative risk [RR], 1.4; CI, 0.74-2.6).

Using reason for treatment (surgery or bleed) as a surrogate marker for the strength of danger signals results in a classification that is too heterogeneous, since danger signals elicited by major bleeding after trauma are much stronger that those of minor surgery with little tissue damage. Hence, the independent effect of danger signals cannot be discerned when using solely reason for treatment as determinant for inhibitor risk.

In order to study the effect of danger signals in a clinical setting a large observational study is needed in which detailed information about all factors that may result in immunological danger signals are collected for each exposure day and graded according to severity. These factors include: surgery type, trauma type, bleed location and severity, medication (type and dose), infection (type and severity, supported by results of laboratory markers e.g. C-reactive protein, leucocyte count), inflammation (type and severity), malignancy (type and staging) and other co-morbidities causing tissue damage or inflammation. We believe that the combination of all these factors may approximate the strength of danger signals more than dichotomizing for bleed or surgery. Such a study will enable comparison of more homogenous clinical settings and will thereby improve the understanding of the causal role of danger signals and factor VIII dose on inhibitor risk.
Nonsevere hemophilia A

For patients with nonsevere hemophilia A it is essential to recognize that exposure to factor VIII concentrates occurs far more infrequently than in severe hemophilia A and always takes place in the context of tissue damage (e.g. minor/major trauma or surgery). Therefore, it is extremely challenging to design a study comparing intensive treatment episodes to other treatment episodes that are comparable for all clinical characteristics except for the intensity of treatment (high factor VIII load in short time span) or the presence of danger signals.

Several studies assessed the association between intensive treatment and inhibitor development in nonsevere hemophilia A patients. In all these studies periods of intensive factor VIII treatment were compared to periods when little or no factor VIII had been administered. Factor VIII is a necessary cause of the development of inhibitors against factor VIII. In the absence of factor VIII it is impossible to develop inhibitors. Thus, a comparison of intensive factor VIII exposure to periods without factor VIII exposure obviously leads to a biased estimate. This is illustrated in one of our previous studies in which we reported a hazard ratio of 18.6 (CI, 25-1403) for inhibitor development following surgery. This extremely high hazard ratio is simply the result of the statistical model in which periods with factor VIII were compared with periods without factor VIII exposure. When we reanalyse the same dataset comparing the inhibitor risk of patients that receive intensive factor VIII treatment for surgery with patients that receive factor VIII for a bleeding episode, the results are far less pronounced. Of the 138 patients, 63 underwent intensive treatment, 41 for surgery and 22 for bleeding. Eight patients developed an inhibitor, seven after surgery (absolute risk, 7/41 = 17%) and one after bleeding (absolute risk, 1/21 = 5%). In comparison to bleeding, the risk of inhibitor development for surgery was not statistically significant (RR, 3.7; CI, 0.5-28.6). To note, an important confounder to address in this infrequently treated group is the number of previous exposure days to factor VIII. We acknowledge that this univariate analysis was not adjusted for prior cumulative factor VIII exposures and other potential confounders. In order to study the separate effects of factor VIII dose and the presence of immunological danger signals on inhibitor risk in nonsevere patients a robust well-designed study is necessary in which very detailed treatment data for each exposure day is collected (see section about severe hemophilia above) to enable appropriate adjustment for potential confounders.

CONCLUSION AND FUTURE PERSPECTIVES

This article considers the methodological aspects that affect the design and interpretation of studies that assess the risk of intensive treatment for inhibitor development in hemophilia A. Future studies addressing the effect of intensive treatment on inhibitor development should take both factor VIII load and the strength of danger signals into account. In order to assess
a causal claim for an independent determinant it is essential to compare two groups of patients that differ only for the determinant under investigation and are similar with respect to all other clinical features that may contribute to the risk. This will ultimately help us to understand the pathophysiology of intensive treatment causing inhibitor development and may enable further research on preventive strategies.
REFERENCES


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