Low-Dose Immune Tolerance Induction in Hemophilia A Patients With Inhibitors

By Eveline P. Mauser-Bunschoten, H. Karel Nieuwenhuis, Goris Roosendaal, and H. Marijke van den Berg

In patients with hemophilia A and inhibitory alloantibodies against factor VIII, various dosage schedules are used to obtain immune tolerance. In this study, we have evaluated the results of 13 years of low-dose immune tolerance induction and factors that are predictive of a positive result. The effect of immune tolerance induction in relation to age at inhibitor development, number of exposure days, age at start of therapy, maximum inhibitor titer, factor VIII products involved, and virologic status were determined. We evaluated 24 patients with severe hemophilia A and inhibitors who were treated with regular infusions with low-dose (25 U/kg every other day) factor VIII to obtain immune tolerance. In 21 of 24 patients (87%), immune tolerance induction was successful. The response time was determined by two factors: the highest inhibitor level and the age at inhibitor development. In patients with maximum inhibitor levels of less than 40 Bethesda units (BU)/mL, immune tolerance was obtained sooner than in patients with inhibitor levels exceeding 40 BU/mL (P = .005). Patients in whom an inhibitor developed before the age of 2.5 years also tended to have a quick immune response (P = .014). Immune tolerance with low-dose factor VIII is often successful in hemophilia A patients with inhibitors. Young children and patients with maximum inhibitors of less than 40 BU/mL show a relatively rapid response.

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an inhibitor, antibody tests are performed as well. When an antibody
titer of 1 BU/mL or more is measured, blood samples for repeated
antibody testing are taken, and a factor VIII recovery study is per-
formed. Patients are considered to have a type A inhibitor when a
recovery of 50% or less is measured, with or without clinical evi-
dence of an inhibitor. Patients are considered to have transient (type
B) inhibitors when the antibody titer of the second sample is less
than 1 BU/mL and a normal recovery is found.18 Patients with type
B inhibitors were excluded from the study. In the period from 1966
to 1980, persistent inhibitors (type A) were detected in 25 patients,
whereas in the period from 1981 to 1993, in eight patients a persistent
inhibitor was found. These 33 patients with persistent antibodies
were eligible for the study. They form the majority (estimated at
90%) of inhibitor patients in The Netherlands. Nine patients were
excluded from the study because they refused immune tolerance
induction. Reasons for refusal were low bleeding frequency (less
than two bleeding episodes per year), fear of viral transmission,
problems with venous access, or psychological instability. Twenty-
four patients were treated with immune tolerance induction. In-
formed consent was obtained from all patients. In eight patients,
factor VIII administration was never stopped once an inhibitor was
detected, whereas in 16 patients, factor VIII was discontinued. In
15 patients the inhibitor was detected before 1981 (during the time
when the policy was to stop factor VIII); in one patient, in whom
an inhibitor was detected in 1988, factor VIII prophylaxis was tem-
porarily stopped because of technical problems in obtaining venous
access.

**Dosage Regimen**

When factor VIII treatment was started because of an operation
or a life-threatening bleeding and the inhibitor level was less than
10 BU/mL, an initial high dose of factor VIII was given to neutralize
the antibodies. The neutralizing dosage was calculated as follows:

\[
2 \times \frac{\text{BW} \times (100 - \text{Ht})}{100} \times 1,
\]

where BW is body weight in kilograms; Ht, hematocrit; and I, inhibi-
tor in BU/mL.

In this group of patients, the initial high dose was followed by
infusion of 25 U factor VIII per kilogram body weight (U FVIII/
kg) twice daily for 1 to 2 weeks, depending on the clinical status
of the patient and the anamnestic response to factor VIII. In patients
in whom factor VIII treatment was started or continued with the
sole aim of obtaining immune tolerance, the factor VIII dosage was
25 U/kg every other day or three times per week, independent of
the inhibitor titer. In very young children in whom venous access
was difficult, factor VIII was injected twice weekly.

When the antibody level decreased and factor VIII recovery im-
proved, or when an anamnestic response was lacking, factor VIII
was gradually tapered down each time the absolute factor VIII recov-
er was higher than 30%, until a standard prophylactic dosage of
10 to 15 U FVIII/kg was obtained.

**Definition of Success**

Immune tolerance induction was considered to be clinically suc-
cessful when the inhibitor decreased to less than 2 BU/mL, with a
factor VIII recovery of at least 50% of normal, a factor VIII half-
life time of 6 hours or more,19 and the absence of an anamnestic
response after infusion with factor VIII.

**Choice of Factor VIII Product**

The choice of factor VIII therapy varied with time. Different
factor VIII products were used: cryoprecipitate and intermediate
purified factor VIII, both virus-inactivated and nonvirus-inactivated,
as well as monoclonal purified factor VIII.

**Statistical Analysis**

Probabilities of disappearance of the inhibitor over time were
estimated with the product limit method of Kaplan and Meier and
were compared using the log rank statistic. The time lapse until
disappearance of the inhibitor was also examined by univariate and
stepwise Cox regression analyses. All variables found to have a P
value of less than .10 in univariate analysis were considered can-
didate variables for multivariate analysis.

**RESULTS**

The study group consisted of 24 patients with severe he-
mophilia A. The patient data are summarized in Table 1. The median age at inhibitor development was 5 years (range,
1 to 23 years). The median age at the onset of immune
tolerance induction was 12 years (range, 1 to 43 years).
Until 1980, cryoprecipitate was used in The Netherlands for
replacement therapy. Inhibitors in the older patients devel-
oped after infusion of this product. Since then, inhibitors
were seen after infusion of all kinds of factor VIII products.
Inhibitors developed after a median of less than 40 transfu-
sions (range, 8 to 53). During immune tolerance induction,
patients were seen at least every month. We checked the
diaries kept by the patients against the amount of factor VIII
supplied to them. Based on these data, the compliance was
almost 100%. None of the patients was lost for follow up
during immune tolerance induction.

Immune tolerance induction was successful in 21 of 24
patients (87%). The success was obtained within 0.5 to 28
months (median, 1 year). Figure 1 shows a Kaplan-Meier
plot of the presence of inhibitor for the whole group. The
plot is almost linear in the first 2 years, indicating a constant
chance of disappearance of inhibitor. Even after 2 years of
therapy, there is a chance that the inhibitor will disappear.
So far, immune tolerance induction has failed in three pa-
tients after a follow up of 36, 48, and 50 months, respec-
tively. In two patients (patients 23 and 24), therapy failed
even when therapy with high-dose intravenous gamaglobu-
lin and cyclophosphamide as described by Nilsson et al20 was
added. Patient 23 received three courses of this regimen
without success, and factor VIII therapy was discontinued
after 3 years; patient 24 received one course without success.
In one patient (patient 8), immune tolerance induction has
not yet been completed.

Table 2 shows the bleeding frequencies before and after
immune tolerance induction, as well as factor VIII recoveries
and half-life times. In 18 of 21 treated patients, the bleeding
frequency has decreased. In three patients, a rather high
bleeding tendency occurred, probably as result of traumata.
In all patients, bleedings were less severe and disabling than
before, and the bleeding tendency was quite comparable with
hemophilia A patients without inhibitors. Nine patients
underwent 15 surgical interventions without bleeding compli-
cations, and the amounts of factor VIII given were similar
to those given to patients who never had an inhibitor.

Univariate Cox regression analysis (Table 3) and stepwise
Cox regression analysis revealed two factors that were inde-
Table 1. Course of Inhibitor Before, During, and After Immune Tolerance Induction

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>ITI*</th>
<th>1st Inhibitor Titer (BU/mL)</th>
<th>Highest Inhibitor Titer Before ITI (BU/mL)</th>
<th>Inhibitor at Onset ITI (BU/mL)</th>
<th>Highest Anamnestic Response at ITI (BU/mL)</th>
<th>Time to Success (mo)</th>
<th>Last Inhibitor (BU/mL)</th>
<th>Follow-up Since Start of ITI (mo)</th>
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<tr>
<td>1</td>
<td>2</td>
<td>1.3</td>
<td>8.7</td>
<td>8.7</td>
<td>None</td>
<td>6</td>
<td>0.6</td>
<td>168</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>1.1</td>
<td>1.1</td>
<td>1.1</td>
<td>None</td>
<td>2</td>
<td>0.5</td>
<td>132</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>8.1</td>
<td>8.1</td>
<td>4.0</td>
<td>11</td>
<td>6</td>
<td>0.5</td>
<td>108</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>2.5</td>
<td>2.5</td>
<td>2.2</td>
<td>68</td>
<td>24</td>
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<td>120</td>
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<tr>
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<td>2</td>
<td>3.9</td>
<td>3.9</td>
<td>3.9</td>
<td>None</td>
<td>3</td>
<td>0.8</td>
<td>144</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>4.2</td>
<td>4.5</td>
<td>4.5</td>
<td>4.6</td>
<td>8</td>
<td>0.4</td>
<td>96</td>
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<td>7</td>
<td>3</td>
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<td>24</td>
<td>6</td>
<td>0.4</td>
<td>36</td>
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<td>8.0</td>
<td>8.0</td>
<td>220</td>
<td>Ongoing</td>
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<td>58</td>
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<tr>
<td>9</td>
<td>2</td>
<td>Pos</td>
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<td>2.3</td>
<td>0.5</td>
<td>0.4</td>
<td>48</td>
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<tr>
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<tr>
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<td>Pos</td>
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<td>0.6</td>
<td>72</td>
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<tr>
<td>14</td>
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<td>Pos</td>
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<td>2.5</td>
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<td>156</td>
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<td>15</td>
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<td>156</td>
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<td>16</td>
<td>1</td>
<td>Pos</td>
<td>164</td>
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<td>108</td>
</tr>
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<td>52</td>
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<td>132</td>
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<td>Pos</td>
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<td>5.7</td>
<td>73</td>
<td>18</td>
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<td>96</td>
</tr>
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<td>19</td>
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<td>0.3</td>
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<td>144</td>
</tr>
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<td>2</td>
<td>5.0</td>
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<td>7.3</td>
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<tr>
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<td>1.1</td>
<td>None</td>
<td>2</td>
<td>0.8</td>
<td>48</td>
</tr>
<tr>
<td>22</td>
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<td>4.3</td>
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<td>12</td>
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<tr>
<td>23</td>
<td>1</td>
<td>Pos</td>
<td>33</td>
<td>2.8</td>
<td>450</td>
<td>FVIII stopped</td>
<td>20</td>
<td>74</td>
</tr>
<tr>
<td>24</td>
<td>2</td>
<td>Pos</td>
<td>25</td>
<td>0.6</td>
<td>44</td>
<td>FVIII stopped</td>
<td>11</td>
<td>52</td>
</tr>
</tbody>
</table>

Abbreviations: ITI, immune tolerance induction; Pos, positive; FVIII, factor VIII.

*1, initial high doses of FVIII to neutralize the antibodies, followed by twice daily 25 U/kg FVIII for 1 to 2 weeks; 2, FVIII 25 U/kg, three times per week or every other day; 3, FVIII 25 U/kg, two times per week.

Independent predictive of the response to therapy: the highest inhibitor level [stepwise analysis, \( P = .005 \); risk ratio, 0.977; 95% confidence interval (CI), 0.962 to 0.992] and the age (years) at inhibitor development (\( P = .014 \); risk ratio, 0.797; 95% CI, 0.668 to 0.950). Patients with low maximum levels responded sooner. Figure 2 shows a Kaplan-Meier analysis of the response time for patients with low (\( n = 12 \)) versus high (\( n = 12 \)) inhibitor levels. The median response time was 6 months for patients with a maximum inhibitor level less than 40 BU/mL, as compared with 19 months for patients with maximum inhibitor levels \( \geq 40 \) BU/mL (log rank \( P < .0001 \)). Patients who developed an inhibitor at an early age tended to respond more quickly (Fig 3). Whether therapy was started directly or many years after inhibitor development did not seem to make much difference. Treatment with a neutralizing dose at the start of immune tolerance induction did not improve the results. Furthermore, no difference in success was seen between HIV-positive (\( n = 4 \)) and HIV-negative (\( n = 19 \)) patients, and no influence of hepatitis B or C infection could be observed. Moreover, the type of factor VIII used to obtain immune tolerance did not affect the results.

Follow-up After Successful Immune Tolerance Induction

In 20 patients, factor VIII was continued on a prophylactic basis once tolerance was obtained, and one patient was treated on a demand basis. In this patient, no anamnestic response of the inhibitory activity was seen, suggesting that immune tolerance was really obtained.

Figure 4 shows the probability of recurrence of the inhibitor after successful immune tolerance induction. The median time of follow-up after successful therapy is 99 months (range, 4 to 162 months). In only one patient, recurrence of
Table 2. Bleeding Frequency, Recovery, and Factor VIII Half-Life Time in Immune-Tolerized Patients

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Before ITI</th>
<th>After ITI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Bleedings/yr</td>
<td>No. of Surgical Interventions</td>
</tr>
<tr>
<td>1</td>
<td>25-50</td>
<td>0-5</td>
</tr>
<tr>
<td>2</td>
<td>5-25</td>
<td>0-5</td>
</tr>
<tr>
<td>3</td>
<td>5-25</td>
<td>0-5</td>
</tr>
<tr>
<td>4</td>
<td>5-25</td>
<td>0-5</td>
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<tr>
<td>5</td>
<td>0-5</td>
<td>0-5</td>
</tr>
<tr>
<td>6</td>
<td>0-5</td>
<td>10-25</td>
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<td>5-25</td>
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<td>8</td>
<td>5-25</td>
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<td>0-5</td>
</tr>
<tr>
<td>14</td>
<td>5-25</td>
<td>0-5</td>
</tr>
<tr>
<td>15*</td>
<td>5-25</td>
<td>5-25</td>
</tr>
<tr>
<td>16</td>
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<tr>
<td>22</td>
<td>25-50</td>
<td>0-5</td>
</tr>
</tbody>
</table>

* Patient treated on-demand.

inhibitory activity was seen after 48 months, and this was easily managed by giving a second course of immune tolerance induction.

DISCUSSION

This study shows that immune tolerance induction in inhibitor patients (1) has a high success rate and (2) can be successfully achieved with a low-dose regimen, and (3) that the duration of treatment is determined by the maximum inhibitor level and the age at inhibitor development.

To gain insight into the nature of treatment effect, the criteria of response consisted of inhibitor titer, factor VIII recovery and half-life times, and clinical data of response, such as bleeding frequency and the outcome of surgical interventions. A complete response was obtained in the majority of patients. In some patients, a suboptimal response was obtained, as evident from the factor VIII recoveries and half-lives. The treatment in these patients was considered clinically effective as they had a factor VIII recovery of more than 60%, had no anamnestic response of the inhibitor titer, and could be adequately treated for bleeding episodes and surgical interventions. They were included in the group of successfully treated patients.

Since the introduction of immune tolerance induction in the beginning of the 1980s, success has been achieved with different dosage regimens. Mostly high-dosage regimens are recommended, with daily dosages of 100 U FVIII/kg or more, with or without the addition of corticosteroids, immunosuppressive drugs, or inhibitor adsorption by plasmapheresis. The success rate of these high-dosage regimens is around 60-80%, with a maximum inhibitor level of <40 BU/mL as compared with the group (n = 12) with a high level (>40 BU/mL). Tick marks represent censored data: two patients who stopped therapy and one patient who still is on therapy.
regimens varied from 76% to 89%, and success was obtained after 1 to 18 months. The success rate in our group of patients is 87%. In comparison with the high-dose regimens, similar results are obtained at considerably lower costs. The estimated costs for 12 months of immune tolerance induction with the low-dose regimen are about $5,000.00 per kilogram of body weight.

Our low-dose schedule is also successful in patients with anamnestic responses over 40 BU/mL. Of 9 of 12 patients, 75%; however, the time needed before success is obtained is substantially longer (12 to 28 months) compared with the group with low-titer inhibitors (3 to 9 months). At 3 years after start of therapy, no success was obtained in three patients. In two patients, factor VIII was stopped. The Kaplan-Meier analysis suggests that continuation of factor VIII may eventually lead to success, although a long period of time may be needed before response is obtained.

Definitive conclusions about the schedule of preference can only be drawn after prospective, randomized, controlled studies. A low-dose schedule could be part of such studies. Until such studies are performed, low-dose schedules may be indicated in individual patients, especially in those patients in whom immune tolerance induction has not been attempted because of the high costs of the other schedules.

Inhibitors develop mostly in young patients, and in some of them, regular venous access may cause such technical problems that regular factor VIII therapy must be temporarily interrupted. These patients can be treated later because no differences in success rate were found in this study between patients in whom factor VIII was continued and patients in whom factor VIII administration was interrupted.

Because in some patients, especially those with inhibitors of less than 5 BU/mL, the inhibitor would have disappeared spontaneously or with on-demand therapy, immune tolerance induction may not be required for all newly detected inhibitors. This could be a reason to postpone the start of regular factor VIII therapy. However, the period of time before success is obtained will be uncertain and, in the case of on-demand therapy, will depend on bleeding episodes. This will introduce a greater risk of severe and disabling bleedings in the majority of patients. Therefore, the immediate start of immune tolerance induction in all patients with newly detected, persistent inhibitors should be considered.

Low-dose immune tolerance induction is often successful in hemophilia A patients with inhibitors. Time needed before success is obtained is related to the maximum inhibitor level and to the age at inhibitor development. Therefore, we postulate that all patients with inhibitors should be treated with low-dose factor VIII to obtain immune tolerance. In patients in whom no response is seen during the first year, this regimen should be continued for a longer period of time.

**Fig 3. Probability of the presence of a clinically relevant inhibitor according to age at inhibitor development. Patients in whom an inhibitor developed at an older age tended to respond slower to immune tolerance induction. Age less than 2.5 years, n = 7; age from 2.5 to 5 years, n = 10; age greater than 5 years, n = 7.**

**Fig 4. Probability of recurrence of inhibitor in patients in whom immune tolerance induction was successful (n = 21).** Tick marks represent the follow-up period (median, 99 months; range, 4 to 162 months) after successful therapy in each individual patient.

**Figures 3 and 4.**

**REFERENCES**


Low-dose immune tolerance induction in hemophilia A patients with inhibitors

EP Mauser-Bunschoten, HK Nieuwenhuis, G Roosendaal and HM van den Berg