INHIBITORS IN HEMOPHILIA A AND B

An Introductory Discussion

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**What are inhibitors?**

Inhibitors are antibodies that neutralize the activity of a clotting factor. Inhibitors to factor VIII or IX may arise as allo-antibodies in patients with hemophilia A or B, respectively, who have been treated with exogenous factor VIII or IX. Inhibitor antibodies to factor VIII may arise as auto-antibodies in non-hemophilic persons. Non-neutralizing antibodies also may arise and may be detected with suitable tests.

**Incidence and Prevalence**

The number of new inhibitors in a population in a given period of time (incidence) reflects transient inhibitors as well as the more common enduring ones. If inhibitor testing is frequent, a few more transient inhibitors are detected. The number of inhibitors present in a population at any given time (prevalence) primarily reflects long-standing inhibitors. The incidence of inhibitors to factor VIII (FVIII) in patients with severe hemophilia A within the first few years of life is reported as 20-30%. The prevalence is lower, and nowadays it may reflect the success of specific treatment to eradicate inhibitors. The incidence of inhibitors in severe hemophilia B is about 4%. Clinically-apparent FVIII auto-antibodies are uncommon; the incidence is difficult to assess because patients are not centralized. Some observers estimate one clinically-apparent case per million population per year. Factor IX auto-antibodies are rare.

**Predisposing influences**

The level of risk for inhibitor formation is influenced by the following: (1) the patient’s hemophilia mutation, (2) his race, (3) his immune system and (4) the nature of his exogenous factor replacement product. Patterns of factor replacement (i.e. intensity of treatment, age of prophylaxis onset) may also have some influence.

The first three issues are genetic, so it should not be surprising that strong inhibitors often arise after only a few exposures to exogenous clotting factor. In prospective studies of previously-untreated patients (“PUPs”, mostly babies), those developing inhibitors did so after a median of 8-9 exposure-days. In one registry, half of patients with severe hemophilia who developed inhibitors did so under age 5 years.

The mutation causing hemophilia determines whether some of the clotting factor is produced, and thus is accessible to the thymus during fetal life, to be recognized as “self”. Large deletions and nonsense mutations causing premature stop codons are associated with the highest incidence of inhibitors. In the common intron-22 inversion mutation, most of the protein is produced intracellularly but does not reach the plasma; presence in cells suffices for thymic recognition. Missense mutations allow a full-length protein to be made and circulated although it has a mistake in it. The incidence of inhibitors in patients with missense mutations is low, but a few specific missense mutations are associated with a high frequency of inhibitors. (One such mutation prevents binding of T cells to FVIII, thus interfering with tolerance development in fetal life.)

The patient’s race/ethnicity also influences the propensity for inhibitor development, but the reasons are not yet known. Patients who are of black African descent, or are Latin-American, or native-American have a higher incidence of inhibitors than do Caucasians. As yet, no study has compared persons of Asian descent to Caucasians.

The immune system of the patient is doubtless involved in his propensity to inhibitor formation. Some variants of immune competent genes and HLA phenotypes are more common than others in patients with inhibitors. The tendency for inhibitors to run in families may be related to inheritance of immune system characteristics.

The role of the specific concentrate
has been questioned. Inhibitors are somewhat less likely to develop with the use of plasma-derived concentrate, compared to recombinant products. (Recombinant clotting factors are not identical to naturally-occurring ones.)

Two plasma-derived FVIII concentrates made in Europe in the early 1990’s, however, did provoke inhibitors in some previously-heavily-treated patients (PTPs) thought to be at low-risk. One such product, made in by the controlled-pore-silica fractionation method had not elicited new inhibitors in PTPs when viral-inactivated with dry heat but did so when pasteurized. The other product became more antigenic when pasteurization was added to a solvent-detergent viral inactivation process. The FVIII in these products was denatured slightly in production.

**Non-hemophilic persons**

Inhibitors occasionally arise as auto-antibodies in post-partum women, in patients with auto-immune disorders or other illnesses, or in older adults without obvious underlying disorders.

**Immune response to FVIII**

The initiation of an immune response and formation of antibodies requires endocytosis of the “foreign” FVIII molecule by antigen-presenting cells and presentation of antigen-derived peptides via the HLA class II molecules on the cell surface to the CD4 T cells. In addition, for the CD4 T cells to become activated, and acquire the ability to stimulate antigen-specific B cells, additional triggers, “danger signals”, are required, such as are released by inflammation, stress or tissue damage. T-regulatory cells have a modulating effect.

Inhibitors are mainly IgG antibodies of the IgG1 and IgG4 subtypes. Anti-FVIII allo-antibodies bind primarily with epitopes in the FVIII A2 or C2 domains and auto-antibodies bind with the C2 domain.

The union of FVIII with its inhibitor is not associated with allergic reactions. The union of factor IX with its inhibitor, however, may cause severe allergic reactions including anaphylaxis. Such reactions can occur with the first infusion of exogenous factor IX given after the inhibitor develops, that is, before it has been diagnosed. Factor IX-inhibitor complexes can cause nephrotic syndrome. Children with hemophilia B are treated cautiously at a clinic, not at home, during the first 50 or so exposure-days.

The reaction of FVIII with its inhibitor is time-dependent both *in vitro* and *in vivo*, a fact relevant to measurement and to clinical treatment. The higher the level of the inhibitor, the more rapidly it inactivates FVIII. If a patient’s inhibitor level is low to moderate, a high therapeutic dose of FVIII may have a chance to take part in coagulation before being neutralized.

Two patterns of reaction kinetics are seen (Figure 1). Inhibitors with “Type 1” or “simple” kinetics completely neutralize FVIII and are neutralized themselves in the reaction. Most inhibitors in hemophilia patients are Type 1. Inhibitors with “Type 2” or “complex” kinetics do not completely neutralize FVIII and, after reaction, retain some ability to neutralize additional FVIII. Although some FVIII may remain measurable in the plasma in the presence of type 2 inhibitors, the patient may bleed as profusely as if he had no FVIII at all. Type 2 reaction kinetics are more common in auto- than in allo-antibodies.

**Clinical Presentation**

In hemophilia, an inhibitor is suspected when a patient’s hemorrhage is not controlled with his usual dose of clotting factor. The presence of an inhibitor does not change the typical site, frequency or severity of bleeding. The inhibitor makes control of hemorrhages more difficult.

Patients with auto-antibodies to FVIII
Figure 1. Simple and complex reaction kinetics.

*Simple reaction kinetics:*

\[
FVIII \text{ Ag} + \text{inhibitor Ab} = \text{AgAb}; \quad (\text{no factor VIII activity, no inhibitor activity})
\]

*Complex reaction kinetics:*

\[
FVIII \text{ Ag} + \text{inhibitor Ab} = \text{Ag (low factor VIII activity)} + \text{Ab (diminished inhibitor activity)}
\]

Figure 2. “Low-responders” and “high-responders”. When repeated doses of FVIII are given to a low responder, as depicted by the solid line, anamnesis is not seen. The first dose of FVIII, given to a high responder (dashed line) when his inhibitor level is low, does provoke anamnesis but subsequent doses, given while the inhibitor level is still high, at the patient’s current maximum production, may not provoke further rises in the inhibitor level.
present much differently. They may have severe bleeding in multiple sites, especially in muscles and soft-tissue, with extensive bruising. “Elder abuse” may be suspected. The startling pattern of bleeding in these patients is unexplained.

**Course in hemophilia**

The immune response to exogenous FVIII (or IX) may be weak or robust. Most inhibitors are “high responders”, that is, within a few days of repeated exposure to the exogenous clotting factor, the inhibitor level rises briskly (“anamnesis”), peaking within a month. If there is no further exposure to the exogenous factor, the inhibitor level gradually falls (in most patients) and, after years, may be undetectable.

A few low-level inhibitors in hemophilia patients are “low responders”, that is, their levels do not rise notably after exposure to the exogenous factor. Such patients can continue treatment with the factor, in a higher-than-usual dose (Figure 2.)

Auto-antibodies usually do not rise after exposure to exogenous clotting factor.

**Laboratory Diagnosis**

A common laboratory screening test for inhibitors is an activated partial thromboplasnin time (APTT) on an equal mixture of patient and normal plasma incubated together for two hours at 37°C. After incubation, in the presence of an inhibitor, the APTT is prolonged compared to controls without inhibitor, as in the actual examples in Figure 3.

<table>
<thead>
<tr>
<th>INCUBATION MIXTURE:</th>
<th>APTT at outset of incubation. seconds:</th>
<th>APTT after two hours, seconds:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal plasma alone</td>
<td>32</td>
<td>40</td>
</tr>
<tr>
<td>Factor VIII deficient plasma alone</td>
<td>90</td>
<td>95</td>
</tr>
<tr>
<td>Normal plasma plus factor VIII deficient plasma with No inhibitor</td>
<td>37</td>
<td>45</td>
</tr>
<tr>
<td>1 Bethesda unit</td>
<td>37</td>
<td>53</td>
</tr>
<tr>
<td>5 Bethesda units</td>
<td>43</td>
<td>64</td>
</tr>
<tr>
<td>20 Bethesda units</td>
<td>54</td>
<td>92</td>
</tr>
</tbody>
</table>

**Figure 3 : APTTs with plasmas containing various FVIII inhibitor levels.**
In a person with hemophilia, a prolonged APTT of a mixture of patient and normal plasma strongly suggests the presence of an inhibitor to the deficient clotting factor. In a non-hemophilic person, however, such a result suggests the presence of some inhibitor, but not the identity of the antigen. Similar results might be seen with an anti-phospholipid ("lupus") inhibitor. Specific tests for such inhibitors can be performed.

Assays of the antigenic clotting factor are low whether the patient’s plasma is tested at a small or a great dilution. However, assay systems for other clotting factors may be affected by the inhibitor.

If a patient plasma is low in FVIII and also contains antibody to FVIII, the effects on the FVIII and other clotting factor assays might be as shown in Figure 4. These results show the apparent levels of four clotting factors assayed in an actual plasma sample containing an inhibitor to FVIII. Each clotting factor was measured in a one-stage APTT-based assay. The apparent level of FVIII is similarly low whether the patient plasma is slightly or greatly diluted in buffer. The apparent levels of factors IX, XI and XII increase as patient plasma is diluted. As the inhibitor in the patient plasma is "diluted out", it has a decreasing detrimental effect on the FVIII present in the reagent plasmas deficient in factor IX, in factor XI or factor XII. Each reagent plasma is deficient in one factor and has a normal amount of all other clotting factors, including FVIII.

If plasma from the patient in Figure 4 is assayed at only two dilutions, for example, 1:5 and 1:10, the laboratory might report that the patient has a severe deficiency of FVIII and a mild deficiency of other clotting factors.

Inhibitors are quantified by the "Bethesda" test, introduced in 1975. Normal pooled plasma (as a source of FVIII) is incubated with an equal amount of undiluted patient plasma for two hours at 37 degrees C and then assayed for residual FVIII. A control consists of normal plasma incubated with buffer. One inhibitor unit (Bethesda Unit, BU) is defined as the amount that destroys half the FVIII in the incubation mixture, corrected for the deterioration of FVIII in the control mixture. The assay can be modified to measure inhibitors to factor IX. Inhibitors with simple reaction kinetics are quantified easily by the Bethesda test but those with complex kinetics can be quantified only roughly.

In the "Nijmegen" modification of the Bethesda test, introduced in 1995 and now widely used, the control consists of normal plasma incubated with immune-depleted FVIII deficient plasma instead of with buffer, and the normal plasma is buffered with imidazole to pH 7.4.

<table>
<thead>
<tr>
<th>Patient plasma, diluted:</th>
<th>Factor VIII</th>
<th>Factor IX</th>
<th>Factor XI</th>
<th>Factor XII</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:5</td>
<td>&lt;1</td>
<td>28</td>
<td>20</td>
<td>38</td>
</tr>
<tr>
<td>1:10</td>
<td>&lt;1</td>
<td>36</td>
<td>29</td>
<td>47</td>
</tr>
<tr>
<td>1:20</td>
<td>&lt;1</td>
<td>42</td>
<td>38</td>
<td>54</td>
</tr>
<tr>
<td>1:50</td>
<td>&lt;1</td>
<td>60</td>
<td>51</td>
<td>72</td>
</tr>
<tr>
<td>1:100</td>
<td>&lt;1</td>
<td>74</td>
<td>63</td>
<td>84</td>
</tr>
</tbody>
</table>

Figure 4. Apparent clotting factor concentration in four clotting factor assays at various dilutions of a plasma sample containing an inhibitor to FVIII.
Figure 5. The reference graph for the Bethesda test.

If the residual FVIII, after incubation, is 100% of the level in the control incubation mixture, then the inhibitor level is zero. If the residual FVIII is 50% of that of the control, then the inhibitor level is one Bethesda unit. The graph is used between 25 and 75% (solid line). Results of more than 75% are within the error of the assay.

If the result is less than 25%, then the patient plasma is tested at various dilutions until the result can be read off the graph. The result then is multiplied by the dilution to assign Bethesda units. For example, if a plasma sample is diluted 1:5 before incubation and the residual FVIII then is 50%, or one unit, $1 \times 5 = 5$ Bethesda units.
MANAGEMENT OF HEMORRHAGES

Hemorrhages in the presence of inhibitors are managed by lowering inhibitor levels (available only in a few countries), by raising plasma levels of the deficient factor or by “bypassing” the need for the deficient factor.

Lowering inhibitor levels

It is possible to lower inhibitor levels, but not practical in the USA due to the complexities of health insurance.

If equipment is available and the situation is not urgent, the level of an inhibitor may be lowered in order to make it feasible to neutralize the remaining circulating inhibitor and raise the plasma clotting factor level. Exchange plasmapheresis can be performed with continuous-flow cell-separation centrifuges which can replace a liter of plasma in 1/2 to one hour in a typical adult, if venous access is good. Replacement of 3 to 4 liters of plasma in typical adults temporarily reduces plasma inhibitor levels by 40% or more. If time permits, e.g. before an urgent but not emergency surgical operation, exchanges may be performed on 2 or 3 consecutive days, if needed. Anamnesis is likely to be provoked within a few days of exposure to normal plasma, thus, FVIII (or IX) concentrate should be given promptly after the final plasmapheresis to achieve a maximal peak plasma level. Any surgery should be timed to co-incide with that peak.

In well-equipped centers, the efficacy of plasmapheresis can be improved by pouring the plasma, while outside the body, through columns containing protein A sepharose, which binds most IgG. Over a period of 6 or more hours, some 5 to 9 liters of patient plasma can be processed with reduction of inhibitor levels by two-thirds or more. This technology, pioneered in Sweden, is primarily available in northern Europe.

Intravenous infusion of normal human gamma globulin in high doses, by itself or after plasmapheresis, has been reported to lower inhibitor levels immediately, to some extent perhaps by the action of anti-idiotypic antibodies. Some degree of long-term inhibitor suppression also has been reported.
Figure 6. Avoiding inhibitor stimulation for all but critical hemorrhages.

The hemophilia A patient with a high-responding inhibitor whose course is illustrated above received bypassing agents in the first few years after inhibitor diagnosis for “routine” hemorrhages to help control bleeding and to avoid stimulating further production of inhibitor antibodies. On two occasions, years apart, when his inhibitor level was low, life-threatening hemorrhages were controlled with infusions of FVIII concentrate that raised his plasma FVIII level to 30%. He gained many years of life, but the third life-threatening hemorrhage occurred not long after the second, while his inhibitor level still was high. A bypassing agent was ineffective and he bled to death.
**Raising plasma factor levels**

**DDAVP (desmopressin)**

DDAVP mediates the release of FVIII and von Willebrand factor from cellular storage sites into the plasma. Its use may elevate plasma FVIII levels temporarily in those inhibitor patients who have the fundamental capacity to make FVIII, that is, non-hemophilic patients or those with mild hemophilia A, in the presence of a low-level inhibitor (less than five Bethesda units). In these special and unusual circumstances, DDAVP may release enough FVIII to neutralize the circulating inhibitor and raise the plasma level of FVIII slightly, sufficient to stop minor bleeding or allow minor surgical procedures.

**Exogenous human factors**

A large bolus of human FVIII (for hemophilia A) or human factor IX (for hemophilia B) may be infused directly, if the inhibitor level is not too high (under 5 BU, sometimes higher) to try to achieve a plasma level of 30 U/dl or more. (Factor IX concentrate should not be used in those patients whose factor IX inhibitors provoke allergic reactions.) The amount of concentrate needed is only roughly correlated with the inhibitor level. A suggested initial FVIII dose is 20-40 U/kg plus 20 U/kg per BU. The factor level should be measured immediately after infusion so that further concentrate can be given if needed. Once an inhibitor is neutralized with boluses of concentrate, the dose needed for subsequent infusions the same day and over the next few days will be lower, until anamnesis supervenes.

Some clinicians infuse FVIII in the presence of an inhibitor without attempting to attain measurable plasma FVIII levels, with some success. If the inhibitor level is not very high, FVIII may have time to act in coagulation before it is neutralized. In one such protocol, patients with FVIII inhibitors of less than 30 BU receive a bolus of 70-140 FVIII U/kg followed by a continuous infusion of 4-14 U/kg/hr. Hemostasis often is achieved although measurable FVIII levels are not always reached.

**Porcine factor VIII**

Porcine FVIII interacts well in the human coagulation sequence but attaches poorly to anti-human-FVIII antibodies. Inhibitors arising in persons with hemophilia A neutralize, on average, only 20-25% as much porcine FVIII as human FVIII; the range is wide (see Figure 7.). Cross-reactivity of auto-antibody inhibitors tends to be even lower that that of hemophilic allo-antibodies. For many years, porcine FVIII made from the plasma of pigs was available as “Hyate-C®”. It was discontinued in 2004. Recently, a recombinant porcine B-domain-deleted FVIII, “Obizur®”, was licensed for use in patients with auto-antibodies to FVIII.

Porcine FVIII is given to patients with low anti-porcine-FVIII inhibitor levels, if such assays are available, in doses sufficient to neutralize that inhibitor activity and achieve a therapeutic circulating FVIII level. If such inhibitor levels are not available, an initial dose of 200 U/kg is recommended, with further dosing depending on plasma FVIII levels or clinical response.

Some patients develop antibodies to porcine FVIII after several days of treatment or after several episodes of treatment, and are no longer responsive (see Figure 8).

**Bypassing agents**

Activated clotting factors such as Xa or VIIa may trigger some degree of coagulation in the absence of factor VIII or IX or in the presence of an inhibitor to one of them. Thus, such activated factors may “bypass” the need for factor VIII or IX (see Figure 9.)

Prothrombin complex concentrate (PCC) contains factors II (prothrombin), VII, IX and X, which travel together in plasma fractionation. PCC was developed to treat
Figure 7. Comparison of anti-human and anti-porcine inhibitor levels in 29 patients.

The above graph depicts anti-human-FVIII-inhibition (solid bars) and anti-porcine-FVIII-inhibition (grey bars) in my laboratory in the first 29 inhibitor patients studied. Patients with lower levels of antibody to human FVIII are shown in the upper graph and those with higher levels on the lower graph. Inhibition of porcine FVIII was lower than that of human FVIII in all instances. The difference was dramatic in some instances.
A man with a high-responding inhibitor needed emergency surgery. At the time, his inhibitor level was 8 BU. The first doses of porcine FVIII neutralized the inhibitor and his plasma FVIII level was adequate for surgery. By post-op day 5, his level of anti-human FVIII began to rise. By post-op day 7, his level of anti-porcine FVIII was rising. By post-op day 6, porcine FVIII was no longer effective. His later course included some oozing from the incision which was controlled with infusions of prothrombin complex concentrate.
hemophilia B. It has been superceded, for that purpose, by concentrates of factor IX alone. A portion of the factors in PCC activate spontaneously during processing. Activation can be enhanced deliberately to create "activated" prothrombin complex (APCC), also known as anti-inhibitor coagulant complex. PCC and APCC were used to treat bleeding in patients with inhibitors starting in the early 1970's. Nowadays, PCC is no longer widely available. Of the two APCCs once manufactured, Autoplex® was discontinued in 2005 but FEIBA® is still widely used.

A single factor can be separated and activated. A concentrate of plasma-derived activated factor VII was developed in the early 1980’s and used successfully in patients with inhibitors in Scandinavia. A recombinant factor VIIa (rVIIa) concentrate was then prepared in Denmark (NovoSeven®). FVIIa may need tissue thromboplastin to initiate the activation of factor X. Tissue thromboplastin is present at sites of injury but is not abundant elsewhere, thus, the coagulant effect of exogenous FVIIa may be focused on sites of injury, a probable safety advantage. Activated factor X also has been used for hemostasis but only experimentally.

Whereas the potency of PCC was described in factor IX units, the potency of APCCs is described in factor VIII correctional or bypassing units, unique to each brand. The dose of FEIBA® recommended in the package insert is 50-100 units/kg at intervals of 6 to 12 hours. The potency of rVIIa is expressed in weight in micrograms. A dose of 90 mcg/kg, which can be repeated at intervals of 2.5 to 3 hours, is recom-

**Figure 9. A simplified coagulation scheme.**

Factor X may be converted to activated factor X either by the contact-activation ("intrinsic") sequence on the left, or the factor VII-tissue thromboplastin ("extrinsic") sequence on the right. (These sequences are depicted as separate for clarity; in reality, they interact.) Activated factor X is one of the essential catalysts of the conversion of prothrombin to thrombin. Activated factor VII, in the presence of thromboplastin, may trigger some formation of factor Xa in the absence of factor VIII or IX.
mended in the package insert. Many patients use double or triple that amount as an initial dose. The European Union approved single doses of 270 mcg/kg as an alternative to repeated smaller doses.

Patients with inhibitors who bleed frequently may benefit from prophylaxis, that is, a dose of APCC or rVIIa given daily or every other day. Sometimes FEIBA® and NovoSeven® are given alternatively, for treatment of bleeding or for prophylaxis. A patient often feels that one or the other agent is more effective in his case, or that alternating doses is helpful. In some institutions, measurement of “global” coagulation, as by thromboelastography, may be used to follow a patient, but there is no specific laboratory test to confirm that hemostasis is being promoted. One must observe the patient clinically.

Other brands of rVIIa concentrate, and modifications of rVIIa to prolong its half-life, are under development.

**Efficacy**

The efficacy of bypassing agents has been difficult to establish. How do we know just when internal bleeding (e.g., in a joint) stops? Diminution of pain is the first, albeit subjective, indicator. Range of motion improves more slowly. By the late 1970’s, doubts had arisen that PCC was effective at all, so comparison to albumin placebo was justified. The first controlled trial of PCC for joint hemorrhages in persons with hemophilia and inhibitors (see Figure 10) confirmed that a single dose of PCC was associated with clinical improvement within a few hours in about half of instances. Trials of Autoplex® (an APCC) versus Proplex® in the USA, and of FEIBA® versus Prothromblex® (a PCC) in Europe gave similar results. Reports of the efficacy of repeated doses of APCC, in uncontrolled studies, with evaluation performed after two or three days, showed a higher success rate.

Clinical trials of NovoSeven® all featured repeated doses at intervals of 2.5 to 3 hours. In rough summary, two doses of rVIIa successfully controlled about half of joint hemorrhages. In a trial of a single standard dose of FEIBA® versus two standard doses of NovoSeven®, outcomes were similar. Some clinicians give double or triple doses of Novo-Seven® at the outset, rather than giving it in repeated doses.

Summaries of the most comparable completed trials may be found in Figure 10.

**Side-effects**

An occasional side-effect of PCC or APCC, seen only in a few sensitive patients with hemophilia A, is an anamnestic rise in the FVIII inhibitor. These plasma-derived products may contain residual FVIII. Such patients are better served by the use of rVIIa.

Patients with inhibitors to factor IX may sometimes have allergic reactions when treated with plasma-derived products which contain factor IX, so they, too, are better served by the use of rVIIa.

Other adverse side-effects of bypassing agents, seen predominantly after intensive use, may include disseminated intravascular coagulation (DIC), deep vein thrombosis (DVT) and, in a few rare patients, including youths, receiving PCC or APCC, myocardial infarction. Autopsies revealed hemorrhagic myocardial infarction without coronary thrombosis. Because of this mysterious but catastrophic complication, closely-spaced doses of PCC or APCC are avoided. Fewer instances of DIC and DVT have been reported with rVIIa than with PCC and APCC. The frequency of adverse events with these products is the subject of ongoing controversy, and vigilance is essential.
Figure 10. Key trials of bypassing agents for joint hemorrhages with a FVIII inhibitor

<table>
<thead>
<tr>
<th>Lusher et alia, 1980, observed at six hours after a single infusion</th>
<th>“effective” (subjective)</th>
<th>Range of motion (ROM) improved 10° or more</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemophilia A, no inhibitor, open-label, usual dose of factor VIII</td>
<td>100%</td>
<td>65%</td>
</tr>
<tr>
<td>Hemophilia A, inhibitor, double-blind</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Konyne (PCC) 75 U/kg</td>
<td>48%</td>
<td>34%</td>
</tr>
<tr>
<td>Proplex (PCC) 75 U/kg</td>
<td>53%</td>
<td>33%</td>
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<tr>
<td>Albumin placebo</td>
<td>29%</td>
<td>18%</td>
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<th>ROM improved 10° or more</th>
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<tr>
<td>Hemophilia A, inhibitor, double-blind</td>
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</tr>
<tr>
<td>Proplex (PCC) 75 U/kg</td>
<td>50%</td>
<td>43%</td>
</tr>
<tr>
<td>Autoplex (APCC) 50 U/kg</td>
<td>56%</td>
<td>48%</td>
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<tr>
<td>Autoplex (APCC) 75 U/kg</td>
<td>52%</td>
<td>52%</td>
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<tr>
<th>Sjamsoedin et alia, 1981, observed at 24 hours after a single infusion</th>
<th>“effective” (subjective)</th>
<th>ROM improved 30° or more</th>
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<tbody>
<tr>
<td>Hemophilia A, inhibitor, double-blind</td>
<td></td>
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<tr>
<td>Prothromblex (PCC) 48 U/kg</td>
<td>46%</td>
<td>7%</td>
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<tr>
<td>FEIBA (APCC) 88 U/kg</td>
<td>66%</td>
<td>25%</td>
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<tr>
<th>Key et alia, 1998, USA</th>
<th>Subjective efficacy</th>
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<tr>
<td>Hemophilia A, inhibitor, open label, home treatment</td>
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</tr>
<tr>
<td>NovoSeven, 90 ug/kg, every 3 hours until effective</td>
<td>Median 2.2 doses needed</td>
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<th>Santagostino et alia, 1999, Italy</th>
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<td>Hemophilia A, inhibitor, open label, home treatment</td>
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<tr>
<td>NovoSeven, 90 ug/kg, every 3 hours until effective</td>
<td>Median 2 doses needed</td>
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<table>
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<tr>
<th>Astermark et alia, 2006, international</th>
<th>Effective at 2 hrs (before 2nd dose rVIIa)</th>
<th>Effective at 6 hrs</th>
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<tbody>
<tr>
<td>Hemophilia A, inhibitor, open-label, home treatment</td>
<td></td>
<td></td>
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<tr>
<td>NovoSeven, target 105 mcg/kg, 2 doses, second at 2 hrs</td>
<td>60%</td>
<td>79%</td>
</tr>
<tr>
<td>FEIBA 85 U/kg, one dose</td>
<td>75%</td>
<td>81%</td>
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</tbody>
</table>
Surgical operations

Both types of bypassing agents have been used to promote hemostasis for emergency surgical operations. NovoSeven® has been used for elective surgical operations, with very frequent dosing. Reports and guidelines mention occasional “oozing”, that is, hemostasis may not be quite normal. Some surgeons who are willing to undertake operations on soft tissue under cover of rVIIa are not willing to implant a “total” joint because imperfect hemostasis with a resulting small collection of blood at the site of the implant may set the stage for late infection. Such infections are more common in persons with hemophilia than in non-hemophilic patients, perhaps due to the provision of good bacterial breeding grounds in small hematomas.

IMMUNE TOLERANCE INDUCTION

Bonn (and similar) protocols

Tolerance to the deficient clotting factor can be achieved in a large majority of patients with allo-antibodies to FVIII by giving them frequent, usually daily, large doses of FVIII over a prolonged period of time, a protocol pioneered in Bonn, Germany. Anamnesis usually occurs in the first month of treatment but inhibitor levels typically fall dramatically in the second month. Thereafter, they may fall more slowly. Complete tolerance consists of a sustained zero inhibitor level and a normal half-life of infused FVIII.

Tolerance is then maintained with low-level prophylaxis. Without prophylaxis, inhibitors may recur, but typically at lower levels, easily suppressed by resuming the tolerance regimen. Some patients achieve and maintain partial tolerance, that is, their inhibitor does not disappear completely but persists at a low level, behaving as a low-responding inhibitor.

The ideal dosage is highly debated. Doses used range from 300 FVIII U/kg/day in Bonn to 25 FVIII U/kg every other day in The Netherlands. In the USA, the first dose tried was 50 U/kg/day but nowadays a dose of 100 U/kg/day is commonly used. Some centers add short courses of low-dose corticosteroids if the fall in the inhibitor level is sluggish. Immunosuppressive drugs alone, without FVIII, are not helpful.

Reports agree that the lower the inhibitor level at the outset of tolerance attempts, and the lower the inhibitor level in the patient’s past history, the more likely he is to achieve tolerance, and the faster the process will be. A trial comparing the low Dutch dose with the high German dose showed that success could be attained with the lower dose but a longer period of treatment was needed. A registry maintained by Prof. Guglielmo Mariani of Italy (Figure 11) showed that higher doses are correlated with a greater chance of success. (He also reported that higher doses were associated with shorter periods of treatment.)

<table>
<thead>
<tr>
<th>Historic highest inhibitor level, Bethesda units:</th>
<th>Under 50 units / kg</th>
<th>50 – 200 units / kg</th>
<th>Over 200 units/ kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Under 50</td>
<td>38.7 %</td>
<td>77.8%</td>
<td>88 %</td>
</tr>
<tr>
<td>50-500</td>
<td>25%</td>
<td>51.9%</td>
<td>83%</td>
</tr>
<tr>
<td>Over 500</td>
<td>Nil</td>
<td>6.3%</td>
<td>56%</td>
</tr>
</tbody>
</table>

Figure 11. Prof. Guglielmo’s Registry as of 1993:
Figure 13. Induction of immune tolerance with daily factor VIII (50 U/kg).

The course of three actual patients on induction of immune tolerance, 50 FVIII units/kg/day, is depicted. The lower, solid line depicts a patient whose inhibitor level rose sharply during the first month, fell sharply in the second and then gradually fell to zero, a response seen in most patients with a low inhibitor level at the outset. The middle, dashed line depicts a patient whose inhibitor also rose in the first month and fell in the second but thereafter fell slowly, with some inhibitor still detected after two years. The upper, dotted line depicts a patient whose inhibitor never fell notably and who later proved to have a large factor VIII gene deletion. A relative with hemophilia and an inhibitor also failed tolerance induction at another institution.
There is some evidence that plasma-derived FVIII containing von Willebrand factor is more effective in inducing tolerance than is recombinant FVIII. Nowadays, tolerance programs usually are started as soon as an inhibitor is diagnosed. Typically such programs start with whatever FVIII concentrate the patient has been receiving. If tolerance is difficult to achieve, the patient may be switched to plasma-derived concentrates. In Bonn, only plasma-derived products containing von Willebrand factor are used for tolerance.

The probability of success in inducing tolerance is related to the underlying hemophilia-causing mutation. Patients with mutations, such as large deletions, associated with a very high likelihood of inhibitor development also are the patients least likely to respond to tolerance protocols. They may require high doses, such as are used in Germany, and a prolonged period of treatment.

**Malmö protocol**

In Malmö, Sweden, an intensive two to four week inpatient tolerance protocol was used for many years. The inhibitor level was lowered with plasmapheresis, then FVIII was given in large doses to maintain a low-normal plasma level, and cyclophosphamide and intravenous gamma globulin were administered. Tolerance was achieved in more than half of patients with the first round of treatment and in 70% with one or more additional rounds. The protocol was appropriate for patients with inhibitors to factor IX, because plasmapheresis removed the inhibitor antibody that tends to cause allergic reactions; tolerance was achieved in 86%.

Once all existing inhibitor patients in Sweden had been treated, new inhibitor patients tended to be babies, for whom the Bonn protocol is more suitable.

**Tolerance for factor IX inhibitors**

Attempts to induce tolerance with the Bonn-style protocol have sometimes resulted in a nephrotic syndrome. Variants of the Malmö protocol, with immunosuppressive agents suitable to the age of the patient, are sometimes successful and are safer.

**Autoimmune inhibitors**

Autoimmune inhibitors arising in postpartum women respond quickly to corticosteroids. Most autoimmune inhibitors arise in elderly persons, idiopathic in more than half of instances, or associated with autoimmune or malignant disorders. Suppression of autoimmune inhibitors may be achieved in about three-quarters of patients with corticosteroids alone or a combination of such steroids with cyclophosphamide. Other immunosuppressive agents such as rituximab have sometimes been used successfully.
Recent reviews


Kruse-Jarres, R. Current controversies in the formation and treatment of alloantibodies to factor VIII in congenital haemophilia A. Am Soc Hematol Educ Program 2011; 407-412


Incidence and Prevalence


Data from 8 studies on 451 patients with severe hemophilia A followed from birth with continuous supervision are combined. The cumulative incidence of high-responding inhibitors was 10% by age 3 years and 20% by age 18 years. This report covered an era when plasma-derived concentrates were used.


In Frankfurt, Germany, from 1976 to 1992, (an era when plasma-derived FVIII concentrates were used), inhibitors developed in 14/27 (52%) of boys with severe hemophilia A followed prospectively. (This incidence is the highest ever reported and is often quoted, note that the number of subjects is small.)


German patients from several centers were evaluated over a 23-year period. Inhibitors developed in 22/72 (32%) of all hemophilia A patients after a median of 15 exposure days. Among patients with severe hemophilia, 43% developed inhibitors. No difference in incidence was seen in this small sample between patients on plasma-derived vs. recombinant concentrate.


The cumulative risk of inhibitor development in an era of treatment with plasma-derived concentrates was 16% at age 5 years and 36% at age 75 years in severe hemophilia A. The cumulative risk of inhibitor development was 6% at age 5 years and 8% at age 75 years in severe hemophilia B. In patients with severe hemophilia, without HIV infection, inhibitor development doubled mortality in the years 1977-92 but did not affect mortality rates in 1993-99 perhaps due to more treatment options.


Between 1990 and 2009, 315 new inhibitors were reported in 2528 patients with severe hemophilia A in the UK. The age of highest incidence was under 5 years, with 51% of the inhibitors arising at that time. Inhibitors could arise throughout life, and were less likely to arise in HIV-infected patients.

In Canada, 34 of 99 severe hemophilia A PUPs developed inhibitors (24 of high-titer) at median age 13.6 months. Six of seven aboriginal (native American) patients developed inhibitors.


In subjects with severe hemophilia A, inhibitors were reported in 56/307 white non-Hispanic subjects, in 11/33 black non-Hispanic subjects and 11 of 28 Hispanic subjects. The differences between whites and non-whites were significant. Only 5% of subjects with severe hemophilia B had inhibitors.


Among 5651 males with severe hemophilia A, the prevalence of high-titer inhibitors among Hispanic participants was 24.5% compared to 16.4% for white non-Hispanic patients. This report and that above come from a USA registry and the lower overall prevalence of inhibitors reflects, in part the exclusion of low-titer inhibitors, and, perhaps, less intensive surveillance.


New inhibitor formation in persons with hemophilia A and more than 150 lifetime exposures to FVIII concentrate is rare, occurring between 1.55 and 3.8 per 1000 person-years. Previously-treated patients (PTPs) are the first to receive new FVIII concentrates in clinical studies under the rationale that a product with increased antigenicity is more likely to be detected because of a low baseline inhibitor risk compared to PUPs.


The authors searched for studies of at least 25 previously-treated patients who were treated with any type of FVIII concentrate. They found 33 cohorts with 4323 subjects who had 43 de novo inhibitors, for an incidence of three such inhibitors per 1000 person-years.


Inhibitors were present in 28/735 patients with severe hemophilia B (3.8%), 1/644 with moderate hemophilia B and none in mild hemophilia B


In an international registry of 94 persons with hemophilia B and inhibitors, the median age at inhibitor detection was 19.5 months with a median of 11 exposure-days. (Anaphylaxis was reported in 56 patients. Large gene deletions and null mutations conveyed the greatest risk of anaphylaxis. Of 39 patients who underwent some form of induction of immune tolerance, only five were completely successful.


Over a five-year period (2008-2012), participating centers reported 7,969 patients with mild-moderate hemophilia A of whom 37 developed inhibitors (0.43/100 treatment-years) and 1,863 patients with mild-moderate hemophilia B, of whom one developed an inhibitor (0.05/100 treatment years.) Inhibitors occurred at a median age of 35 years after a
median of 38 exposure-days.


In an international survey of 1112 non-severe hemophilia A patients, 59 were found to have an inhibitor (5.3%) after a median of 24 exposure-days. Among 214 different F8 missense mutations, 19 were associated with inhibitor development.

**F8—F9 genotypes and inhibitors**


The inhibitor risk in hemophilia A is greatest (88%) with large deletions covering multiple domains. The most common hemophilia mutation, intron 22 inversion, was associated with 21% inhibitors. Missense mutations in the C1/C2 domain were associated with a 10% inhibitor incidence whereas missense mutations elsewhere had only a 3% risk.


Data from 30 studies on 5383 patients, of whom 1029 had inhibitors, showed that the risk was highest with large deletions and nonsense mutations and lowest with small deletions/insertions and missense mutations.


**Missense mutations (374 different ones)** were identified in 1135 hemophilia A patients; of whom 46% had mild hemophilia. Of the 720 whose inhibitor status was known, only 5% had inhibitors. Inhibitor prevalence was four-fold higher for severe hemophilia than for milder types (perhaps due to less exposure to exogenous clotting factors in milder hemophilia). Mutations associated with inhibitor formation were especially clustered within the C1/C2 domain (8.7% inhibitors vs. 3.6% for non-C1/C2 domain.) Inhibitor risk is higher is the amino acid substitution belongs to a different physico-chemical class.


**A patient with mild hemophilia A and a missense mutation in the A3 domain developed an antibody that recognized a C2 epitope. It neutralized allogeneic but not autogenous FVIII. This is not the only example of an inhibitor in mild hemophilia A that solely inhibits exogenous FVIII.**


Six wild-type FVIII proteins were found and designated haplotypes 1-6 (H1-6). H1 and H2 were found in all racial groups and were the only types found in white people. H1 is the basis of Kogenate and H2 of Recombinate/Advate recombinant FVIII concentrates. H3, H4 and H5 (which have substitutions in the immunogenic regions of the protein) were found only in black people. (H6 was found in some Chinese persons.) Among 78 black hemophilia A patients, 24% had a background H3 or H4 haplotype. The odds ratio for prevalence of inhibitors in H3-4 patients was 3.6 vs. those with an H1-2 background. Perhaps standard FVIII concentrate is mismatched to the H3-4 patients. The preponderance of the haplotypes H3-4 among black inhibitor patients has not been substantiated in later studies, but all studies suffer from small numbers of subjects.

Total gene deletions and major gene derangements were found in the majority. In 30 patients, anaphylaxis developed at a median of 11 exposure-days. Hemorrhages are best managed with recombinant activated factor VII. Attempts at immune tolerance in such patients were rarely successful and sometimes complicated by the nephrotic syndrome.


Mutations in patients with hemophilia B with known inhibitor status from an international database were classified. Inhibitors were associated with large deletions of more than 50 basepairs (39%) but not with smaller deletions. Inhibitors were associated with 60% of nonsense mutations if synthesis was terminated before the first 100 amino acids but only 23% if synthesis was terminated later. Inhibitors were associated with 27% of frameshift mutations. Inhibitors were associated with only 1.2% of missense mutations, the type of mutation which accounts for 56% of hemophilia B.

Immunology


A purebred miniature Schnauzer with severe hemophilia A was bred to produce hemophilic descendants. He was mated to a normal Brittany spaniel; six of her nine hemophilic descendants developed significant inhibitors after treatment with canine cryoprecipitate. The dog also was mated to his sister and only one of their 16 treated hemophilic descendants had an inhibitor, at a trace level, present only transiently. These results suggest that the two batches of the founder dog’s grandsons inherited their grandmother’s immune systems, one system conveying susceptibility to inhibitors, and one not.


In 260 patients with severe hemophilia, patients with inhibitors were matched with patients with the same mutation without inhibitors. Significantly higher frequencies of the DRB1*15 and DQB1*0602 alleles were found in inhibitor patients. The A allele of the 308G>A polymorphism of TNF-alpha was almost twice as frequent in the inhibitor group as in case controls. High production of TNF-alpha/IL-10 was found in 12% of inhibitor patients and only 3% of non-inhibitor patients.

Bafunno V, ... Peyvandi F, Margaglione M. Polymorphisms in genes involved in autoimmune disease and the risk of FVIII inhibitor development in Italian patients with haemophilia A. Haemophilia 2010, 16:469-473

Distributions of single-nucleotide polymorphisms (SNPs) in the CRLA4, PTPN22, IL10, TNFα, FOXP3 and IRF5 genes, all previously reported to be associated with autoimmune disorders, were not found to differ between 115 hemophilia A patients with 328 patients without inhibitors in Italy.

Caucasians in the USA, interleukin haplotypes in 302 patients with inhibitors of more than one BU were compared to those in 633 patients with no inhibitor. Haplotypes associated with an increased inhibitor risk were found in IL10, IL12, and IL1α. Protective haplotypes were found in IL2 and IL1β. One rare F8 haplotype was seen in 3.1% of patients with inhibitors and only 0.25% of non-inhibitor subjects.


In a study of 833 hemophilia A patients, of whom 457 had a current inhibitor or a history thereof, 53 of 13,331 single nucleotide polymorphisms (SNPs) in primary immune response and immune modifier genes predicted inhibitor status. Of the 13 most highly-significantly-related SNPs, 8 showed protective effects and 5 indicated increased risk; the latter were located on genes CD44, CSF1R, DOCK2, MAPK9 and IQGAP2.

Whelan SFJ, Hofbauer CJ...Reipert BM et alia. Distinct characteristics of antibody responses against factor VIII in healthy individuals and in different cohorts of hemophilia A patients. Blood 2013; 121:1039-1048

Non-neutralizing FVIII antibodies were found in 116 of 600 normal blood donors (19%) (not the first such report, but the largest numerically). Most appeared to be directed against the B-domain. Such non-neutralizing antibodies also were found in 34% of persons with hemophilia who did not have neutralizing antibodies. Only patients with neutralizing antibodies had IgG4 antibodies.

Plasma-derived concentrates with high antigenicity


The Dutch Red Cross produced FVIII concentrate by controlled pore silica fractionation. In 1990, viral inactivation was changed from dry heat to pasteurization. Dutch patient surveillance showed that in the previous 27 months on dry-heated concentrate, only 4 inhibitors developed, all in newly-exposed patients, that is, the most vulnerable. During 18 months of use of pasteurized concentrate, inhibitors were reported in 11 patients, all but two of whom were previously-heavily-exposed patients, those believed to have low vulnerability.


The above pasteurized Dutch concentrate also was used in Belgium starting in 1990. Five of 50 patients with severe hemophilia A, all heavily transfused in the past, developed new high level inhibitors. Belgium then switched totally to solvent-detergent-treated (SD) concentrate.


In 1995, Belgium replaced the SD concentrate mentioned above with a concentrate doubly viral inactivated with both SD and pasteurization. Eight of 140 previously-heavily-transfused patients with severe hemophilia A developed inhibitors shortly after changing products.


Investigations of the above event showed that the combination of SD plus pasteurization induced changes in FVIII not found after other single or double viral inactivation processes.

Josic D, Buchacher A, Kannicht C, et alia. Degradation products of factor VIII which can lead to increased immunogenicity. Vox Sang 1999; 77 (suppl 1): 90-99
On investigation, plasma obtained from certain blood banks was found to already contain FVIII breakdown products. (Plasma may not have been separated from red cells promptly.) Concentrate produced from these sources was associated with inhibitor formation. The reason for the increased antigenicity of this product was debated; this report is from the manufacturer.

Recombinant concentrates and inhibitors

Below are a few of the many papers comparing inhibitor rates in PUPs treated with plasma-derived vs. recombinant concentrates. Results showed either equal risk, or greater risk with recombinants.


In this meta-analysis of 24 studies covering 1965 PUPs treated with plasma-derived FVIII and 420 with recombinant FVIII, inhibitor incidence was 14.3% with plasma-derived FVIII and 27.4% with recombinant FVIII.


In 574 consecutive PUPs born between 2000 and 2010, inhibitors developed in 177 (32.4%). Plasma-derived and recombinant FVIII concentrates were associated with similar risks, but second-generation full-length recombinants were associated with a higher risk of inhibitors.


Inhibitors developed in 118 of 407 (29%) PUPs with severe hemophilia A born in the UK between 2000-9011 after a median of 16 exposure-days. All patients were on recombinant products. (The UK had had a recent “mad cow disease” epidemic and feared transmission of abnormal prions in plasma products.) A second-generation recombinant FVIII concentrate, Kogenate/Helixate, was associated with a higher incidence (35%) of inhibitors than a third-generation product, Advate (24%). This report and the previous one cast suspicion on second generation products, but another report disputed the association.

Peyvandi F, Mannucci PM...Rosendaal RF et alia. Source of factor VIII replacement (PLASMATIC OR RECOMBINANT) and incidence of inhibitory alloantibodies in previously untreated patients with severe hemophilia A: The multicenter randomized Sippet study. American Society of Hematology, 57th annual meeting, December 2015, Abstract # 5.

Between 2010 and 2014, 251 PUPs from five continents were randomized to receive a VWF-containing plasma-derived FVIII concentrate vs. recombinant FVIII. Inhibitors developed in 76 patients, of whom 50 had high titers. Recognized risk factors were equally divided between the two product groups. Recombinant FVIII was associated with 87% higher inhibitor incidence compared to plasma-derived FVIII. The difference persisted even when subjects on second generation full-length recombinant concentrates were excluded from analysis.


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Binding of VWF to the C2 domain of FVIII may reduce immunogenicity by steric hindrance. Normally, some 94% of FVIII in the circulation is bound to VWF. In recombinant products, some 20% of FVIII may remain unbound, which may facilitate an immune response. The authors suggest a few other mechanisms by which VWF may exert a protective effect.


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See also:


Other treatment-related issues


Thirteen hemophilia centers reported a total of 1079 episodes of treatment with continuous infusion of concentrate for surgery or major hemorrhages. Inhibitors developed in 0.45% of 659 patients with severe hemophilia A and in 7.2% of patients with mild hemophilia. (One presumes that those with mild hemophilia had less prior exposure than those with severe hemophilia; previous to this report, there had been suspicion that continuous infusion was a risk factor.)


In a multicenter study of 606 consecutive PUPs born between 2000 and 2010, the incidence of inhibitor development was 32% (high titer 22%). High-dose intensive FVIII treatment for bleeding or surgery was associated with an increased risk of inhibitor formation. During the first 20 exposure-days, patients using on-demand treatment vs. prophylaxis had an equal risk of inhibitor development but thereafter prophylaxis was associated with a decreased risk. The authors suggest that some patients, based on their genetics, are highly unlikely to ever develop inhibitors whereas others are at very high risk, but the group in the middle is most susceptible to such influences as the mode of treatment.

Aznar JA, Moret A, Ibáñez F et alia. Inhibitor development after switching of FVIII concentrate in multitransfused patients with severe hemophilia A. Haemophilia 2014; 20:624-9

In Spain, adequate medical records were available on 97 hemophilia A patients who had more than 150 exposure days at the time their concentrate brand was switched some time between 1970 and 2007. Multiple plasma-derived and recombinant products were used in this era and switches often were multiple. Nine patients developed inhibitors (9%), all low-level. Neither the number of different FVIII products nor switching seemed to influence the risk. This paper and the next are included to refute the widespread “old wives’ tale” that switching concentrate brands promotes inhibitor formation.


Inhibitors in healthy persons


Inhibitor activity against FVIII, found in 85/500 (17%) of plasma samples from healthy blood donors, ranged from 0.4 to 2 BU. Mean levels of FVIII activity were not different in persons with and without these antibodies.


A higher frequency of anti-idiotypic antibodies to FVIII inhibitors was found in donors in older age groups and in multiparous women (reflecting the population who tend to acquire auto-antibodies to FVIII). These donor groups may be good sources of such antibodies for therapeutic IV gamma globulin.

An immunoassay for the quantitation of anti-FVIII antibodies was developed. This test captures neutralizing and non-neutralizing antibodies. Detectable non-neutralizing antibodies were found in 4/150 healthy donors and in 13/39 inhibitor-free hemophilia A subjects.


Antibody affinity was studied using a competition-based ELISA test. FVIII-specific antibodies found in inhibitor patients (with congenital and acquired hemophilia) had an up to 100-fold higher affinity than those found in non-inhibitor hemophilia patients and healthy persons.

Characteristics of inhibitor antibodies


Type 1 antibodies completely destroy FVIII when antibody is present in high concentration, and may react with antigenic determinants near sites for procoagulant activity. Type 2 antibodies do not completely inactivate factor VIII; they react with more distant sites.


In this classic article, “high-responding” patients were defined as those in whom “the antibody titers increased after each antigenic stimulation or persisted for years in the absence of transfusion” whereas “low-responding” patients were those in whom antibody titers remained low, and in whom there was no significant difference in individual titers before and 8-10 days following transfusion.

Laboratory Diagnosis


The Bethesda test and “Bethesda” units are described and defined. The committee of U.S. hematologists agreed to use it henceforth. Prior to this time, each lab tested inhibitors by their own method and assigned their own unitage.


The time-dependence of the reaction of factor VIII inhibitors with factor VIII, and its effect on APTT screening tests, is described.


False-positive Bethesda tests were encountered in The Netherlands and attributed to rising pH and to FVIII activation during incubation. Two test modifications were introduced (and have since become popular) including buffering of the normal plasma in incubation mixes with imidazole buffer and using, as a control, immuno-depleted FVIII-deficient plasma instead of buffer.


In studies of 95 adults with severe haemophilia A, who had not been transfused in the previous few days, the early phase T 1/2 of infused FVIII, as concentrate, was measured at 3.25 hours. The early T ½ was used as a sensitive, routine pre-operative screen for inhibitor.

**Plasmapheresis**


Inhibitor levels were lowered with plasmapheresis alone using older equipment.


Inhibitors levels were lowered in 10 patients on 19 occasions with plasmapheresis coupled with extracorporeal adsorption of antibodies given over a treatment period of 1-6 days. In all instances, it was possible either to eliminate the inhibitor totally or to reduce it to low levels at which remaining inhibitor was neutralized easily with concentrate infusions.


An excellent description is provided of the use of plasmapheresis with extracorporeal adsorption of antibodies in 13 patients with FVIII inhibitors measuring 18 to 540 Bethesda units, treated on a total of 89 occasions. At each session, lasting about 3.8 hours, about seven liters of plasma were processed with a mean reduction in inhibitor level of about 72% per session.

**DDAVP**


A patient with a FVIII auto-antibody of 1.8 Bethesda units was given DDAVP on two occasions and had a seven- to nine-fold rise in plasma FVIII (to over 50 FVIII U/dL). Plasma FVIII levels remained in the hemostatic range for three hours after each infusion, allowing dental procedures to be performed.


The authors present an excellent summary of the use of DDAVP in 22 patients with auto-antibodies to FVIII. Successful hemostasis was correlated with low inhibitor levels.

**Human factor VIII**


Stimulated by in vivo observations, the authors incubated seven brands of FVIII concentrate in vitro with seven plasmas with different inhibitors to FVIII. Several inhibitors caused notably less neutralization of FVIII when it was present together with VWF (in concentrates “Humate-P” and “Koate HP”) than when it was presented as FVIII alone, in plasma-derived or recombinant concentrates. The authors suggest that some inhibitor patients may respond better clinically to concentrates containing FVIII with VWF.

**Porcine factor VIII**


A polyethylene-glycol fractionated porcine FVIII concentrate was first used in London in 1980. The chance of hemostasis after infusion was related to the ability to
raise plasma FVIII to a measurable level, and that response was related to a low anti-porcine-FVIII inhibitor level. Some patients had no anamnesis after infusions and could use the concentrate repeatedly.


Median cross-reactivity was 32%. Anamnesis occurred after 9/22 (41%) of treatments. Severe thrombocytopenia was seen in two instances.


In 65 patients with auto-antibodies to FVIII, treated with porcine FVIII for a total of 74 acute hemorrhages, there was a good to excellent response in 78%. These authors did not find a relationship between the probability of hemostasis and the initial antibody level or plasma FVIII level attained after infusion.


A retrospective international survey showed that the median cross-reactivity in 137 patients was 15%. No rise in antibodies to porcine factor VIII after infusion was seen in 29% of recipients, an intermediate response was seen in 40% and a brisk response in 31%. Seven patients who did not have anamnesis were treated on-demand at home for 1.5 to 13 years (median 6.2 years) and another 23 patients were treated regularly with porcine FVIII in the hospital for 2-7 years (median 3). These non-anamnestic patients used porcine FVIII for 2000 bleeding episodes. The risk of transfusion reaction was dose-related. Such reactions were rare in the home setting, where dosage was low. A post-infusion fall in platelet count was common but usually transient and clinically insignificant. Occasional marked falls in platelet counts were seen, usually with intensive replacement therapy.


A recombinant, porcine B-domain deleted factor VIII concentrate (subsequently trade-named “Obizur”) was successful in controlling hemorrhages in 24/28 subjects with acquired FVIII inhibitors. Ten patients had baseline neutralizing antibodies against porcine FVIII; all but one responded well to treatment. No thrombocytopenia occurred. A standard initial dose of 200 units/kg was used, after which dosage was titrated to maintain desired plasma FVIII levels. Five patients developed de novo anti-porcine antibodies. The product currently is licensed for use in acquired hemophilia only.

Bypassing agents


In this first controlled study of bypassing agents, patients with severe hemophilia A and inhibitor in the USA were treated for acute joint hemorrhages. As a preliminary, 20 non-blind control patients who did not have inhibitors were treated with a single dose of factor VIII and observed the same way as inhibitor patients; at six hours, all had symptomatic improvement and 65% had improved joint range of motion (ROM) of at least 10 degrees. None had any change in joint circumference. In the double-blind controlled group, 51 patients with inhibitors were treated for 157 joint hemorrhages with a single dose of either Konyne® (a PCC) 75 factor IX U/kg, Proplex® (a PCC) 75 factor IX U/kg, or an intravenous albumin placebo. Six hours after the infusion, patients judged the treatment efficacious in 29% of hemorrhages treated with albumin, 48% with Konyne®, and 53% with Proplex®. Range of motion was improved in 18% on placebo, 34% on Konyne® and 33% on Proplex®. Results with the two PCCs were significantly better than with albumin.

In a trial of similar design to that above, acute joint hemorrhages were treated with single doses either of Proplex® (75 factor IX units/kg) or Autoplex® (either 50 or 75 “factor eight correctional units” per kg). Six hours after the infusion, patients judged the treatment efficacious in 50% of hemorrhages treated with Proplex®, 56% with lower-dose Autoplex®, 52% with higher-dose Autoplex®. Differences were not significant.


In this European trial, a large majority of hemorrhages treated were into joints. Hemorrhages were treated with single doses either of Prothromplex® (a PCC, 48 factor IX units/kg) or FEIBA® (88 factor eight inhibitor bypassing units/kg). At 24 hours, Prothromplex® was effective or partly effective in 46% and FEIBA® in 66% of instances. A 30-80% improvement in range of motion was seen in 7% of hemorrhages treated with Prothromplex® and in 25% of those treated with FEIBA®.


Various doses of infused activated factor X and phospholipids were given to hemophilic and to normal dogs to determine the therapeutic dose (shortening of the cuticle bleeding time) and toxic dose (causing DIC). (Other experimental bypassing agents containing Xa have been proposed, but none has been licensed.)


Purified factor VIIa from human plasma was given in doses of 9-20 ug/kg (equivalent to 700-1000 U/kg). Plasma levels of factor VII were 330% to 610% 15 minutes after infusion. With the lowest dose level, a second dose clearly was needed for hemostasis. As dose levels went up, a second dose was not always clearly needed.


Doses of 35 or 70 ug of rVIIa/kg were given at intervals of 2.5 hours, up to a maximum of 6 doses, for treatment of 119 joint hemorrhages in 33 patients with hemophilia A (of whom 27 had an inhibitor) or B (of whom half had an inhibitor). Response was judged by assessing subjective pain and measuring decrease in size of hemorrhage at 8-14 hours after initiating treatment. In 144 hemorrhages (20 of which were in non-inhibitor patients), treatment was judged effective or excellent in 71% of those given 35 ug/kg and 71% of those given 70 ug/kg. The median number of doses given for effective or excellent responses was two.


NovoSeven®, rVIIa, 90 ug/kg, was given every three hours for a maximum of four doses. Once the patient judged it effective, one more dose was given for “maintenance” and then treatment was stopped. In about 47-48% of the courses judged effective, a median of 2.2 doses had been given prior to the maintenance dose.


NovoSeven®, 90 ug/kg, was given every three hours for a maximum of four
doses for hemorrhages treated within an hour of onset. The authors state: “two infusions were sufficient to achieve a successful outcome in more than half of the bleeding episodes.”


In an international, non-blinded, randomized crossover study of 96 joint hemorrhages in 48 patients, treatment was either of a single dose of FEIBA® at about 85 U/kg, or NovoSeven® at about 105 U/kg given twice, the second dose two hours after the first. At 6 hours, FEIBA® was effective in 81% and NovoSeven® in 79%.


In three young hemophilia patients with inhibitors, 95/114 hemorrhages responded to a single dose of NovoSeven® at 300 U/kg. A second dose was required in 19 episodes. This protocol gave faster relief than two doses of 90 U/kg.


In France, FEIBA® was used between 1978 and 1993 in 433 bleeding episodes in 60 patients. In joint hemorrhages, bleeding was controlled with one dose in 50.7% of instances, 2 doses in 31.2 %, 3 doses in 7.4 %, and more than three in 10.7% of instances. In five of six major surgeries, FEIBA® provided effective hemostasis; the sixth patient had excessive bleeding but a favorable outcome. Adverse events included anamnesis (more than a 50% rise in inhibitor level) after 31.5% of evaluable treatments, three instances of DIC and one myocardial infarct. The authors do not encourage the use of FEIBA® in elective orthopedic surgery.

Schneiderman J, Rubin E, Nugent DJ, Young G. Sequential therapy with activated prothrombin complex concentrates and recombinant FVIIa in patients with severe haemophilia and inhibitors: update of our previous experience. Haemophilia 2007; 13:244-248.

In four patients, during a total of 35 admissions, activated prothrombin complex concentrate and rVIIa were given within six hours of each other for hemorrhages that had not responded to one of those agents given at home. After failing to respond to a median of three days of therapy with a single agent, hemorrhages resolved in a median of three days on combined therapy. No thrombotic events were seen but D-dimer elevations over 5 ug/ml were noted in 42% of courses lasting more than three days.


In patients refractory to either NovoSeven® or FEIBA® used alone, these agents have been used together, either simultaneously or in sequence. The authors review 17 reports in the literature of such use (in mixed doses) in 49 patients. Among nine patients with acquired hemophilia, one patient developed deep vein thrombosis (DVT) and pulmonary embolism (PE), one developed fatal cerebral thrombosis and three developed DIC. Among 40 patients with congenital hemophilia, one had a fatal myocardial infarction, another had a fatal PE, another recovered from PE, one recovered from DVT and one recovered from DIC.


Five hemophilia A patients with high inhibitor titers were treated with a combination of 30 to 70 ug/kg FVIIa and 20-30 U FEIBA. This combination was effective in most hemorrhages and no adverse events occurred.

Holmstrom M, Tran HTT, Holme PA. Combined treated with APCC (FEIBA) and tranexamic acid in patients with haemo-

FEIBA® was given in combination with tranexamic acid in 6 hemophilia A patients with inhibitors and one patient with an acquired inhibitor. Hemostasis was excellent in 10/11 bleeding episodes. No thrombosis developed and thromboelastography and thrombin generation assays showed no signs of hypercoagulability.


Reports of small series of patients receiving bypassing agents plus tranexamic acid, in an attempt to gain a synergistic effect, are reviewed. There has been no increased incidence of thrombotic events. The authors conclude that it might be tried in patients who fail with one bypassing agent.

Prophylaxis with Bypassing Agents


In a prospective, randomized cross-over study, patients with high-titer inhibitors were either treated for hemorrhages (on demand) with about 85 U of FEIBA® /kg or given prophylaxis three days a week with the same dose. On prophylaxis, patients had 62% fewer hemorrhages than with on demand therapy. Target joint bleeding had been described in 70% of patients and such bleeding was reduced by 72%. No thrombotic complications were reported.


Sixteen children under age 13 years with hemophilia A and inhibitors were given FEIBA prophylactically at doses of 75U/kg three times a week (in USA) or 70-100 U/kg every day or every other day (Germany), adjusted as needed. Median number of joint hemorrhages per year decreased from four to one. No thrombotic complications were seen.


In Brazil, Ukraine, Russia and New Zealand, a total of 36 child and adult patients with hemophilia A or B and inhibitors who had a history of 12 or more hemorrhages in the past year were randomized to on-demand vs. prophylactic FEIBA (the latter dose was 85 U/kg every other day.) The median annual bleed rate in subjects on demand was 28.7 and in those on prophylaxis was 7.9. No thrombotic complications were seen.


Patients with hemophilia A or B and inhibitors of 2 BU or more, from 11 countries, who had at least two hemorrhages per month during a 3-month preliminary observation period, were randomized to receive either 90 or 270 ug/kg rVIIa, double-blind, once daily for three months. Each arm contained eleven patients. Prophylaxis with 90 ug/kg rVIIa reduced bleeds from 5.6 to 3.0 per month, and prophylaxis with 270 ug/kg reduced bleeds from 5.3 to 2.2 per month. The reduction in bleeds was a significant difference, but the effects of two dosages were not significantly different. No thrombotic complications were encountered. A very careful study.


In a retrospective study, data was reviewed from 86 patients with hemophilia and inhibitors in 14 countries who had used prophylaxis with rVIIa. The median dose fre-
quency in children was 7 times/week, in adolescents 5.5 times/week and in adults 3 times/week. Individual doses varied. Overall, the frequency of bleeding was reduced from 1.37 to 0.74 bleeds/month. In patients who had had especially frequent hemorrhages, the rate was reduced from 2.1 to 1.01 bleeds/month.

Surgery with Bypassing Agents

NovoSeven® was given in doses of 35 ug/kg or 70 ug/kg (double-blind) pre-operatively, intra-operatively and for 48 hours post-operatively, every 2 hours as needed. If hemostasis was inadequate, an open-label “escape” dose of up to 180 ug/kg could be given. After the first 48 post operative hours, NovoSeven® was given at the same dose at intervals of two to six hours, as determined by the investigator, for another three days. After post-operative day 5, a dose of 90 ug/kg, open-label, was given as often as the investigator determined. The 70ug/kg initial dose proved adequate for 8 minor and 6 major surgeries through post-operative day 4. The 35 ug/kg dose was less satisfactory. Six patients left the study, five as treatment failures who were subsequently managed on FVIII or FEIBA®, and the sixth because of thrombosis of the internal jugular during central venous catheter placement.

Data from clinical trials and published reports was combined. It covered 395 procedures, of which 261 were surgical, including 100 orthopedic procedures. In the latter, hemostasis was judged effective in 72, partially effective in 9, ineffective in 13 and not reported in 6. The incidence of thrombotic events was low (0.4% of patients.)


Of 40 surgical procedures in Argentina, 15 were major (but none was a “total” joint replacement); rVIIa was the hemostatic cover in all major cases. Bleeding was greater than usually encountered in nine of the major procedures. With minor surgical procedures, bleeding was no greater than expected.


In the UK, eight surgical procedures (including six total knee replacements) were performed on four inhibitor patients under
cover of rVIIa together with tranexamic acid for the first 2–6 postoperative days and FEIBA for the remaining period. FEIBA was used to permit less frequent dosing at this period of recovery and to reduce costs. Five episodes of postoperative bleeding occurred, four in the same patient in connection with four different operations.


Six of 20 patients with high-responder inhibitors to FVIII occasionally had anamnestic inhibitor responses after treatment with PCC or APCC. FVIII antigen, measured in vials of one PCC and the two APCCs, was 11.7, 14.1 and 117 units/vial, respectively. FVIII was present predominantly as the light chain. Only small amounts of von Willebrand factor were found, and only in one APCC. Thus, the factor VIII present in these concentrates is modified, degraded and dissociated from vWF.


In the USA, FVIII inhibitor levels were measured before and after prothrombin complex concentrate treatment of 261 bleeding episodes in 75 patients with hemophilia and inhibitor. A rise in inhibitor level to twice baseline or more was seen in 13.5% of episodes, in 27 patients. (The plasma-derived concentrate contained traces of factor VIII.)


Complications of bypassing agents

Green, D. Complications associated with the treatment of haemophiliacs with inhibitors. Haemophilia 1999: 5(suppl 3), 11-17. Review

Dr. Green reviews reports of anamnesis, DIC and thrombosis after bypassing agents and of thrombocytopenia and severe allergic reactions after porcine factor VIII.


In the USA, FVIII inhibitor levels were measured before and after prothrombin complex concentrate treatment of 261 bleeding episodes in 75 patients with hemophilia and inhibitor. A rise in inhibitor level to twice baseline or more was seen in 13.5% of episodes, in 27 patients. (The plasma-derived concentrate contained traces of factor VIII.)


Sixteen published instances of myocardial infarct after use of PCC or APCC are reviewed.


A 72 year old patient was given a bo-
lus of rVIIa at 102 ug/kg and then a total of 1 mg by continuous infusion, as well as tranexamic acid, prior to a dental extraction. Immediately after the extraction, he had a myocardial infarct. The patient survived so no pathologic report is available. The authors wonder whether tissue factor, exposed on atherosclerotic plaques, attracts activated factor VII and thus leads to myocardial infarct.


Typical doses of rVIIa or of FEIBA® were infused into rabbits with isolated vein segments. Both drugs were associated with minor thrombus formation after 10 minutes and definite thrombus formation at 30 minutes. At three hours, FEIBA® was associated with a significant decrease of platelet counts and fibrinogen levels whereas rVIIa was not.


For the most recent 10 year period of post-marketing surveillance of FEIBA®, 16 thrombotic adverse events were documented, an incidence of 4.05 per 105 infusions. Included were 7 instances of DIC and 5 of myocardial infarction. Known risk factors were present in 13/16 instances, including overdose in half of instances.


Safety data from licensure of NovoSeven® to April 2003 revealed 25 thromboembolic events from over 700,000 doses given. The current report covers May 2003 through 2006. A total of 30 thromboembolic events, six of them fatal, occurred from about 800,000 doses. The drug may be more dangerous when “used in off-label indications and when activated coagulation is present or when coagulation parameters are normalized during the process of restoring hemostasis.”


A total of 431 adverse events reports about recombinant VIIa were received by the FDA, of which 168 described 185 thromboembolic events. Seventeen events occurred in patients with hemophilia, 59 occurred in non-hemophilic patients enrolled in post-licensure trials, and, in the remainder, the drug had been used for off-label indications. In such instances, with many pre-existing medical conditions, arterial and venous thrombosis often resulted in serious morbidity and mortality. A close temporal association was noted between the dose of rVIIa and the thromboembolic event in many instances where it could be assessed.


Data from the FDA, supplemented by case reports, were used in conjunction with estimates of the total number of infusions to assess comparative incidence of thrombotic adverse events in patients using NovoSeven® vs those using FEIBA® in the period April 1999 through June 2002. Incidence rates were 24.6 per 100,000 infusions for NovoSeven® vs 8.24 per 100,000 infusions for FEIBA®. (This report does NOT segregate recipients with hemophilia from others who may have serious pre-existing medical conditions predisposing to thrombosis; NovoSeven® has been used more often in such “off-label” situations than has FEIBA®.)

Induction of Immune Tolerance: Bonn Protocol and Similar Programs

The first deliberate induction of tolerance at Bonn, Germany, is described. An excellent graph shows that the inhibitor rose in the first few days of intensive treatment and then fell, reaching one unit by three months.


In this excellent study, performed because of doubts about the success of immune tolerance, 18 patients were evaluated, most from the Bonn center, who had completed induction of tolerance. Twelve no longer had detectable inhibitor and six had questionable or very low inhibitors (maximum 2.4 Bethesda units) behaving as low-responders. All now could be treated with factor VIII.


In early years, the Bonn protocol consisted of two phases. In phase one, 100 FVIII U/kg and 50 FEIBA® U/kg were given twice daily until the inhibitor level fell to less than one BU and FVIII could be measured in the plasma after infusion. Thereafter, in phase two, 150 FVIII U/kg was given daily without FEIBA® until the inhibitor disappeared completely and the T ½ of infused FVIII was normal. In recent years, Bonn patients are put on the second phase dosage immediately and do not receive FEIBA®.

In 60 patients (36 high responders and 24 low responders) induction of tolerance was either achieved (# 52, 86.7%) or stopped.(# 8). In 41 patients whose treatment was continuous, the median time until the inhibitor was less than one BU was 3 months and the median time to a normal T ½ was 11.6 months. In 11 patients whose tolerance programs were interrupted, the median time until the inhibitor was less than one BU was 18.8 months and the median time to a normal T ½ was 39.9 months. They did achieve complete tolerance in five patients whose inhibitor at the outset was over 100 BU. Central lines were used in five patients and all lines became infected.

Ewing NP, Sanders NL, Dietrich SL, Kasper CK. Induction of immune tolerance to factor VIII in hemophiliacs with inhibitors. JAMA 1988; 259: 65-68

In this first Western Hemisphere series, nine of 12 patients achieved complete tolerance in less than 11 months on a dose of 50 FVIII units/kg/day. The three unsuccessful patients included one with a large gene deletion who did not respond at all, and two who had had intensive FVIII treatment ending two months before the tolerance regimen was started. Those two patients did respond, but slowly, over years.


In The Netherlands, 18 patients with hemophilia A and inhibitors under 9 BU were given 25 FVIII units/kg every other day. Treatment was stopped if the inhibitor level peaked at more than 80 BU. At the time of publication, 12 patients had achieved tolerance.


The Dutch study experience is updated. Over 26 years, 21 patients were treated with 25-50 units FVIII/Kg every other day or three times a week. Tolerance was achieved in 18 patients. A low inhibitor level at the outset and a low (under 40 BU) maximum rise during tolerance both were associated with faster time to tolerance.


This international registry included 204 patients who underwent immune tolerance. Of these, 82.3% were high responders with historic inhibitor peaks of more than 10 BU. Higher rates of success were seen in patients with lower inhibitor levels at the outset
and in patients in whom higher doses of FVIII, over 100 U/kg/day, were used.


Data on 188 courses of tolerance induction were collected from 1993-99. The success rate was 70% for hemophilia A and 31% for hemophilia B. Lower historical peak inhibitor titers, pre-induction inhibitor titers, and peak titers on induction all were predictive of success in hemophilia A. An inverse correlation between daily FVIII factor dose and success was found, surprisingly, with less success using higher doses, however, the range of American doses tends to be narrow, and lower than German ones. In hemophilia B, allergic events were common and were the major reason for tolerance failure. Three/17 subjects developed nephrotic syndrome.


The “German” dose style of 200 FVIII U/kg/day was compared prospectively with the “Dutch” style of 50 U/kg three times a week; 66 inhibitor patients completed the protocol. Success did not differ between these treatment arms but the time to achieve a negative titer and normal in vivo recovery was shorter with the high dose arm.


“A longitudinal study of ITI at our center showed a significantly decreased success rate since the introduction of high purity plasma derived and recombinant FVIII products using the Bonn protocol. In inhibitor patients who showed an unsatisfactory response to treatment with FVIII concentrates with very little or no VWF the change to concentrates containing high amounts of VWF increased success rates up to 90%. These observations raise the question of whether VWF plays an important role in the induction of immune tolerance.”


In 49 patients with inhibitors, refractory to standard immune tolerance induction, reported in 29 studies, rituximab was used to aid induction of tolerance, and was successful in 53%. No severe adverse reactions to rituximab were reported.


In 86 patients, the success of induction of tolerance was related to the underlying mutation: 17% success with large deletions (n=6), 33% with splice site mutations (n=3), 48% with inversions (n=50), 64% with nonsense mutations (n=11), 71% with small deletions (n=7), 87% with small insertions (n=87), 100% with missense mutations (n=1).


In Europe, using plasma-derived VWF-containing FVIII concentrate, primary immune tolerance induction was completely or partly successful in 87% of children and 89% of adults with hemophilia A and inhibitors. Use of that concentrate as “rescue” immune tolerance, when programs with other products had failed, resulted in 70% success in children and success in one adult who tried it. Nearly all subjects received concentrate daily. There was no clear relationship between the daily dose and the rate of success. The median time to complete success was about 18 months for primary protocols and 26 months in the rescue protocol. Favorable results with plasma-derived VWF-containing FVIII concentrate (as opposed to recombinant FVIII which contains no VWF) continue to be reported, both for primary and for rescue immune tolerance. Induction of immune tolerance is expensive, but, over a lifetime, projected to be about 40% less costly than on-demand ther-
apy with bypassing agents, and about half as costly as prophylaxis with bypassing agents.


Induction of immune tolerance over a 30-year period was analyzed. Among those with a high-responding inhibitor who started immune tolerance within one month of detection (n=23), 96% succeeded although 13/23 had an inhibitor titer of 10 or more BU at the outset. Among those with a high-responding inhibitor who started immune tolerance more than 6 months after diagnosis of an inhibitor, 64% succeeded. Thus, an inhibitor titer of 10 BU should not be an impediment to starting tolerance (as had been previously suggested.)

Malmö Protocol for Tolerance Induction


In 9 of 11 patients with hemophilia A and inhibitor, the inhibitor disappeared after 2-3 weeks of an intensive inpatient regimen of treatment. Tolerance appeared to be stable on follow-up. Once such course of treatment sufficed in seven cases and two such courses were required in two patients. Two had inadequate responses. The protocol was defined as follows:

(1) inhibitors of more than 10 BU were reduced by plasmapheresis with extracorporeal adsorption of antibodies;
(2) FVIII was given to neutralize remaining circulating inhibitor and raise the plasma FVIII level to 40-100 U/dL and maintain it on subsequent days at 30-80 U/dL;
(3) Cyclophosphamide was given just prior to the first dose of FVIII, at a dose of 12-15 mg/kg intravenously on each of two days, followed by 2-3 mg/kg orally for 8-10 days.
(4) Intravenous gamma globulin, 2.5 to 5 grams, was given immediately after the first dose of FVIII and then, starting day 4, at-doses of 0.4 grams/kg for 5 days.

After the intense course of treatment, FVIII was continued prophylactically to maintain suppression. The total amount of FVIII used was equivalent to that used in a month or less on the Bonn protocol.


Results of induction of tolerance with the Malmö protocol are updated. Tolerance was achieved in 10 of 16 patients with hemophilia A and inhibitors to FVIII and in 6 of 7 patients with hemophilia B and inhibitors to factor IX. (One of the patients with hemophilia B relapsed at six months and another course of treatment did not re-induce tolerance.) Overall, 12 patients achieved tolerance after one course of the protocol and four more achieved tolerance after two or more courses. The chance of success was best in those with low inhibitor levels at onset, histories of low inhibitor levels and a long interval since previous replacement therapy. Actual duration of treatment in successful courses ranged from 13 to 39 days. The average FVIII use over a mean of 20 days was 162,000 units. The average factor IX use over a mean of 23 days was 219,000 units. (Note that the rate of success in inducing tolerance in hemophilia B was good, in stark contrast to outpatient regimens of daily infusions.)


At 6-19 days into tolerance induction in four patients with hemophilia B and inhibitors, a new non-neutralizing IgG4 antibody appeared, which complexed with infused factor IX.


A new non-neutralizing IgG4 antibody
was found in six patients who had undergone induction of tolerance. The antibody fused with infused FVIII but did not neutralize it or reduce its half-life.


Two patients with hemophilia A and inhibitor were followed during induction of immune tolerance. Anti-idiotypic antibodies were elicited which neutralized the inhibiting capacity of anti-FVIII antibodies. The authors contend that anti-FVIII inhibitors have not disappeared after induction of tolerance, but are disarmed by anti-idiotypic antibodies.

**Acquired factor VIII inhibitors**


In this international survey, incidence was about equal in males and females. More than half of patients were over 50 years of age. Inhibitors were post-partum in 7.3%, associated with recognized auto-immune disease in 18%, and not associated with any underlying disorder in 46.1%. Others had a variety of possibly-associated conditions. Major hemorrhages occurred in 87% of patients and 22% died.


A prospective, randomized multicenter trial was conducted with 31 patients who had auto-antibodies to FVIII. All patients were treated with prednisone, 1 mg/kg for 3 weeks, and, if inhibitors still persisted, then were randomized either to continue prednisone, or to taper off it and begin cyclophosphamide 2 mg/kg, or to continue prednisone and add cyclophosphamide. Inhibitors disappeared in 10 patients during initial prednisone therapy, in 3 of 4 randomized to continue on prednisone, in 3 of 6 continued on cyclophosphamide alone, and in 5 of 10 continued on both agents.


**Post-partum inhibitors occur most common after the first pregnancy, within three months of delivery. They may disappear spontaneously, over months or years. Retrospectively, the authors compared no treatment to treatment with corticosteroids and to use of other immunosuppressive drugs with or without corticosteroids. Use of immunosuppressive drugs with or without corticosteroids resulted in remission sooner than use of corticosteroids alone. No difference was seen in the rate of remission in patients treated with corticosteroids alone versus those not given medication.**


**Nine consecutive patients, ages 50-79 years, with autoantibodies to factor VIII were given daily cyclophosphamide,100-200mg orally, and prednisone 50-60 mg, until inhibitors disappeared. Remission was achieved in all patients, with a median treatment time of 12 weeks, and a maximum time of 270 days. (Contains excellent graphs.)**


**In the major referral center in London, 24 patients with auto-antibodies to factor VIII were seen over a 28 year period. Two-thirds were female. The median age was 69 years. (This rate of acquired inhibitors, from a catchment area of millions of people, needing diagnosis or therapy in the foremost referral center of the area, is not high.)**

Delgado J, Jimenez-Yuste V, Hernandez-Navarro F, Villar A. Acquired haemophilia: Review and meta-analysis fo-

Results of 20 surveys are summarized. The median age was 64 years, and 55% of patients were female. Related conditions included pregnancy in 15%, malignancy in 18%, autoimmune disorders in 11% and unidentified or other in 58%. The over-all mortality rate was 20%; the inhibitor-related mortality rate was 11% (but only one of 32 patients with a post-partum inhibitor died.) The rate of complete remissions was 74%.


Auto-antibodies were suppressed successfully in three patients given rituximab together with other immunosuppressive agents and also in patient with mild hemophilia who had both an auto-antibody to his own FVIII and an allo-antibody to exogenous FVIII. The allo-antibody persisted longer than the auto-antibody. The authors believe that addition of rituximab, a monoclonal antibody against CD 20 T cells, to their other immunosuppressive drugs either improved a sluggish response or resulted in a quicker response than would have been expected.


Four non-hemophilic patients with peak inhibitor levels of 249 to 508 Bethesda units who had failed to respond to cyclophos-phamide, vincristine and prednisone were then treated with four weekly rituximab infusions. Each patient had an initial partial response but all but one relapsed thereafter. One patient sustained a partial response for 13 months.


Two patients with auto-antibodies treated with intravenous IgG had a rapid and prolonged but not total suppression of their inhibitors. Intravenous IgG was ineffective in two hemophilia A patients with inhibitors.


A 25% or more reduction of the levels of autoimmune factor VIII inhibitors was achieved in 6 of 16 patients given high-dose intravenous IgG.


Thirty-five patients with high-titer acquired inhibitors were treated with immunoabsorption for inhibitor elimination, FVIII substitution, intravenous immunoglobulin and immunosuppression with cyclophosphamide and prednisolone. Bleeding was rapidly controlled during one or two apheresis sessions and no subsequent bleeding episodes occurred. Inhibitor levels decreased to undetectable levels within a median of three days, and factor substitution was stopped within a median of 12 days, and treatment was completed within a median of 14 days. On long-term follow-up, if cancer patients were excluded, the complete remission rate was 97%.


The European registry collected information on 501 patients from 117 centers between 2003-2008. Patients’ median age was 73.9 years. Data on immunosuppression was available on 331 patients. Steroids plus cyclophosphamide resulted in a more stable complete remission (70%) than steroids alone (48%) or rituximab-based regimens (59%). The median time to complete remission was about five weeks for steroids with or without
cyclophosphamide but was about twice as long with rituximab. Immunoglobulin administration did not improve outcome. The likelihood of achieving stable remission was not affected by underlying etiology but was influenced by the presenting inhibitor titer and FVIII level.


The incidence over 12 years in South Australia was 1.2 cases/million population/year, with a median age of 78 years and equal sex ratio. Of the total of 25 patients, 13 required hemostatic agents. Of 18 patients treated with immunosuppressive agents, 15 achieved remission. (Note that not all patients required a hemostatic agent, the condition may be diagnosed and treated before a major hemorrhage occurs.)


In Europe between 2003 and 2008, 501 patients with acquired hemophilia from 13 countries were entered in a registry. The median age was 73.9 years. Among the non-pregnancy-related cases, 57% were male. The condition was idiopathic in 51.9%, related to malignancy in 11.8% and related to autoimmune conditions in 11.6%. Of the 501 patients, 474 had presented with bleeding. Of the 477 patients who had immunosuppressive therapy, 72.6% achieved complete remission.


In the European registry of acquired hemophilia cases, 42/501 were peri-partum. Ante-partum inhibitors were evident in eight women (and two babies had post-natal bleeding.) Mean time to diagnosis after delivery was 89 days (range 21-120). First-line immunosuppressive therapy provided complete remission in 74% of cases. All women survived.


In the European registry, data on immunosuppressive therapy in 331 patients could be analyzed. Steroids combined with cyclophosphamide resulted in more stable complete remission (70%) than steroids alone (48%) or rituximab-based regimens (59%). The median time to complete remission was about 5 weeks for steroids with or without cyclophosphamide; rituximab-based regimens took about twice as long. Immunoglobulin administration did not improve outcome.


In the European registry, not all the patients had a bleeding episode, and not all such episodes required a hemostatic agent. Among 307 patients, 174 received rVIIa, 63 received activated prothrombin complex (APCC), 56 received factor VIII and 14 received DDAVP. Bleeding was controlled in 79.6% of patients with initial therapy. Bleeding control was similarly successful with rVIIa versus APCC, and more successful with those bypassing agents (93%) than with FVIII or DDAVP (68.3%). Thrombotic events occurred in 2.9% of patients on rVIIa and 4.8% on APCC.


In a prospective study in multiple centers in Austria and Germany, data on 102 patients with acquired hemophilia who were treated with an agreed standard protocol were analyzed. The primary end point was time to achieve a partial remission, i.e. FVIII activity restored to more than 50U/dl and no active bleeding. Prednisolone alone (75-150 mg/day according to body weight) was given for three weeks and if PR was not achieved,
then cyclophosphamide (100-200 mg/day according to weight) was added in weeks 4 to 6, and in week 7, prednisolone and rituzimab (4 weekly doses of 375 mg/sq meter) were given. As soon as PR was achieved, cyclophosphamide or rituximab were stopped (if the patient was on them) and prednisolone tapered. Remission was achieved with prednisolone alone in 47% of patients, of whom some two-fifths had a partial relapse during the steroid-tapering phase, and nearly all responded to increasing the steroid dose. Of the 44 patients requiring second-line therapy, 35 received cyclophosphamide (and 21 achieved partial remission) and 9 received rituximab.

Overall, partial remission was achieved less frequently and over a longer time in patients whose baseline FVIII level had been below 1 U/dL. There was a trend towards a lower partial remission rate with higher baseline inhibitors but it was not statistically significant. Age, gender and underlying condition did not affect the probability of success.