

# DIAGNOSIS AND MANAGEMENT OF INHIBITORS TO FACTORS VIII AND IX

**An Introductory Discussion for Physicians**

**Carol K. Kasper**

University of Southern California  
Hemophilia Center, Orthopaedic Hospital  
California, U.S.A.

Published by the World Federation of Hemophilia (WFH)

© World Federation of Hemophilia, 2004

The WFH encourages redistribution of its publications for educational purposes by not-for-profit hemophilia organizations. In order to obtain permission to reprint, redistribute, or translate this publication, please contact the Communications Department at the address below.

This publication is accessible from the World Federation of Hemophilia's web site at [www.wfh.org](http://www.wfh.org). Additional copies are also available from the WFH at:

World Federation of Hemophilia  
1425 René Lévesque Boulevard West, Suite 1010  
Montréal, Québec H3G 1T7  
CANADA  
Tel. : (514) 875-7944  
Fax : (514) 875-8916  
E-mail: [wfh@wfh.org](mailto:wfh@wfh.org)  
Internet: [www.wfh.org](http://www.wfh.org)

The *Treatment of Hemophilia* series is intended to provide general information on the treatment and management of hemophilia. The World Federation of Hemophilia does not engage in the practice of medicine and under no circumstances recommends particular treatment for specific individuals. Dose schedules and other treatment regimes are continually revised and new side-effects recognized. WFH makes no representation, express or implied, that drug doses or other treatment recommendations in this publication are correct. For these reasons it is strongly recommended that individuals seek the advice of a medical adviser and/or to consult printed instructions provided by the pharmaceutical company before administering any of the drugs referred to in this monograph.

Statements and opinions expressed here do not necessarily represent the opinions, policies, or recommendations of the World Federation of Hemophilia, its Executive Committee, or its staff.

Treatment of Hemophilia Monographs  
Series Editor  
Dr. Sam Schulman

## Table of Contents

Introduction.....	1
Incidence and Prevalence .....	1
Issues Affecting Incidence in Hemophilia.....	1
Characteristics of Inhibitor Antibodies.....	2
Clinical Presentation .....	2
Laboratory Diagnosis .....	3
Methods of Management of Hemorrhages .....	5
Lowering inhibitor levels .....	5
Raising plasma factor levels.....	5
Bypassing agents .....	6
Induction of Immune Tolerance .....	9
Bonn protocol and similar regimens.....	9
Malmo Protocol (Intensive, Inpatient Regimen) .....	10
References .....	12



---

---

# Diagnosis and Management of Inhibitors to Factors VIII and IX

## An Introductory Discussion for Physicians

Carol K. Kasper

---

---

### Introduction

Inhibitors are antibodies that neutralize the activity of a clotting factor. Inhibitor antibodies to factor VIII or IX may arise as allo-antibodies in patients with hemophilia A or B who have been transfused with exogenous factor VIII or IX, respectively. Inhibitor antibodies to factor VIII may arise as auto-antibodies in non-hemophilic persons but such inhibitors will not be discussed here.

### Incidence and Prevalence

The number of new inhibitors in a population in a given period of time (incidence) reflects transient inhibitors as well as enduring ones. As the frequency of testing increases, more transient inhibitors are identified and the apparent incidence of inhibitors increases. 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 in patients with severe hemophilia A within the first few years of life has been reported in various series as 10-50% (typically, 20-30%). The prevalence is lower, 10-20%. The prevalence of inhibitors in severe hemophilia B is about 4%.

### Issues Affecting Incidence in Hemophilia

**Genetic factors** strongly influence the probability of inhibitor formation. Inhibitors arise much more commonly in severe hemophilia than in mild or moderate hemophilia. If the fetus is exposed to some of the relevant clotting factor, even if it is non-functional or incomplete, the factor is likely to be recognized as "self" and the fetus becomes tolerant.

Inhibitors are especially frequent (30-40%) in patients with mutations that prevent formation

of the clotting factor, such as deletions of large portions of the gene, nonsense mutations causing premature stop codons, and inversion of the factor VIII gene. With other, less drastic mutations, a trace amount or a section of the relevant molecule may be made. A few missense mutations causing mild hemophilia are associated with a high frequency of inhibitors. One such mutation in factor VIII prevents binding of T cells to factor VIII, thus interfering with tolerance development in fetal life.

Genetic predisposition also is suggested by the observation that strong inhibitor antibodies typically arise early in life, after treatment with the exogenous clotting factor on only a few occasions. In prospective studies of previously untreated patients (mostly babies) followed closely, inhibitors arose after a median of 8 to 9 exposure-days. A few inhibitors, predominantly weak ones, may arise later in life, notably after periods of intensive treatment with the clotting factor.

The strong tendency of inhibitors to run in families is due in part to the mutual causative mutation but in part to other, as yet ill-defined, circumstances. Some HLA phenotypes are slightly more common than others in patients with inhibitors. In the USA, the inhibitor incidence in hemophilia A patients of black African descent is twice as high as in white patients.

**The role of concentrate type** has been scrutinized. The medical community was wary of viral inactivation with heat when it was introduced as pasteurization (heating in solution) at the end of the 1970s in Germany and as dry heat (baking) in 1983 in the USA, fearing that some of the factor VIII might be slightly denatured by heat and thus more antigenic. These fears were unfounded: the early viral inactivated concentrates did not provoke excess inhibitors.

Two factor VIII concentrates made in Europe in the early 1990s, however, did provoke new inhibitor development in several patients who were thought to be at low risk of forming new inhibitors because they previously had been heavily treated with factor VIII concentrates. One of these concentrates, made in The Netherlands by the controlled-pore-silica fractionation method, had not elicited an excess number of inhibitors when viral inactivated with dry heat but did so when viral inactivated with pasteurization. The other concentrate, made in Austria, became more antigenic when pasteurization was added to a solvent detergent viral inactivation process. An additional problem in the latter concentrate may have been partial degeneration of factor VIII, possibly due to delay in separating plasma from donated blood.

When recombinant concentrates were introduced in the 1990s, previously untreated patients were studied with frequent inhibitor tests. The incidence of inhibitors was not higher than that reported for previously untreated patients on plasma-derived concentrates.

### Characteristics of Inhibitor Antibodies

Inhibitors are IgG antibodies. Anti-factor VIII antibodies react with active sites of the factor VIII molecule, primarily with epitopes in the A2, A3, C1 and C2 domains. Identification of these epitopes was critical for design of proposed less-antigenic recombinant molecules with alternate amino acid sequences at those sites.

Antibodies to factor VIII are unlikely to fix complement or to precipitate. The union of factor VIII with its inhibitor is not associated with allergic reactions. The union of factor IX with its inhibitor, however, may cause serious allergic reactions, including anaphylaxis. Such reactions can occur with the first infusion of exogenous factor IX given after the inhibitor develops; that is, before the inhibitor has been diagnosed. Factor IX inhibitor complexes can precipitate and eventually cause a nephrotic syndrome.

The reaction of factor VIII with its inhibitor is time-dependent both *in vitro* and *in vivo*, an observation 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 factor VIII may be able to interact in coagulation before being neutralized.

Two patterns of reaction kinetics are seen. Inhibitors with "Type 1" or "simple" kinetics completely neutralize factor VIII and are neutralized themselves in the reaction. Most inhibitors in patients with hemophilia are Type 1. Inhibitors with "Type 2" or "complex" kinetics do not completely neutralize factor VIII and, after reaction, retain some ability to neutralize additional factor VIII. Although some factor VIII may remain measurable in the presence of type 2 inhibitors, the patient may bleed as profusely as if he had no factor VIII at all. The reason for this confusing presentation is unknown. Type 2 reaction kinetics are more common in auto-antibodies than in allo-antibodies.

### Clinical Presentation

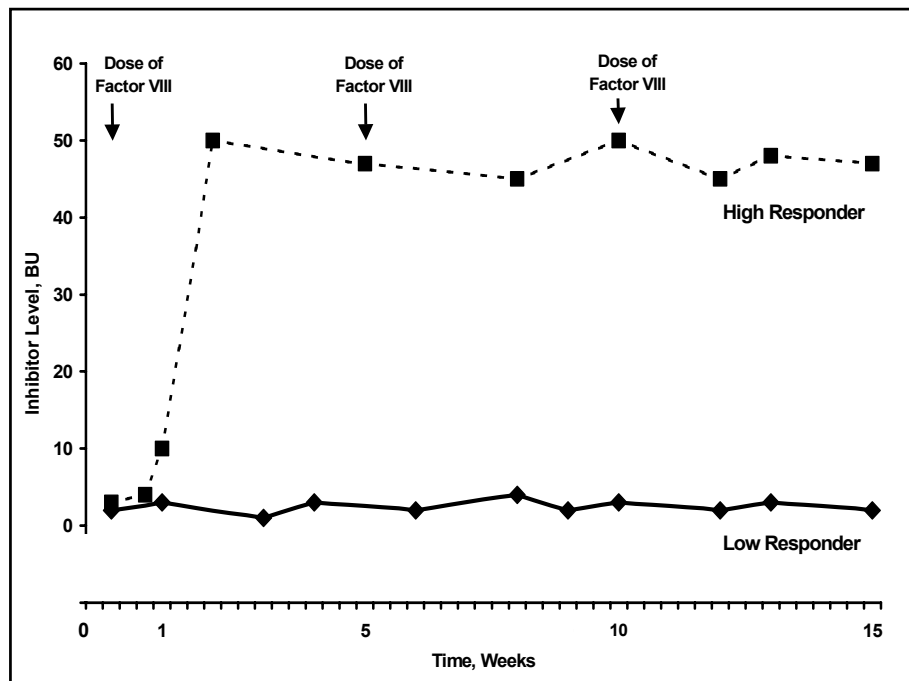
In hemophilia, an inhibitor is suspected when a patient's hemorrhage is not promptly controlled with his usual dose of clotting factor concentrate. The presence of an inhibitor does not change the typical site, frequency, or severity of bleeding. The inhibitor makes control of hemorrhages more difficult.

The immune response to exogenous factor VIII (or IX) may be weak or robust. In hemophilia, most inhibitors are "high responders"; that is, within a few days of exposure to exogenous clotting factor, the inhibitor level rises briskly, peaking within the first month. Without further exposure, the inhibitor level gradually falls in most patients and, after years, may become 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 clotting factor. Thus, such patients can continue treatment with the factor in a sufficiently high dose. Auto-antibodies usually do not rise after exposure to exogenous clotting factor.

**Figure 1**

When repeated doses of factor VIII are given to a low responder, as depicted by the solid line, anamnesis is not seen. The first dose of factor VIII, given to the high responder (dashed line) when the inhibitor level is low, does provoke anamnesis but subsequent doses, given while the inhibitor level still is high (at its own ceiling) provoke no further rise.



### Laboratory Diagnosis

The most common screening test for inhibitors is an APTT on a mixture of patient and normal plasma incubated together for one to two hours at 37° C. In the presence of an inhibitor, the APTT after incubation is prolonged compared to controls without inhibitor, as in Table 1 below.

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 relevant clotting factor. Assays of other clotting factors also may be affected by the

inhibitor and may be low if the patient plasma is not much diluted. In the following example, the levels of four clotting factors were assayed in plasma containing an inhibitor to factor VIII (see Table 2). Each clotting factor was measured in a one-stage APTT-based assay. The level of factor VIII 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 factor VIII in the reagent plasmas deficient in factor IX, factor XI, or factor XII.

**Table 1**

INCUBATION MIXTURE	APTT at outset of incubation, seconds	APTT after two hours, seconds
Normal plasma alone	32	40
Factor VIII deficient plasma alone	90	95
Normal plasma plus factor VIII deficient plasma with		
No inhibitor	37	45
1 Bethesda unit	37	53
5 Bethesda units	43	64
20 Bethesda units	54	92

**Table 2**

Patient plasma, diluted	Factor VIII	Factor IX	Factor XI	Factor XII
1:5	<1	28	20	38
1:10	<1	36	29	47
1:20	<1	42	38	54
1:50	<1	60	51	72
1:100	<1	74	63	84

If plasma from the patient above 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 factor VIII and a mild deficiency of other clotting factors. Laboratories commonly assay plasma at only one or two dilutions unless otherwise instructed.

Inhibitors are quantified by the "Bethesda" test, in which normal pooled plasma (as a source of factor VIII) is incubated with undiluted patient plasma for two hours at 37° C and then assayed for residual factor VIII. One inhibitor unit (Bethesda unit, BU) is defined as the amount that destroys half the factor VIII in that mixture, corrected for the deterioration of factor VIII in a control consisting of normal plasma incubated with buffer. The assay can be modified to

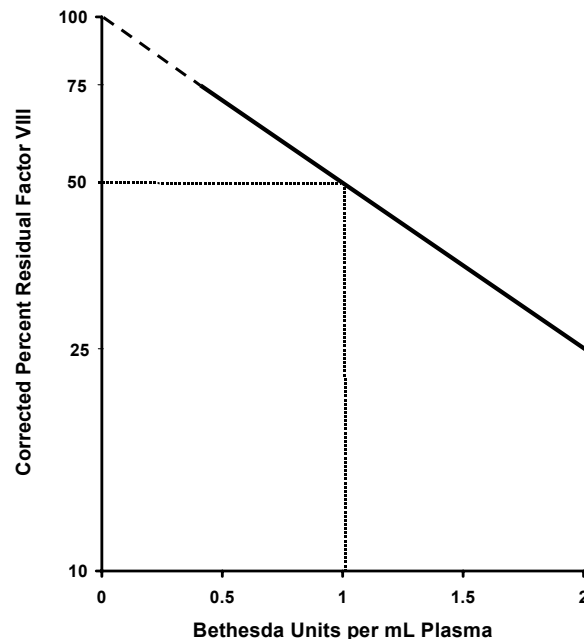
measure inhibitors to factor IX (by assaying for factor IX instead of VIII). Inhibitors with simple reaction kinetics are quantified easily by the Bethesda test but those with complex kinetics can be quantified only roughly.

In the "Nijmegen" modification of the Bethesda test (introduced in 1995, and now in widespread use), the control consists of normal plasma incubated with immuno-depleted factor VIII-deficient plasma instead of with buffer, and the normal plasma used in the incubation mixtures is buffered with imidazole to pH 7.4.

Measurement of the *in vivo* recovery and half-life of a bolus of factor VIII infused into the patient is more sensitive to traces of inhibitor than the above screening tests.

**Figure 2**

This figure illustrates the reference graph for the Bethesda test. If the residual factor VIII, after incubation, is 100% of the level in the control incubation mixture, then the inhibitor level is zero. If the residual factor VIII 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 factor VIII then is 50%, or one unit,  $1 \times 5 = 5$  Bethesda units.



## Methods of Management of Hemorrhages

### Lowering inhibitor levels

If time and circumstances permit, the level of an inhibitor may be lowered in order to make it feasible to give sufficient exogenous clotting factor to neutralize the remaining circulating inhibitor and raise the plasma factor level.

Exchange plasmapheresis can be performed efficiently with continuous flow cell-separation centrifuges which can replace a litre of plasma in a half to one hour in a typical adult. Replacement of three to four litres of plasma in typical adults temporarily reduces plasma inhibitor levels by 40% or more. If time permits, such as before an urgent but not emergency surgical operation, exchanges may be performed on two or three consecutive days, if needed. Anamnesis is likely to be provoked within a few days of exposure to normal plasma, thus, in exchanges over several days, the patient's plasma should be replaced with saline. Factor VIII (or IX) concentrate should be given promptly after the final plasmapheresis to achieve a maximal peak of the factor in the patient's plasma. Any surgery should be timed to coincide with that peak.

In well-equipped centres, 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 six or more hours, some 5 to 9 litres of patient plasma can be processed with reduction of inhibitor levels by two-thirds or more.

Intravenous infusion of normal human gamma globulin, in high doses, by itself, or after plasmapheresis, may lower inhibitor levels immediately, perhaps by the action of anti-idiotypic antibodies. Some degree of long-term immune suppression also may be seen.

### Raising plasma factor levels

#### *DDAVP (desmopressin, Stimate)*

DDAVP mediates the release of factor VIII and von Willebrand factor from cellular storage sites into the plasma. Its use may elevate plasma factor VIII levels temporarily in those inhibitor patients who have the fundamental capacity to make factor VIII, that is, non-hemophilic patients or those with mild hemophilia, in the

presence of a low-level inhibitor (e.g., two Bethesda units). In these special circumstances, DDAVP may release enough factor VIII to neutralize the circulating inhibitor and raise the plasma level of factor VIII slightly, sufficient to stop bleeding, or allow minor surgical procedures.

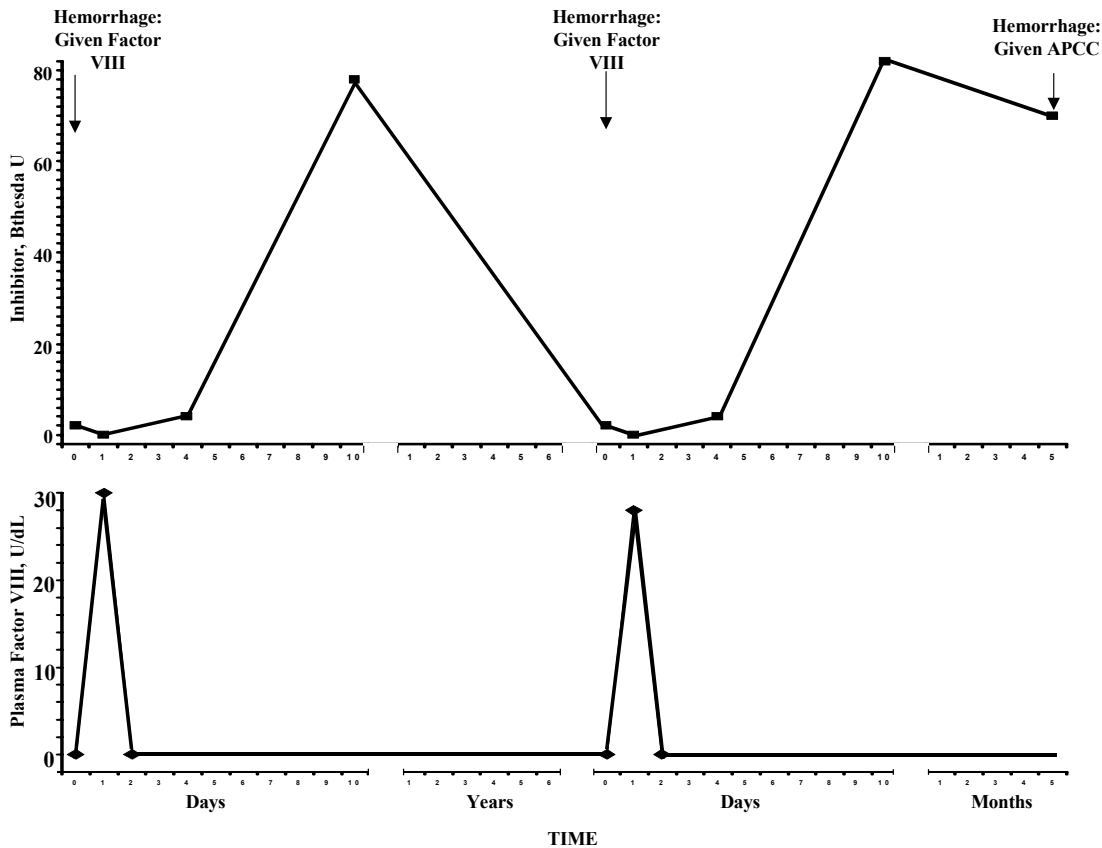
#### *Exogenous human factors*

A large bolus of human factor VIII (for hemophilia A) or purified human factor IX concentrate (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. The amount of concentrate needed is only roughly correlated with the inhibitor level. A suggested initial factor VIII 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 that day and the next few days will be lower until anamnesis supervenes.

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

Some clinicians, who have access to excellent specialized laboratories, check the ability of a patient's inhibitor to neutralize the factor VIII from various clotting factor concentrates and select that type or brand which is least neutralized.

Figure 3



The high-responding patient whose course is illustrated above received prothrombin complex concentrate for "routine" hemorrhages to avoid stimulating his inhibitor level. On two occasions, years apart, life-threatening hemorrhages were controlled with infusions of factor VIII. The third such hemorrhage occurred while his inhibitor level still was high. A bypassing agent was given but it was not sufficiently effective and he bled to death.

#### *Porcine factor VIII*

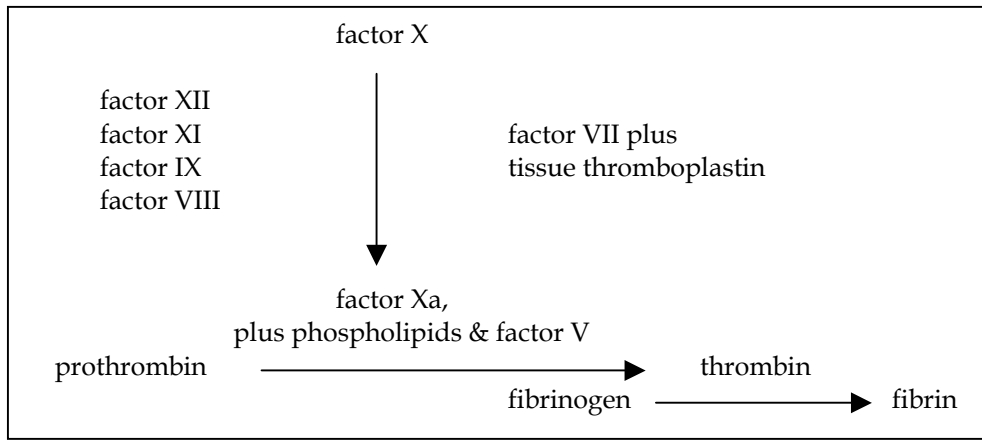
Highly purified porcine factor VIII was available for many years for treatment of bleeding in patients with inhibitors to factor VIII. Porcine factor VIII interacts well in the human coagulation sequence but attaches anti-human-factor VIII antibodies poorly. Inhibitors arising in persons with hemophilia A neutralize, on average, only 20-25% as much porcine factor VIII as human factor VIII. (The range is wide, from zero to 75%). Cross-reactivity of auto-antibody inhibitors tends to be lower than that of hemophilic allo-antibodies. Manufacture of the concentrate was discontinued in 2004 because it proved too difficult to exclude or inactivate porcine parvovirus, which is endemic in pigs. (The virus has never been shown to infect humans.) At the present time, recombinant porcine factor VIII is in clinical trials.

#### **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 they may "bypass" the need for factor VIII or IX. Prothrombin complex concentrate (PCC) contains clotting factors II (prothrombin), VII, IX and X, which travel together in plasma fractionation. A portion of each of these factors activates spontaneously during processing. Activation can be enhanced deliberately to create "activated" prothrombin complex (APCC), also known as anti-inhibitor coagulant complex (AICC). PCC and APCC have been used to treat bleeding in patients with inhibitors since the early 1970s. Nowadays, PCCs are rarely used for this purpose but APCCs are popular.

### Figure 4

In this 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.



A single factor can be separated and activated. A concentrate of plasma-derived factor Xa, with phospholipid, was developed and used successfully in animal trials in the 1980s, but human trials were not attempted. A concentrate of plasma-derived factor VIIa was developed in the early 1980s and used successfully in patients with inhibitors. A recombinant factor VIIa (rVIIa) concentrate was then prepared (“NovoSeven”). Factor VIIa needs 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 factor VIIa may be focused on sites of injury, a probable safety advantage. The potency of PCC is described in factor IX units. The potency of the two APCCs (“Autoplex” and “FEIBA”) is described in terms of factor VIII correctional or bypassing units, which are unique to each brand. The potency of rVIIa is expressed in weight in micrograms and in international units.

#### *Efficacy*

It is difficult to know when internal bleeding (for example, in a joint) stops. Diminution of pain is the first, albeit subjective, indicator. Range of motion improves more slowly. By the late 1970s doubts had arisen that PCC was effective at all, so comparison to albumin placebo was justified. The first controlled trial of PCCs for joint hemorrhages in persons with hemophilia and inhibitors confirmed that a SINGLE dose of PCC was associated with

symptomatic improvement within a few hours in about half of instances. Trials of “Autoplex” (an APCC) versus “Proplex” (a PCC) in the USA, and of “FEIBA” (an APCC) versus “Prothrombex” (a PCC) in Europe gave similar results. Uncontrolled reports of the efficacy of **repeated** doses of APCC, with evaluation performed after two or three days, showed a higher success rate. Clinical trials of NovoSeven (rVIIa) all featured **repeated** dosing at intervals of 2.5 to 3 hours. In rough summary, one or two doses or rVIIa successfully controlled about half of joint hemorrhages. A trial of NovoSeven versus an APCC is under way. Summaries of the most comparable controlled trials are appended.

Some clinicians give one dose of an APCC or of rVIIa daily or every other day as prophylaxis in hemophilic patients with inhibitors who hemorrhage frequently. Anecdotes report reasonable success. NovoSeven has been used effectively, in frequently repeated doses, for a few urgent and elective major surgical operations. APCCs also have been used effectively for a few urgent surgical operations.

An occasional side-effect of PCCs or APCCs, seen only in a few sensitive patients with hemophilia A, is an anamnestic rise in the factor VIII inhibitor. These plasma-derived products may contain residual denatured factor VIII. Other adverse side-effects of bypassing agents, seen predominantly after intensive use, may include DIC, deep vein thrombosis (DVT) and

myocardial infarction. The latter complication has been reported in very young patients. On autopsy, when performed, hemorrhagic myocardial infarction without coronary thrombosis was seen. Because of this mysterious but catastrophic complication, closely-spaced

repeated doses of PCC or APCC are avoided. Early reports suggest that rVIIa may be associated with fewer instances of DIC, DVT, and myocardial infarct than other bypassing agents. Continued vigilance is essential because adverse events often are not even recognized, much less reported.

**Table 3**

Key trials of bypassing agents for joint hemorrhages in hemophilia A are summarized below.

PCC doses are quoted in factor IX units, APCC doses in factor VIII inhibitor bypassing or correctional units, recombinant activated factor VII doses in micrograms. Range of motion (ROM) results are summarized for early trials.

Lusher et alia, 1980, observed at six hours after a single infusion	"effective" (subjective)	ROM improved 10° or more
Hemophilia A, no inhibitor, open-label Usual dose of factor VIII	<b>100%</b>	<b>65%</b>
Hemophilia A, inhibitor, double-blind		
Konyne (PCC) 75 U/kg	<b>48%</b>	<b>34%</b>
Proplex (PCC) 75 U/kg	<b>53%</b>	<b>33%</b>
Albumin placebo	<b>29%</b>	<b>18%</b>

Lusher et alia, 1983, observed at six hours after a single infusion	"effective" (subjective)	ROM improved 10° or more
Hemophilia A, inhibitor, double-blind		
Proplex (PCC) 75 U/kg	<b>50%</b>	<b>43%</b>
Autoplex (APCC) 50 U/kg	<b>56%</b>	<b>48%</b>
Autoplex (APCC) 75 U/kg	<b>52%</b>	<b>52%</b>

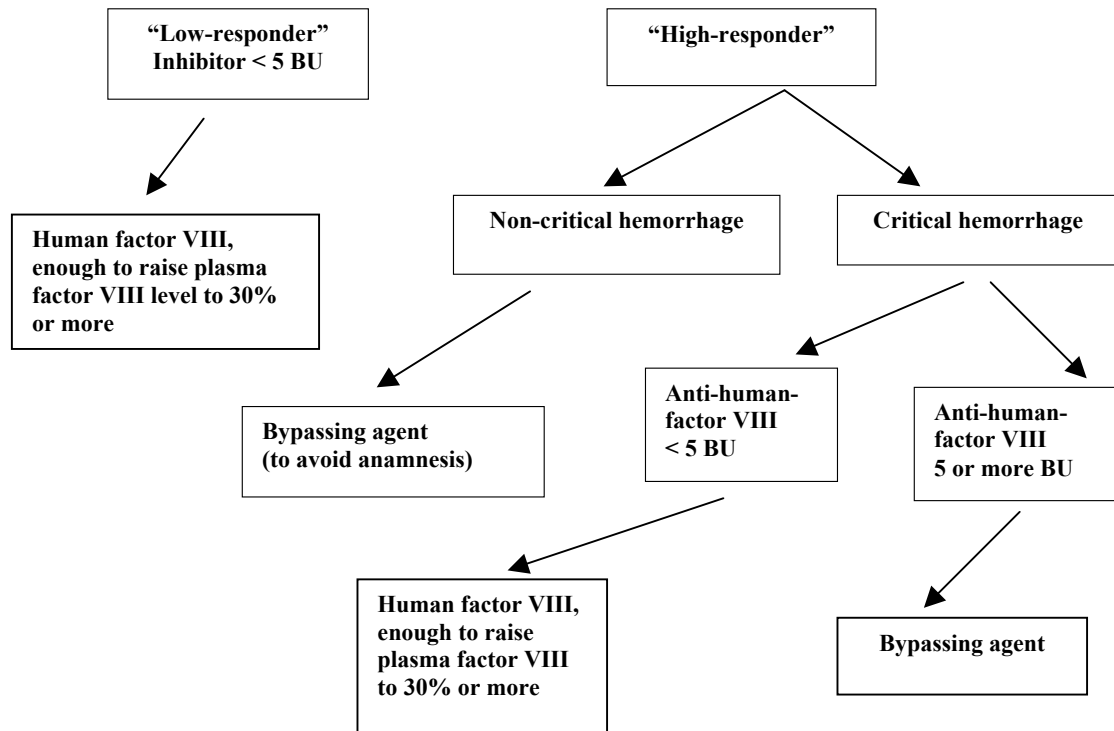
Sjamsodin et alia, 1981, observed at 24 hours after a single infusion	"effective" (subjective)	ROM improved 30° or more
Hemophilia A, inhibitor, double-blind		
Prothrombex (PCC) 48 U/kg	<b>46%</b>	<b>7%</b>
FEIBA (APCC) 88 U/kg	<b>66%</b>	<b>25%</b>

Key et alia, 1998, USA	Subjective efficacy
Hemophilia A, inhibitor, open label, home treatment	
NovoSeven, 90 ug/kg, every 3 hours until effective	<b>Median 2.2 doses needed</b>

Santagostino et alia, 1999, Italy	Subjective efficacy
Hemophilia A, inhibitor, open label, home treatment	
NovoSeven, 90 ug/kg, every 3 hours until effective	<b>Median 2 doses needed</b>

**Figure 5**

A Simple Algorithm for Management of Allo-Antibody (Hemophilic) Inhibitors



## Induction of Immune Tolerance

### Bonn protocol and similar regimens

Tolerance to the deficient clotting factor can be achieved in a large majority of patients with allo-antibodies to factor VIII by giving frequent (usually daily) doses of factor VIII over a prolonged period of time. During the first month of treatment, anamnesis usually occurs, but in the second month, inhibitor levels typically fall rapidly. Thereafter, they may fall more slowly. Complete tolerance consists of a sustained zero inhibitor level and normal T<sub>1/2</sub> of infused factor VIII. The duration of treatment until complete hemostasis is achieved varies from a few weeks to more than a year. In Germany, tolerance is then maintained with the low-level prophylaxis customary there for all patients with severe hemophilia. Without prophylaxis, inhibitors may recur at low levels. Some patients achieve and maintain partial tolerance; that is, their inhibitor does not disappear completely but remains at a low level, behaving as a low-responding inhibitor.

The ideal dosage is highly debated. Doses used range from 300 FVIII U/kg every day in Bonn,

Germany, where tolerance was pioneered, to 25 FVIII U/kg every other day in The Netherlands. In North America, doses of 50-100 U/kg daily have been used. Some centres add short courses of low-dose prednisone or another corticosteroid if the fall in the inhibitor level is sluggish.

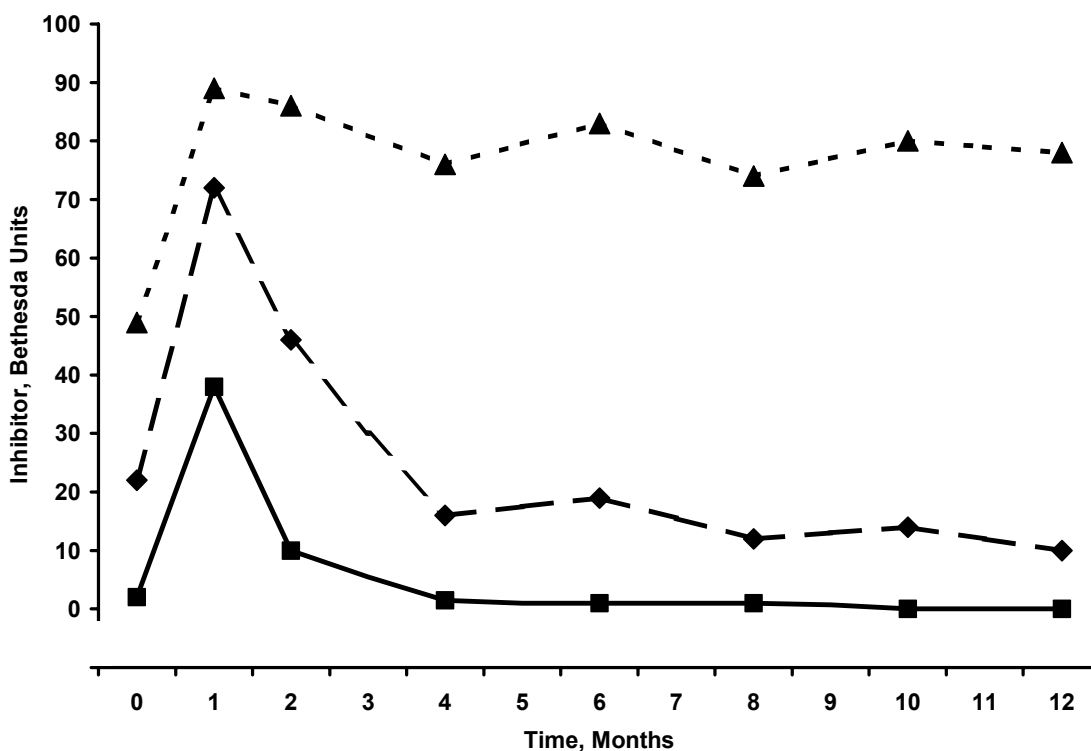
Reports from large registries of patients undergoing induction of immune tolerance agree that the lower the inhibitor level at the outset of tolerance induction, and the lower the inhibitor level in the patient's past, the more likely he is to achieve tolerance. Dosage, on the other hand, is disputed. The registry maintained by Prof. Guglielmo Mariani of Italy (see Table 4) indicates that higher doses are correlated with a greater chance of success (and also with shorter duration of needed treatment, not depicted.). The North American registry, maintained by Dr. Donna DiMichele of New York, does not show a correlation of dose with chance of success. An international clinical trial is under way, comparing the lowest-dose (Dutch) protocol with the highest-dose (German) one.

**Table 4**

Prof. Guglielmo's Registry as of 1993:			
Proportion of patients achieving tolerance on daily factor VIII dose of:			
Historic highest inhibitor level, Bethesda units:	Under 50 units / kg	50 - 200 units / kg	Over 200 units/ kg
Under 50	38.7%	77.8%	88%
50-500	25%	51.9%	83%
Over 500	Nil	6.3%	56%

**Figure 6**

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. 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.



### Malmo Protocol (Intensive, Inpatient Regimen)

In Malmo, Sweden, an intensive two- to four-week inpatient tolerance protocol is used, outlined in Figure 7 below. With this protocol, tolerance was achieved in more than half of patients with the first round of treatment, and in 70% with one or more additional rounds of treatment.

Success is achieved using the Malmo protocol with a lower total dose of factor VIII than with Bonnstyle outpatient protocols. Suppression of factor IX inhibitors in patients with hemophilia B was achieved in 86% of instances, whereas in Bonnstyle outpatient protocols of daily factor IX the rate of success has been low, about 10%, with a high frequency of nephrotic syndrome. A critical difference may be the initial removal of much of the factor IX inhibitor in the Malmo system.

After immune tolerance is induced, patients may develop new circulating antibodies that either complex protectively with infused clotting factors or act as anti-idiotypic antibodies to the inhibitors.

### Figure 7

**The Malmo (Sweden) protocol for inducing immune tolerance:**

1. If the inhibitor is over 10 BU, lower it by means of plasmapheresis and extracorporeal adsorption of antibodies;
2. Give sufficient factor VIII to neutralize remaining circulating inhibitor and to maintain plasma FVIII (or IX) levels at 30-80 U/dL;
3. Give cyclophosphamide 12-15 mg/kg intravenously for two days then 2-3 mg/kg orally for 8-10 days;
4. Give intravenous gamma globulin in a dose of 2.5-5 grams with the first dose of factor VIII and, at each of days 4-9, in a dose of 0.4 grams/kg.

## References

### Incidence and Prevalence

Briet E, Rosendaal FR, Kreuz W, Vasi V, Peerlinck K, Vermylen J, Ljung R, Rocino A, Addiego J, Lorenzo JI. High titer inhibitors in severe haemophilia A : A meta-analysis based on eight long-term follow-up studies concerning inhibitors associated with crude or intermediate purity factor VIII products. *Thromb Haemost* 1994; 71:162-3.

*Raw data from 8 studies on 451 patients with severe hemophilia A followed from birth with regular outpatient visits are combined. At age 3 years, the cumulative incidence of high-responding inhibitors was 10%; by age 18 years, it was 20%.*

Ehrenforth S, Kreuz W, Sharrer I, Linde R, Funk M, Gungor T, Krackhardt B, Kornhuber B. Incidence of development of factor VIII and factor IX inhibitors in haemophiliacs. *Lancet* 1992; 339:594-597.

*In Frankfurt, Germany, from 1976 to date of the article, 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. In a larger study of a more extensive sample in Germany, the proportion was lower.)*

Kreuz W, Ettingshausen CE, Zyschka A, Oldenburg J, Sagner IM, Ehrenforth S, Klingebiel T. Inhibitor development in previously untreated patients with hemophilia A: a prospective long-term follow-up comparing plasma-derived and recombinant products. *Semin Thromb Haemost* 2002; 285-290.

*German patients from several centres were evaluated prospectively over a 23-year period. An inhibitor developed in 22/72 (32%) of patients with hemophilia A after a median of 15 exposure-days. Amongst 46 patients with severe hemophilia A, 43% developed inhibitors. No difference was seen between those treated with plasma-derived concentrates and those treated with recombinant concentrates.*

Scharrer I, Bray GI, Neutzing O. Incidence of inhibitors in haemophilia A patients: A review of recent studies of recombinant and plasma-derived concentrates. *Haemophilia* 1999; 5:145-154.

*All issues influencing incidence, including patient characteristics and treatment history, are reviewed.*

Hay CRM, Ludlam CA, Volcin BT, Hill FHG, Preston FE, Wasseem N, Bagnall R, Peake IR, Berntorp E, Mauser Bunschoten EP, Fijnvandraat K, Kasper CK, White G, Santagostino E. Factor VIII inhibitors in mild and moderate-severity haemophilia A. *Thromb Haemost* 1998; 79:762-766.

*Development of inhibitors is reported in 26 patients with mild to moderate hemophilia A. Inhibitors usually appeared in young adults and usually after intensive therapy. Analysis of mutations causing hemophilia showed that certain missense mutations predispose to inhibitor formation*

Jacquemin M, Lavend'homme R, Vantomme V, Burny W, Chaux P, Lacroix-Desmazes S, Gilles JG, Peerlinck K, Vermylen J, van der Bruggen P, Saint-Remy JM. Mutations scattered in the C1 domain destroy T cell epitopes: A molecular mechanism responsible for the higher incidence of inhibitor in mild/moderate haemophilia A patients with mutations in the C1 domain. *Thromb Haemost* 2001; suppl , abstract # OC1003, XVIII ISTH, July 6-12, Paris.

*Missense mutations in the carboxy-terminal part of the C1 domain predispose to the development of inhibitor antibodies in patients with mild to moderate hemophilia A. At least three such mutations alter T cell epitopes and may prevent T cells from becoming tolerant toward the corresponding epitope in normal factor VIII.*

Tinlin S, Webster S, Giles AR. The development of homologous (canine/anti-canine) antibodies in dogs with haemophilia A (factor VIII deficiency): A ten-year longitudinal study. *Thromb Haemost* 1993; 69: 21-24.

*A purebred miniature Schnauzer with severe hemophilia A was bred to produce hemophilic descendants. Hemorrhages were treated with canine cryoprecipitate. The hemophilic dog 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. A genetic influence other than the hemophilia mutation is suggested.*

Gill JC. The role of genetics in inhibitor formation. *Thromb Haemost* 1999; 82:500-504.

*The author reviews familial incidence of inhibitors and association with mutation type.*

Tuddenham EGD, McVey JH. The genetic basis of inhibitor development in haemophilia A. *Haemophilia* 1998; 4:543-545.

*The incidence of inhibitors is related to mutation type. The highest frequency is with inversions of the factor VIII gene (40% have inhibitors), deletions of large parts of the gene (32% inhibitors) and in-frame stop codons (37% inhibitors) especially if that mutation is in one of six particular exons (60% inhibitors) versus other exons (8% inhibitors). RNA may skip an exon containing a stop codon or a splice site mutation, thus still producing some factor VIII, the presence of which permits tolerance development in fetal life.*

Katz J. Prevalence of factor IX inhibitors among patients with haemophilia B: results of a large-scale North American study. *Haemophilia* 1996, 2:28-31.

*At the time of a survey, inhibitors were present in 28/735 patients with severe hemophilia B, 1/644 with moderate hemophilia B and none of 588 with mild hemophilia B. The overall prevalence was 1.5%, or 3.8% in severe hemophilia B.*

Warrier I. Management of haemophilia B patients with inhibitors and anaphylaxis. *Haemophilia* 1998; 4: 574-576.

*Among over 30 such patients, inhibitors and anaphylaxis developed early (median 11 exposure-days). Total gene deletions and major gene derangements were found in the majority. Hemorrhages are best managed with recombinant activated factor VII. Attempts at induction of immune tolerance in such patients were rarely successful, and sometimes accompanied by nephrotic syndrome.*

### **Factor VIII concentrates with heightened antigenicity**

Rosendaal FR, Nieuwenhuis HK, van den Berg HM, Heijboer H, Mauser-Bunschoten EP, van der Meer J, Smit C, Strengers PFW, Briet E. A sudden increase in factor VIII inhibitor development in multitransfused haemophilia A patients in The Netherlands. *Blood* 1993; 81:2180-2186.

*The Dutch Red Cross produced factor VIII concentrate by a controlled pore silica fractionation method. In 1990, the viral inactivation process 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, that is, those believed to have low vulnerability.*

Peerlinck K, Arnout J, Gilles JG, Saint-Remy J-M, Vermeylen J. A higher than expected incidence of factor VIII inhibitors in multitransfused haemophilia A patients treated with an intermediate purity pasteurized factor VIII concentrate. *Thromb Haemost* 1993; 69:115-118.

*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. Excellent patient surveillance allowed rapid identification of the problem. Belgium then switched totally to solvent-detergent-treated (SD) concentrate.*

Peerlinck K, Arnout J, DiGiambattista M, Gilles JG, Laub R, Jacquemin M, Saint-Remy JMR, Vermeylen J. Factor VIII inhibitors in previously treated haemophilia A patients with a double virus-inactivated plasma derived factor VIII concentrate. *Thromb Haemost* 1997; 77:80-86.

*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.*

Raut S, DiGiambattista M, Bevan SA, Hubbard AR, Barrowcliffe TW, Laub R. Modification of factor VIII in therapeutic concentrates after virus inactivation by solvent-detergent and pasteurization. *Thromb Haemost* 1998; 80:624-31.

*Investigating the above inhibitor outbreak, the authors found that the combination of SD plus pasteurization induced changes in factor VIII not found after other single or double viral inactivation processes.*

Josic D, Buchacher A, Kannicht C, Lim YP, Loester K, Pock K, Robinson S, Schwinn H, Stadler M. Degradation products of factor VIII which can lead to increased immunogenicity. *Vox Sang* 1999; 77 (suppl 1):90-99.

*Investigating the above inhibitor outbreak, these authors found that plasma obtained from certain blood banks already contained factor VIII breakdown products. (The plasma may not have been separated from red cells promptly.) Concentrate produced from these sources was associated with inhibitor formation.*

### Characteristics of inhibitor antibodies

Gilles GG, Jacquemin MG, Saint Remy JMR. Factor VIII inhibitors. *Thromb Haemost* 1997; 78: 641-646.

*The authors review studies of involved epitopes and also review type 1 and 2 kinetics.*

Lacroix-Desmazes S, Misra N, Bayry J, Artaud C, Drayton B, Kaveri V, Kazatchkine MD. Pathophysiology of inhibitors to factor VIII in patients with haemophilia A. *Haemophilia* 2002; 8:273-279.

*The authors review the genesis of inhibitors and describe the involved epitopes on light and heavy chains of factor VIII and the association of inhibitors with anti-idiotypic antibodies.*

Gawryl MS, Hoyer LW. Inactivation of factor VIII coagulant activity by two different types of human antibodies. *Blood* 1982; 60:1103-1109.

*Type I antibodies completely destroy factor VIII when antibody is present in high concentration, and may react with antigenic determinants near sites for procoagulant activity. Type II antibodies do not completely inactivate factor VIII, perhaps due to steric inhibition by VWF. These antibodies react with more distant sites.*

Allain J-P, Frommel D. Antibodies to factor VIII. V. Patterns of immune response to factor VIII in hemophilia A. *Blood* 1976; 47:973-981.

*In this classic article, the authors defined "high responding" patients 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

Kasper CK, Aledort LM, Counts RB, Edson JR, Fratantoni J, Green D, Hampton JW, Hilgartner MW, Lazerson J, Levine PH, McMillan CW, Pool JG, Shapiro SS, Shulman NR, van Eys J. A more uniform measurement of factor VIII inhibitors. *Thrombos Diathes Haemorrh (Thromb Hemost)* 1975; 34:869-872.

*The Bethesda test and "Bethesda" units are described and defined.*

Lossing TS, Kasper CK, Feinstein DI. Detection of factor VIII inhibitors with the partial thromboplastin time. *Blood* 1977; 49:793-797.

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

Kasper CK. Laboratory tests for factor VIII inhibitors, their variation, significance and interpretation. *Blood Coagulation and Fibrinolysis*, 1991; 2:7-10.

*I review the effect of inhibitors of various potencies on inhibitor screening tests, and the effect of factor VIII inhibitors and of "lupus" inhibitors on clotting factor assays. Examples are given of calculation of Bethesda units for inhibitors with simple and with complex reaction kinetics.*

Verbruggen B, Novadova I, Wessels H, Boezeman J, van den Berg M, Mauser-Bunschoten E. The Nijmegen modification of the Bethesda assay for factor VIII:C inhibitors: improved specificity and reliability. *Thromb Haemost* 1995; 73: 247-251.

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

Kasper CK. In vivo recovery and early half-life of infused factor VIII in haemophilia A. *Haemophilia* 1995; 1:14-16.

*In 149 studies on 95 different adults with severe hemophilia A, who had not been transfused in the previous few days, the early-phase T<sub>1/2</sub> of infused factor VIII, as concentrate, was defined as about 3.25 hours. The early T<sub>1/2</sub> was used as a sensitive, routine pre-operative screen for inhibitor.*

### Plasmapheresis

Cobcroft R, Tamagnini G, Dormandy KM. Serial plasmapheresis in a hemophiliac with antibodies to FVIII. *J Clin Path* 1977; 30:763-765.

*The authors describe the lowering of inhibitor levels with plasmapheresis alone using older equipment.*

Freiburghaus C, Berntorp E, Ekman M, Gunnarsson M, Kjellberg BM, Nilsson IM. Immunoabsorption for removal of inhibitors: Update on treatments in Malmo-Lund between 1980 and 1995. *Haemophilia* 1998; 4:16-20.

*The authors describe the lowering of inhibitor levels 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.*

Jansen M, Schmaldienst S, Banyal S, Quehenberger P, Pabinger I, Derfler K, Hoerl WH, Knoebl P. Treatment of coagulation inhibitors with extracorporeal immunoabsorption (Ig-Therasorb). *Br J Haematol* 2001; 112:91-97.

*An excellent description is provided of the use of plasmapheresis with extracorporeal adsorption of antibodies in 13 patients with inhibitors to factor VIII 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.*

De la Fuente B, Panek S, Hoyer LW. The effect of 1-deamino 8 D-arginine vasopressin (DDAVP) in a nonhaemophilic patient with an acquired type II factor VIII inhibitor. *Brit J Haematol* 1985; 59:127-131.

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

Mudad R, Kane WH. DDAVP in acquired hemophilia A: Case report and review of the literature. *Am J Hematol.* 1993; 43: 295-299

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

### Human factor VIII

Berntorp E, Ekman M, Gunnarsson M, Nilsson IM. Variation in factor VIII inhibitory reactivity with different commercial factor VIII preparations. *Haemophilia* 1996; 2:95-99

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

### Porcine factor VIII

Kernoff PBA, Lilley TPA, Matthews KB, Goldman E, Tuddenham EGD. Clinical experience with polyelectrolyte-fractionated porcine factor VIII concentrate in the treatment of hemophiliacs with antibodies to factor VIII. *Blood* 1984; 63:31-41.

*A polyethylene-glycol fractionated porcine factor VIII concentrate was first used in London in 1980. The chance of hemostasis after infusion was related to the ability to raise the plasma factor VIII to a measurable level, and that response was related to a low anti-porcine-factor VIII inhibitor level. One patient had thrombo-cytopenia. Some patients had no immune reaction (no anamnesis) after infusions of porcine factor VIII concentrate.*

Gatti L, Mannucci PM. Use of porcine factor VIII in the management of seventeen patients with factor VIII antibodies. *Thromb Haemost* 1984; 51:379-384.

*The median cross-reactivity in these patients was 32%. Anamnesis occurred after 9/22 (41%) of treatments. Two instances of severe thrombocytopenia were seen.*

Ciavarella N, Antoncetti S, Ranieri P. Efficacy of porcine factor VIII in the management of haemophiliacs with inhibitors. *Brit J Haematol* 1984; 58: 641-648.

*Five patients received a total of 60 infusions. Excellent graphs demonstrate that, a few days after infusion, there was zero to low increase of anti-porcine factor VIII inhibitor but some increase in anti-human factor VIII inhibitor.*

Hay CRM, Lozier JN, Lee CA, Laffan M, Tradati F, Santagostino E, Ciavarella N, Schiavoni M, Fukui H, Yoshioka A, Teitel J, Mannucci PM, Kasper CK. Safety profile of porcine factor VIII and its use as hospital and home-therapy for patients with haemophilia-A and inhibitors: The results of an international survey. *Thromb Haemost* 1996; 75:25-9.

*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, "non-responders", 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-responder 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.*

Barrow RT, Healey JF, Gailani D, Scandella D, Lollar P. Reduction of the antigenicity of factor VIII toward complex inhibitory plasmas using multiply-substituted hybrid human/porcine factor VIII molecules. *Blood* 2000; 95:564-568.

*Porcine sequences were substituted for highly antigenic epitopes of human FVIII, to make potential therapeutic agents, i.e. recombinant hybrid FVIII molecules.*

### **Bypassing agents**

Lusher JM, Shapiro SS, Palascak JE, Rao AV, Levine PH, Blatt PM. Efficacy of prothrombin-complex concentrates in hemophiliacs with antibodies to factor VIII: A multicenter therapeutic trial. *New Engl J Med* 1980; 303:421-425.

*This first controlled study of bypassing agents is the "gold standard" of such studies. All patients had severe hemophilia A and were treated for acute joint hemorrhages. As a non-blind control, 20 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.*

Lusher JM, Blatt PM, Penner JA, Aledort LM, Levine PH, White GC, Warriar AI, Whitehurst DA. Autoplex versus Proplex: A controlled, double-blind study of effectiveness in acute hemarthroses in hemophiliacs with inhibitors to factor VIII. *Blood* 1983; 62: 1153-1158.

*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.*

Sjamsodin LJM, Heijnen L, Mauser-Bunschoten EP, van Geijswijk JL, van Houwelingen H, van Asten P, Sixma JJ. The effect of activated prothrombin-complex concentrate (FEIBA) on joint and muscle bleeding in patients with hemophilia A and antibodies to factor VIII: A double blind clinical trial. *New Engl J Med* 1981; 305:717-721.

*A large majority of hemorrhages were into joints. Hemorrhages were treated with single doses either of Prothrombex (a PCC, 48 factor IX units/kg) or FEIBA (88 factor eight inhibitor bypassing units/kg). Subjective evaluation at 24 hours showed Prothrombex 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 Prothrombex and in 25% of those treated with FEIBA.*

Giles AR, Mann KG, Nesheim ME. A combination of factor Xa and phosphatidylcholine-phosphatidylserine vesicles bypassed factor VIII *in vivo*. *Br J Haematol* 1988; 69:491-497.

*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 (DIC).*

Hedner U, Bjoern S, Bernvil SS, Tengborn L, Stigendahl L. Clinical experience with human plasma-derived factor VIIa in patients with hemophilia A and high titer inhibitors. *Haemostasis* 1989; 19:335-343.

*Purified factor VIIa from human plasma, activated spontaneously during the purification process, 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, a second dose clearly was needed for hemostasis. As dose levels went up, a second dose was not always clearly needed.*

Lusher JM, Roberts HR, Davignon G, Joist JH, Smith H, Shapiro A, Laurian Y, Kasper CK, Mannucci PM. A randomized, double-blind comparison of two dosage levels of recombinant factor VIIa in the treatment of joint, muscle and mucocutaneous haemorrhages in persons with haemophilia A or B, with and without inhibitors. *Haemophilia* 1998; 4:790-798.

*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 patients with hemophilia A (of whom 27/33 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 hemarthroses (20 in non-inhibitor patients), treated 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 those having effective or excellent responses was two.*

Key NS, Aledort LM, Beardsley D, Cooper HA, Davignon G, Ewenstein BM, Gilchrist GS, Gill JC, Glader B, Hoots WK, Kisker CT, Lusher JM, Rosenfield CG, Shapiro AD, Smith H, Taft E. Home treatment of mild to moderate bleeding episodes using recombinant factor VIIa (Novoseven) in haemophiliacs with inhibitors. *Thromb. Haemost.* 1998; 80:912-918.

*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 of treatment judged effective, a median of 2.2 doses had been given prior to the maintenance dose.*

Santagostino E, Gringeri A, Mannucci PM. Home treatment with recombinant activated factor VII in patients with factor VIII inhibitors: the advantages of early intervention. *Brit J Haematol* 1999; 104:22-26.

*NovoSeven, 90 ug/kg, was given every 3 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."*

Shapiro AD, Gilchrist GS, Hoots WK, Cooper HA, Gastineau DA. Prospective, randomized trial of two doses of r VIIa (NovoSeven) in haemophilia patients with inhibitors undergoing surgery. *Thromb Haemost* 1998; 80:773-778.

*NovoSeven was used in doses of 35 ug/kg or 70 ug/kg (double-blind) and given pre-operatively, intra-operatively and for 48 hours post-operatively, every 2 hours as needed. If hemostasis was inadequate, as judged by individual investigators, 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 higher (70ug/kg) 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 factor VIII or FEIBA, and the sixth because of thrombosis of the internal jugular during central venous catheter placement.*

Kenet G, Lubetsky A, Luboshitz J, Varon D, Martinowitz U. Comparison of different treatment regimens with rFVIIa: A single center experience. (Abstract P2548, presented at ISTH Paris July 6-12,2001)

*In an unblinded study, the authors compared the efficacy of three regimens of NovoSeven. In a study of 58 bleeding episodes, a bolus of 90 ug/kg was given, followed with continuous infusion of 15 ug/kg/hour. Satisfactory hemostasis was achieved in 70% of patients. In another study of 72 bleeding episodes, a bolus of 180 ug/kg was given, followed by continuous infusion of 30 ug/kg/hour. Hemostasis was satisfactory in 72% of instances. In a third study, of 84 bleeding episodes, a single dose of 300 ug/kg was given without subsequent continuous infusion. The dose was repeated in 23% of episodes. The large dose (given once or twice) was efficacious in 77% of instances. The authors prefer to use the large dose, without continuous infusion.*

Negrier C, Goudemand J, Sultan Y, Bertrand M, Rothschild C, Lauroua P. Multicenter retrospective study on the utilization of FEIBA in France in patients with factor VIII and factor IX inhibitors. *Thromb Haemost* 1997; 77:1113-1119.

*The authors describe their experience between 1978 and 1993 in France using FEIBA in 433 bleeding episodes in 60 patients. In joint hemorrhages, bleeding was controlled with one dose in 50.7%, 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.*

White GC. Seventeen years' experience with Autoplex/ Autoplex T: Evaluation of inpatients with severe hemophilia A and factor VIII inhibitors at a major haemophilia centre. *Haemophilia* 2000; 6:508-512.

*The first dose effectively controlled bleeding in 10% of 51 bleeding episodes in which number of doses was recorded. Two or three doses controlled another 29% of hemorrhages. Among all 54 bleeding episodes, effective hemostasis was achieved in 85% and partial hemostasis in another 9% within 72 hours. Anamnesis was noted in one patient and no thrombotic events were encountered.*

### Complications of bypassing agents

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

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

Kasper CK. Effect of prothrombin complex concentrate on factor VIII inhibitor levels. *Blood* 1979; 54: 1358-1368.

*In a USA national study, factor VIII inhibitor levels were measured before and after PCC treatment of 261 bleeding episodes in 75 patients with inhibitors to factor VIII. A rise in inhibitor level to twice baseline or more was seen in 13.5% of episodes, in 27 patients.*

Yoshioka A, Kamisue S, Tanaka I, Kato M, Kohmura I, Shima M, Fukui H. Anamnestic response following infusion of prothrombin complex concentrates (PCC) and activated prothrombin complex concentrates (APCC) in haemophilia A patients with inhibitors. *Blood Coagulation and Fibrinolysis* 1991; 2 (suppl 1): 51-58.

*Six of 20 patients with high-responder inhibitors to factor VIII occasionally had anamnestic inhibitor responses after treatment with PCC or APCC. Factor VIII antigen, measured in vials of one PCC and the two APCCs, was 11.7, 14.1 and 117 units/vial, respectively. Factor VIII 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.*

Chavin SI, Siegel DM, Rocco TA, Olson JP. Acute myocardial infarction during treatment with an activated prothrombin complex concentrate in a patient with factor VIII deficiency and a factor VIII inhibitor. *Amer J Med* 1988; 85: 245-249.

*The authors review the first nine such patients reported, of whom six were under age 30 years, and four under age 20. Pathology findings were available for five patients. Myocardial hemorrhages were seen, but no evidence of atherosclerosis or thrombosis. Some patients also had old infarcts. Myocardial infarction tended to be associated with use of high doses of concentrate over several days.*

Hough RE, Hampton KK, Preston FE, Channer KS, West J, Makris M. Recombinant VIIa concentrate in the management of bleeding following prothrombin complex concentrate-related myocardial infarction in patients with haemophilia and inhibitors. *Brit J Haematol* 2000; 111: 974-979.

*A review of 16 published instances of myocardial infarct after use of PCC or APCC is included.*

Peerlinck K, Vermynen J. Acute myocardial infarction following administration of recombinant activated factor VII (NovoSeven) in a patient with haemophilia A and inhibitor. *Thromb Haemost* 1999; 82:1775-6.

*A 72 year old patient was given a bolus of 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.*

Diness V, Bregengaard C, Erhardtson E, Hedner U. Recombinant human factor VIIa (rFVIIa) in a rabbit stasis model. *Thromb Res* 1992; 67:233-241.

*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. FEIBA was associated with a significant decrease of platelet counts and fibrinogen levels at 3 hours whereas rVIIa was not.*

Ehrlich HJ, Henzl MJ, Gomperts ED. Safety of factor VIII inhibitor bypassing activity (FEIBA): 10-year compilation of thrombotic adverse events. *Haemophilia* 2002; 8:83-90.

*For the most recent 10 year period of post-marketing surveillance, 16 thrombotic adverse events were documented, an incidence of 4.05 per 10<sup>5</sup> 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.*

## Induction of Immune Tolerance

### Bonn Protocol and similar outpatient programs

Brackmann HH, Gormsen J. Massive factor VIII infusion in haemophilia with factor VIII inhibitor, high responder. *Lancet* 1977; 2:933.

*The first experience with induction of tolerance at Bonn is described.*

Sultan Y, White GC, Aronstam A, Bosser C, Brackmann HH, Brochier G, Gormsen J, Mariani G, Roberts HR, Scarabin Y, Scharrer I, Scheibel E. Hemophilic patients with an inhibitor to factor VIII treated with high dose factor VIII concentrate: Results of a collaborative study for the evaluation of factor VIII inhibitor titre, recovery and half life of infused factor VIII. *Nouv Rev Fr Hematol* 1986; 28:85-59.

*In this excellent study, performed because of doubts expressed about the success of immune tolerance, the authors evaluated 18 patients, mostly from the Bonn centre, who had completed induction of immune tolerance. Twelve patients 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.*

Oldenburg J, Schwaab R, Brackmann HH. Induction of immune tolerance in haemophilia A inhibitor patients by the 'Bonn protocol': Predictive parameters for therapy duration and outcome. *Vox Sang* 1999; 77 (suppl 1): 49-54.

*In early years of immune tolerance, the Bonn protocol consisted of two phases. Initially, 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 factor VIII could be measured in the plasma after infusion. In the second phase, 150 FVIII U/kg was given daily without FEIBA until the inhibitor disappeared completely and the T<sub>1/2</sub> of infused factor VIII was normal. In recent years, Bonn patients are put on the second phase dosage immediately and do not receive FEIBA.*

*The authors review the response of 60 patients (36 high responders and 24 low responders) in whom induction of tolerance was either completed (# 52) or stopped.(# 8). Tolerance was achieved in 52 patients (86.7%). 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<sub>1/2</sub> 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<sub>1/2</sub> 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 North American series, nine of 12 patients achieved complete tolerance in less than 11 months on a dose of 50 factor VIII U/kg/day. The three unsuccessful patients included one with a large gene deletion (diagnosed at a later date) who did not respond at all, and two who had had intensive factor VIII treatment ending two months before the tolerance regimen was started. Those two patients did respond, but slowly, over years.*

Van Leeuwen EF, Mauser-Bunschoten EP, van Dijken PJ, Kok AJ, Sjamsoedin-Visser EJM, Sixma JJ. Disappearance of factor VIII:C antibodies in patients with haemophilia A upon frequent administration of factor VIII in intermediate or low dose. *Br J Haematol* 1986; 64:291-297.

*These Dutch investigators gave 25 factor VIII U/kg every other day to 18 patients with hemophilia A and inhibitors under 9 BU. They stopped induction of tolerance if the inhibitor level peaked at more than 80 BU. At the time of publication, 12 patients had achieved tolerance.*

Mauser-Bunschoten EP, Nieuwenhuis HK, Roosendaal G, van den Berg HM. Low-dose immune tolerance induction in hemophilia A patients with inhibitors. *Blood* 1995; 86:983-988.

*The authors updated their results over 13 years of low dose induction of immune tolerance, with 25 factor VIII U/kg every other day. Tolerance was achieved in 87% of 24 patients with severe hemophilia A and inhibitors. Excellent tables and graphs are presented.*

Mariani G, Scheibel E, Nogao T, Kasper CK, Ewing NP, Mauser-Bunschoten E, Ghirardini A, Bellocco R, Brackmann HH. Immunotolerance as treatment of alloantibodies to factor VIII in hemophilia. *Seminars in Hematology* 1994; 31 (suppl 4): 62-64.

*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 factor VIII, over 100 U/kg/day, were used.*

DiMichele DM, Kroner BL. Analysis of the North American immune tolerance registry (NAITR) 1993-1997: Current practice information. *Vox Sang* 1999; 77 (suppl 1): 31-32.

*Successful induction of immune tolerance correlated with lower inhibitor levels at the outset. No correlation was found in the dosage range studied, which was <50 to 199 factor VIII U/kg/day (but mostly 50-100 U/kg/day).*

### **Malmö Protocol, intensive inpatient treatment**

Nilsson IM, Berntorp E, Zettervall O. Induction of immune tolerance in patients with hemophilia and antibodies to factor VIII by combined treatment with intravenous IgG, cyclophosphamide and factor VIII. *New Eng J Med* 1988; 318:947-950.

*In 9 of 11 patients with hemophilia A and inhibitor, the inhibitor disappeared after 2-3 weeks of an intensive inpatient regimen of treatment and tolerance appeared to be stable on follow up. One 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. *sufficient factor VIII was given to neutralize remaining circulating inhibitor and raise the plasma factor VIII 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 factor VIII, 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. IV IgG, 2.5-5 grams, immediately after the first dose of factor VIII and then, starting day 4, at doses of 0.4 grams/kg for 5 days. After the intense course of treatment, factor VIII was continued prophylactically to maintain suppression. The authors point out that the total amount of factor VIII used was equivalent to that used in a month or less on the Bonn protocol.

Freiburghaus C, Berntorp E, Ekman M, Gunnarsson M, Kjellberg BM, Nilsson IM. Tolerance induction using the Malmo treatment model 1982-1995. *Haemophilia* 1999; 5:32-39.

*In excellent detail, the authors review results of induction of tolerance with the Malmo protocol. Tolerance was achieved in ten of 16 patients with hemophilia A and inhibitors to factor VIII and in six of seven 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, twelve 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 outset, 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 factor VIII 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.)*

Nilsson IM, Berntorp E, Zettervall O. Induction of split tolerance and clinical cure in high-responding hemophiliacs with factor IX antibodies. *Proc Natl Acad Sci* 1986; 83:9169-9173.

*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.*

Nilsson IM, Berntorp E, Zettervall O, Dahlback B. Noncoagulation inhibitory factor VIII antibodies after induction of tolerance to factor VIII in hemophilia A patients. *Blood* 1990; 75: 378-383.

*The authors demonstrated the presence of a new non-neutralizing IgG4 antibody in six patients with hemophilia A who had undergone induction of tolerance. The antibody fuses with infused factor VIII but does not neutralize it or reduce its T  $\frac{1}{2}$ .*

Gilles JG, Desqueper B, Lenk H, Vermynen J, Saint-Remy JM. Neutralizing anti-idiotypic antibodies to factor VIII inhibitors after desensitization in patients with hemophilia A. *J Clin Invest* 1996; 97:1382-1388.

*The authors followed two patients with severe hemophilia A and inhibitors during induction of immune tolerance. Anti-idiotypic antibodies were elicited which neutralize the inhibiting capacity of anti-factor VIII antibodies. The authors contend that anti-factor VIII inhibitors have not disappeared after induction of tolerance, but are disarmed by anti-idiotypic antibodies.*