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**Factor VIII/von Willebrand Factor Complex  
in Hemophilia A treatment  
Recent Findings, Emerging Major Role**

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**Factor VIII/von Willebrand Factor Complex  
in Hemophilia A Treatment  
Recent Findings, Emerging Major Role**

*Guest Editor*  
**Fernando Hernández**

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## Introduction

FERNANDO HERNÁNDEZ

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With the development of coagulation factor concentrates, the quality of life for hemophilia A (HA) patients has increased dramatically. Today, in addition to plasma-derived factor VIII (FVIII) products, recombinant FVIII concentrates are also available. Several factors play a role in choosing a concentrate from the many products available: viral safety, clinical efficiency, inhibitor induction and efficiency in immune tolerance induction. There has been intense debate on possible differences in safety between products derived from human plasma and products obtained by recombinant technology. Until now, there has been no evidence demonstrating a significant difference in safety between these two technologies. In terms of clinical efficiency, the products have shown similarities, although some pharmacokinetic parameters such as half life and recovery are superior in plasma-derived products.

Some of the high purity plasma-derived FVIII products also contain von Willebrand factor (VWF) in the final product. Recent findings raised the discussion on the importance of VWF in FVIII concentrates. VWF acts as a natural stabilizer of FVIII in the circulation and, as a result, can extend the half life of FVIII. In recent years there has been extensive discussion as to whether there is a difference in the incidence of inhibitor development depending on the presence of VWF in the concentrate used. The question has also been raised as to whether the type of product, and particularly the VWF content, has an influence on the success of immune tolerance (IT) induction. In the following review these emerging topics will be covered.

In the first section, Federici presents an exhaustive description of the biosynthesis, structure and function of FVIII and VWF, two distinct but related glycoproteins, that circulate in plasma forming a stable complex (FVIII/VWF). Recent biochemical studies have demonstrated that VWF is a key partner for FVIII, playing a significant role in FVIII functions, in its production and stabilization, and in its conformation and immunogenicity. FVIII/VWF concentrates are currently used in patients with VWD who are unresponsive to DDVAP. More recently, the presence of the physiologic FVIII/VWF complex is being considered to play an important role in replacement therapy of patients with HA. The use of FVIII/VWF concentrates has been proposed also in the treatment of HA patients with anti-FVIII inhibitors. There are *in vitro* studies that have demonstrated the protective role of

VWF, particularly with respect to the inhibitors directed against the C2 domain of FVIII.

Berntorp describes in greater detail the variation in FVIII inhibitor reactivity depending on the type of FVIII concentrate. Studies have been done *in vitro* and *in vivo* showing that inhibitors reacting against the FVIII light chain C2 domain may display a low inhibitor titer against VWF containing concentrates as compared to VWF-devoid FVIII products. He shows a case in which a patient's recovery with an intermediate-purity concentrate was superior to that with a monoclonal antibody-purified concentrate containing far less VWF. Subsequent *in vitro* testing of a patient's inhibitor against a panel of concentrates having varying purities and contents of VWF may support the assumption that VWF, at least partially, blocked the inhibitor in this case. He also broaches the issue of IT therapy. The presence of VWF and/or phospholipids prevents FVIII degeneration and creates a prolonged antigen presentation to the immune system and thus may be beneficial in terms of immune tolerance induction.

In the last two sections, the authors show that FVIII concentrates rich in VWF result in more consistently successful inhibitor eradication, when inducing immune tolerance in patients with hemophilia A. They describe the immune tolerance achieved after switching patients showing an unsatisfactory response to treatment with pure FVIII concentrates to a concentrate high in VWF content.

As outlined in Kreuz's section, inhibitor development is influenced by a number of variables. In order to investigate them an on-going multicentre PUP study was started in 1993, with 216 patients included. Preliminary results (update February 2002) show a slightly higher inhibitor development in severely affected hemophilia A patients treated with recombinant FVIII concentrates compared to those who received plasma-derived FVIII. To confirm the data, more PUPs must be included and followed up in this study. In the case of inhibitor development, the preferred way to reduce the high risk of bleeding episodes is to rapidly eliminate inhibitors and induce immune tolerance (ITI). Historically different protocols, with various therapeutic regimens and different types of concentrates have been adopted. A study on ITI at the Frankfurt center showed a significantly decreased success rate since the introduction of monoclonal and recombinant FVIII products. In inhibitor patients who showed an unsatisfactory

### Factor VIII/von Willebrand factor complex

treatment outcome with FVIII concentrates with very little or no VWF, the change to concentrates containing high amounts of VWF increased the success rate up to 90%. Therefore it was concluded that the type of concentrate greatly influences the ITI success rate, and raised the question of whether VWF plays an important role in ITI.

Auerswald also shows a higher success rate for immune tolerance treatment with FVIII/VWF concentrates than with high purity FVIII products. In addition, inhibitors can reappear after successful ITT with FVIII/VWF concentrates when switched

back to VWF devoid FVIII concentrates for normal prophylaxis. In conclusion, FVIII/VWF complex concentrates can be used safely in hemophilia A patients. The clinical use of these products needs to be evaluated in more detail to prove their potential advantages. In times of increasing financial problems in the health care environment the cost for replacement therapy demands more attention. Therefore FVIII/VWF concentrates should be considered to be more valuable than only as an alternative therapy option.

## The factor VIII/von Willebrand factor complex: basic and clinical issues

AUGUSTO B. FEDERICI

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Factor VIII (FVIII) and von Willebrand factor (VWF) are two distinct but related glycoproteins that circulate in plasma as a tightly bound complex (FVIII/VWF). Their deficiencies or structural defects are responsible for the most common inherited bleeding disorders, namely hemophilia A (HA) and von Willebrand's disease (VWD). The VWF has a dual role in hemostasis: first it promotes platelet adhesion to thrombogenic surfaces as well as platelet-to-platelet cohesion during thrombus formation; second, it is the carrier for FVIII in plasma. FVIII acts as a co-factor to accelerate the activation of factor X by activated factor IX in the coagulation cascade. After many years of investigations, the molecular mechanisms of FVIII/VWF interactions are now well known and recent biochemical investigations have confirmed that VWF is a key partner for FVIII, playing significant roles in FVIII function, its production and its stabilization, in its conformation and immunogenicity. FVIII and VWF are both present in most plasma-derived FVIII/VWF concentrates used in clinical practice. FVIII/VWF concentrates can be classified into three main categories according to the degree of their purification. Intermediate-high purity plasma-derived concentrates containing FVIII/VWF currently in use since 1987 carry a low risk of transmitting blood-borne infections. Concentrate safety depends on the interaction of two factors: the decrease of viral plasma load and the increase of viral inactivation. These FVIII/VWF concentrates are currently used in type 3 VWD and in type 1 or 2 VWD patients who are unresponsive to desmopressin (DDAVP). More recently the presence of the physiologic FVIII/VWF complex has been considered to play an important role also in replacement therapy for patients with HA. The correct use of FVIII/VWF concentrates in VWD and HA have been reported in several national and international guidelines.

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**Key words:** von Willebrand factor, factor VIII, FVIII/VWF complex, von Willebrand's disease, FVIII/VWF concentrates, hemophilia A.

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Factor VIII (FVIII) and von Willebrand factor (VWF) are two distinct but related glycoproteins that circulate in plasma as a tightly bound complex (FVIII/VWF). Their deficiency or structural defects are responsible for the most common inherited bleeding disorders, namely hemophilia A (HA) and von Willebrand's disease (VWD). A deficiency of FVIII resulting in HA was documented more than 1,700 years ago in the Talmud<sup>1</sup> while another hemorrhagic disorder similar to HA (pseudo-hemophilia) was reported by Eric von Willebrand in 1926.<sup>2</sup> Prior to 1971, the relationship between HA and VWD generated a great deal of confusion because the autosomally-inherited VWD is associated with some degree of FVIII deficiency, although HA is an X-linked disease. The pivotal observation indicating the distinction between FVIII and VWF was made by Zimmerman *et al.* in 1971: using a specific antiserum they demonstrated that patients with HA showed the protein which was missing in VWD.<sup>3</sup> Since then this protein has been indicated as VWF and further investigations demonstrated that VWF shows sites of synthesis and characteristics different from those of FVIII (Table 1). VWF has a dual role in hemostasis: first it promotes platelet adhesion to thrombogenic surfaces as well as platelet-to-platelet cohesion during thrombus formation; second, it is the carrier for FVIII in plasma, prolonging the half-life of this coagulation factor and making possible its function in the generation of  $\alpha$ -thrombin.<sup>4,5</sup> FVIII, once proteolytically activated by thrombin, acts as a co-factor to accelerate the activation of factor X by activated factor IX (IXa) in the coagulation cascade. Since FVIII and VWF form a tightly bound, non-covalent complex in plasma, both glycoproteins are copurified when isolated from plasma.<sup>6,7</sup> VWF represents 99% of the molecular mass of the FVIII/VWF complex since the plasma concentrations of VWF and FVIII are 5-10  $\mu$ g/mL and 50-100 ng/mL, respectively.<sup>7,8</sup> Binding of FVIII to VWF is essential for the survival of FVIII *in vivo*.<sup>7,8</sup> FVIII is protected from phospholipid-dependent proteolysis, from activated protein C and activated factor X.<sup>6</sup> A binding site for FVIII has been located at the amino terminus of VWF<sup>7</sup> and a new VWD variant (type 2N) was identified in 1981 characterized by a mutant VWF that is structurally and functionally normal, except that it does not bind to and stabilize FVIII.<sup>9</sup> This review summarizes basic concepts on the FVIII/VWF complex and describes their impact on the correct use of plasma FVIII/VWF concentrates in clinical practice.

## Factor VIII/von Willebrand factor complex

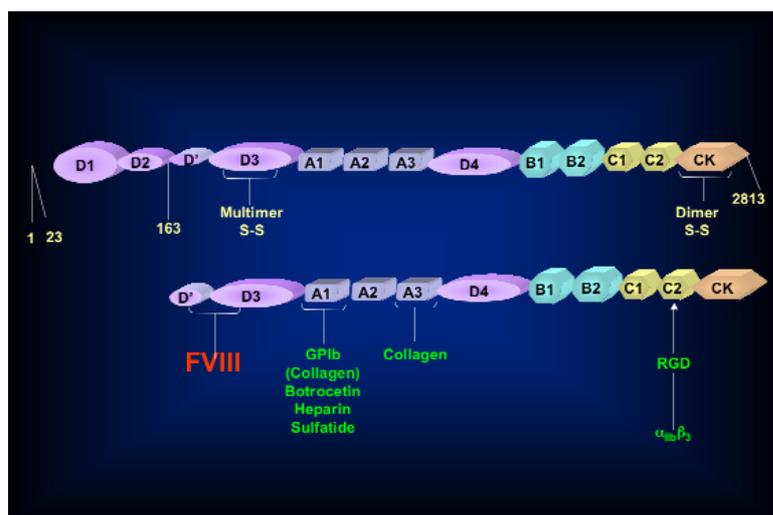
**Table 1. Characteristics of the factor VIII/von Willebrand complex.**

	<i>Factor VIII</i>	<i>Von Willebrand Factor</i>
Clinical deficiency	Hemophilia A	Von Willebrand's disease
Chromosome location	X	12
Plasma concentration	150 ng/mL (1 nml/L)	8 µg/mL (50 nmol subunits/L)
Site of synthesis	Hepatocytes and reticuloendothelial cells	Endothelial cells and megakaryocytes
Molecular mass	240 kDa	270 kDa/subunit
Subunit composition	Cu(I)-linked heterodimer	S-S bonded multimers extending up to 20×10 <sup>6</sup> daltons
Function	Co-factor for factor IXa activation of factor X	Stabilizes factor VIII in plasma Platelet adhesion to damaged vessel
Interactive sites	Residues 1670-1689 Residues 2248-2312	Residues 1-270 of mature VWF
Modification required for complex formation	Sulfation at Tyr 1680 increases affinity 5-fold	Pro-peptide cleavage Multimer formation increases affinity 6-fold
Platelet binding site	Unknown protein and/or phospholipid	Receptor GP Ib and α <sub>IIb</sub> β <sub>3</sub>

### **Biosynthesis, structure and function of VWF**

VWF is synthesized in endothelial cells and either stored in intracellular organelles known as Weibel-Palade bodies or secreted constitutively. VWF is also synthesized in megakaryocytes and stored in α-granules.<sup>4,5</sup> The gene coding for VWF is 178,000 bases long and is located on chromosome 12 and divided over 52 exons (Table 1). VWF is synthesized as a large precursor protein (360 kDa, 2813 amino acids) that consists of a 22-amino acid signal peptide, a pro-polypeptide (100 kDa, 741 amino acids) also known as VWF antigen II and a mature subunit (270 kDa, 2050 amino acids). Dimers are formed in the endoplasmic reticulum by covalent dimerization of the subunits at their C-termini. Multimers are formed in the Golgi apparatus and the secretory vesicles by covalent multimerization of these dimers at the D3-domains (Figure 1). The pro-peptide of VWF is required for normal multimer formation and is cleaved off by furin, a dibasic paired amino acid cleaving enzyme. VWF is released from endothelial cells as very large multimers and circulates in the plasma as a series of multimers of very high molecular weight (500 to 20,000 kDa). Proteolysis is involved in the generation of these multimers and shear stress enhances the susceptibility to proteolytic cleavage. About 1% of plasma VWF contains the pro-peptide, pos-

sibly due to incorrect processing. Recently, it was shown that human plasma contains a VWF degrading enzyme and that the cleavage site of this enzyme is located in the A2 domain. Each VWF subunit shows a characteristic pattern of homologous A, B, C and D domains which are independent building blocks in many other proteins. The pro-peptide contains a D1 and D2 domain. The mature subunit consists of D'-D3-A1-A2-A3-D4-B1-B2-B3-C1-C2 domains and a C-terminal part of 151 amino acids that has no internal homology. Functional domains of VWF are all included inside its subunit (Figure 1). VWF acts as an adhesive glycoprotein and mediates platelet adhesion to sub-endothelium through its binding sites for the platelet receptor GpIb-α-IX and collagen and platelet-platelet interactions through its binding site for platelet GpIIb/IIIa. Additional binding sites are those for heparin and sulphatides. Apart from its adhesive functions, VWF serves as a carrier protein for factor VIII. By the non-covalent interaction between the two proteins, factor VIII is protected against binding to membrane surfaces and to proteolytic attack by a variety of serine proteases, including activated protein C.<sup>7,8</sup> In this review, devoted to the FVIII/VWF complex, we have decided to focus only on the interactions between VWF and FVIII, without describing the adhesive properties of VWF.



**Figure 1. Domain structure and processing of VWF.** The domain structures (A–D) of pre-pro-VWF and mature VWF are shown. In the endoplasmic reticulum, carboxy-terminal disulfide bonded dimers are formed.

Disulfide bonds that result in multimerization occur late in the secretory pathway. The two platelet receptors Gp Ib and IIb/IIIa are indicated, where the latter contains the integrin tripeptide binding site (ArgGlyAsp). Other molecular interactive sites are also depicted (adapted from reference #4).

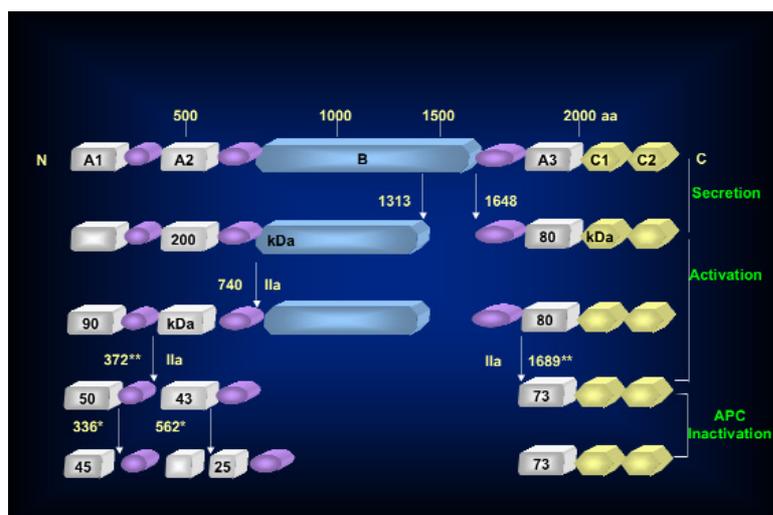
### **Biosynthesis, structure and function of FVIII**

The cellular site of synthesis of factor VIII has long been sought. The mRNA for factor VIII has been detected in isolated human hepatocytes, spleen, lymph nodes and kidney.<sup>6</sup> The factor VIII gene is located on the tip of the long arm of the X-chromosome, is 186,000 bases long and the information for factor VIII is spread among 26 exons. Both DNA sequence analysis and amino acid sequence analysis of the protein indicate that factor VIII is expressed in mammalian cells as a 2351-amino acid precursor from which a 19-amino acid *signal peptide* is cleaved during translation (Table 1). Structurally, factor VIII contains three distinct domains (A, B, C) arranged in the order A1–A2–B–A3–C1–C2 (Figure 2). The A domains are homologous to regions in clotting factor V and to ceruloplasmin, a copper binding protein in plasma, indicating that these proteins evolved from a common ancestor. The C domains exhibit homology with the sequence of the discoidin lectins from *Dictyostelium*, which are able to bind negatively charged phospholipids. The B domain is dispensable for procoagulant activity and VWF-binding. It is not homologous to any other known protein, and contains 18 of the 25 potential asparagine (N)-linked glycosylation sites within factor VIII. Interestingly, B-domainless factor VIII is processed and secreted more efficiently in medium conditioned by the transfected cells. Factor VIII contains two acidic regions, the first is located between the A1 and A2 domains (amino acids 336–372) and is important for procoagulant activity. The second is located

between the B and A3 domains (amino acids 1648–1689) and is required for the association of factor VIII with VWF. Factor VIII contains six sites of tyrosine sulphation, a process which is known to affect the biological activity, binding affinities, and secretion of proteins. The presence of post-translationally sulphated tyrosine 1680 is required for high affinity interaction with VWF. Although synthesized as a single chain, factor VIII purified from blood consists of a collection of heterodimers. This is due to proteolysis at residue 1648 which results in the formation of a light chain with a molecular mass of 80 kDa. Further proteolysis between the A2 and B domains at residue 740, or at different sites within the B domain, results in the formation of variably sized heavy chains, with molecular masses ranging from 90 to 180 kDa. Factor VIII binds to phospholipids via its light chain, and more specifically with regions within residues 2303 to 2332 in the C2 domain.<sup>6–8</sup>

### **Proteolytic degradation of VWF**

Proteolytic degradation of plasma VWF is a normal event. The largest VWF multimers have enhanced thrombogenic potential due to the presence of a greater number of constituent subunits and of multiple interaction sites for both platelets and structure of the vessel wall. When *unusually large* VWF multimers appear in plasma, such as after acute release from endothelial cells following infusion of desmopressin, their presence is transient and they are rapidly cleaved.<sup>5</sup> This finding supports the existence of a physiologic regulatory



**Figure 2. Domain structure and processing of factor VIII (FVIII).** The structural domains of FVIII are depicted: A1 domain (1-336), A2 domain (372-740), B domain (740-1648) and C domain (2020-2332). Three regions rich in amino acid residues lie between domains A1 and A2, A2 and B, and B and A3. The thrombin cleavage sites are shown. The two cleavages required for thrombin activation (\*\*) and the two cleavages for inactivation by activated protein C (\*) are indicated (adapted from reference #8).

process that normally limits the accumulation of the largest multimers in the circulation. Such mechanisms may become efficient only after birth, as shown by the presence of *unusually large* multimers in fetal and neonatal blood. In the last years, two independent studies have subsequently provided strong evidence that normal plasma contains a metallo-proteinase acting specifically on VWF multimers, cleaving the subunit at the bond Tyr-842 and Met 843.<sup>10</sup> The estimated molecular mass of this enzyme varied from 200 to 300 kilodaltons. The relevance of this protease is illustrated by several reports showing that plasma of patients with chronic relapsing thrombocytopenic purpura – a disease caused by diffuse thrombotic occlusion of small vessels associated with the presence of unusually large multimers in plasma – contains decreased VWF cleaving activity.<sup>10</sup>

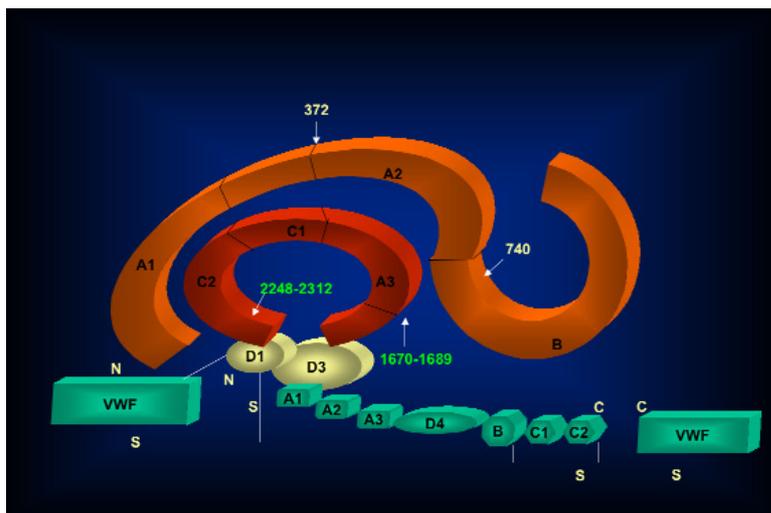
#### **Proteolytic activation and inactivation of factor VIII**

Proteolytic activation of factor VIII is necessary for the generation of the intrinsic pathway factor X activation complex<sup>6-8</sup> The activation of factor VIII by thrombin is associated with cleavages at Arg 372 in the heavy chain which forms the A1 and A2 subunits, at Arg 740 in the heavy chain which releases the B domain, and at Arg 1689 in the light chain which produces the A3-C1-C2 subunit (Figure 2). The A2 domain is required for procoagulant activity but not for thrombin cleavage. Light chain cleavage at Arg 1689 releases factor VIII from VWF. VWF itself may be a co-factor in this cleavage. The resulting factor VIIIa is a 160-kDa heterotrimer consisting of two heavy-chain-derived fragments

(containing amino acids 1-372 and 373-740), and a light-chain-derived fragment (containing amino acids 1689-2332). FVIIIa can be inactivated by activated protein C, thrombin, and factor Xa. The binding site for activated protein C on factor VIII has been localized on the light chain, between amino acid residues 2009 and 2018. Activated protein C cleaves the heavy chain of factor VIII at Arg 336, at Arg 562 bisecting the A2 domain, and at Arg 740 at the A2-B junction. Modification of the arginine to either an isoleucine or a lysine at residue 336, resulted in a factor VIII molecule with increased procoagulant activity, possibly due to resistance to inactivation by activated protein C.<sup>6-8</sup>

#### **Factor VIII/VWF interactions**

After many years of investigations, the molecular mechanisms of FVIII/VWF interactions are now known (Figure 3). On the VWF, a major FVIII-binding site resides within the amino-terminal part of VWF, from residues 1-272.<sup>5,7</sup> On the FVIII, a major VWF-binding site is localized on the amino-terminal region of the FVIII light chain corresponding to the A3, C1 and C2 domains: several studies with monoclonal antibodies that inhibit the binding have further localized this domain to residues 1670-1689.<sup>6-8</sup> Recently antibody inhibition data indicated that epitopes included within amino acids 2248-2312 within the C2-domain at the carboxy-terminus of the light chain also play a role in the VWF interaction: a monoclonal anti-FVIII antibody recognizing the C2 domain epitopes 2248-2312 inhibited FVIII binding both to VWF and phosphatidyl-serine.<sup>11</sup>



**Figure 3. Model of the FVIII/VWF interactions.** The binding of FVIII to VWF is mediated by two binding sites on the FVIII light chain. The model is based on the available data that indicate that the acid region on A3 and the carboxy-terminal region on C2 are in close proximity and form one high affinity binding site for the amino-terminal part of VWF, containing domains D1 and D2 (adapted from reference #7).

### The role of VWF in FVIII plasma levels and activity

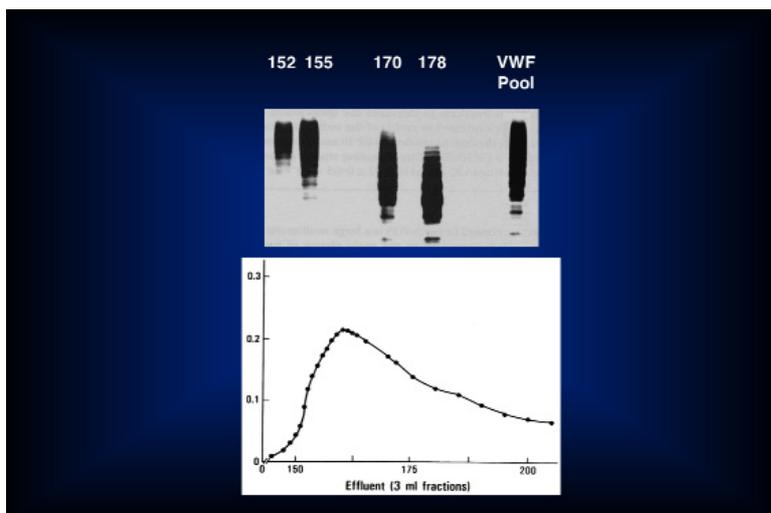
Recent biochemical investigations have demonstrated that VWF is a key partner for FVIII<sup>8,11</sup>: in fact VWF plays a key role in FVIII function, in FVIII production and stabilization, in FVIII conformation and in FVIII immunogenicity (Table 2). First, VWF can protect FVIII from useless degradation while keeping it in a high degree of reactivity by (a) increasing FVIII susceptibility to thrombin cleavage, (b) by decreasing FVIII susceptibility to activated protein C, and (c) factor Xa inactivation and by preventing phospholipid binding. Second, VWF is not only essential for the production of FVIII, as shown by data from *in vitro* FVIII biosynthesis,<sup>8</sup> but also for the stability of FVIII in plasma. VWF could also inhibit the clearance of FVIII by competing with lipoprotein-related receptors. There are several clinical observations confirming the key role of VWF in stability of FVIII in plasma. Type 3 VWD with absent VWF in circulation is associated with markedly reduced plasma FVIII levels (1–10%): after infusion of FVIII concentrates in patients with this condition, the half life of FVIII is dependent on the presence of VWF in the concentrate, and monoclonally purified or recombinant FVIII concentrates, containing no or only minimal amounts of VWF, are ineffective in patients with severe type 3 VWD.<sup>12</sup> In type 2N NormandyVWD, showing an impaired VWF binding site for FVIII, the plasma levels of FVIII are always lower than those of VWF.<sup>9</sup> Moreover, it has been shown that the half-life of FVIII in HA patients treated with FVIII concentrates is related to pre-infusion levels of their VWF, i.e. higher VWF levels are associated with longer half-lives.<sup>13</sup> Since VWF

is lower in patients with blood group O, some authors showed that the half-life of infused FVIII is shorter in HA patients with blood group O than in those with blood group A.<sup>14</sup> When increasing pre-infusion VWF levels by intranasal desmopressin, other authors could prolong the half-life of FVIII in HA patients treated with recombinant FVIII.<sup>15</sup> Third, VWF can modify FVIII conformation by altering the 3-dimensional structure of FVIII: therefore, some antibodies recognize FVIII only in the presence of VWF. Fourth, VWF modulates the immunogenicity of FVIII by interfering with both B- and T-cell epitopes: in fact, antibodies compete with VWF for binding to the A3 and C2 domains of FVIII (B-cell epitopes) and a major T-cell-epitope is located

**Table 2. The role of VWF in FVIII plasma levels and activity.**

VWF plays a key role in:

- FVIII function
  - by increasing FVIII susceptibility to thrombin cleavage;
  - by decreasing FVIII susceptibility to activated protein C and to activated Factor X;
  - by preventing phospholipid binding
- FVIII production and stabilization
- FVIII conformation
- FVIII immunogenicity



**Figure 4.** The chromatographic profile of purified VWF obtained from cryoprecipitate is described. *Lower panel.* The optical density (OD, 280 nm) of the effluent divided into 3 mL fractions collected from a 4B-CL chromatographic column is described: higher OD values correspond to higher VWF protein concentrations (mg/ml). *Upper panel.* The multimeric profile of the VWF purified fractions is reported. Fractions 152 and 155 obtained in the left part of the curve are characterized by relatively higher molecular weight multimers. Fractions 170 and 178 show loss of high molecular weight multimers with a significant increase of intermediate-low molecular weight multimers. The pool of fractions 152-178 contains all set of multimers.

within the C1/C2 domains of FVIII.<sup>11</sup> Several reports have demonstrated that human antibodies directed to FVIII C1 and C2 domains are less inhibitory to FVIII complexed to VWF<sup>16-18</sup>

#### **FVIII/VWF concentrates**

Coagulation factor concentrates can be classified into three main categories according to the degree of their purification and are also referred as part of the following three different generations: first generation concentrates (fresh-frozen plasma and cryoprecipitate); second generation (low-intermediate purity concentrates); third generation (high purity concentrates). Intermediate-high purity plasma-derived concentrates containing FVIII/VWF in use since 1987 carry a low risk of transmitting blood-borne infectious agents. Concentrate safety depends on the interaction of two factors: the decrease of viral plasma load and the increase of viral inactivation.<sup>19</sup> Major efforts are being made to select low-risk plasma donors screened by the most sensitive serologic assays. Another step further is the adoption of polymerase chain reaction (PCR)-based virus detection methods, which became obligatory in Europe in 1999. However, it is mainly the adoption of virucidal methods during or at the end of the production process that has dramatically improved the safety of concentrates. Virucidal methods include the following: terminal heating of concentrates in the lyophilized state at high temperatures ( $\geq 80^{\circ}\text{C}$ ; known as *dry-heating*) or at  $60^{\circ}\text{C}$  in solution (pasteurization); exposure to hot vapor under high pressure; addition of a mixture composed of an organic solvent and a detergent; and nano-filtration of the final product through small pores (35 nm) that retain mechanically infectious agents but not coagulation factors.<sup>19</sup>

Once obtained all the purified FVIII/VWF concentrates should be validated by studying the FVIII/VWF activities in pharmacokinetic (PK) studies in patients who lack these proteins. All FVIII/VWF activities in FVIII/VWF concentrates strictly depend on three major factors: the starting materials (plasma or cryoprecipitate), the method of purification, and the methods used to reduce the risk of blood-borne infections. FVIII/VWF can be purified by cryoprecipitate also in low scale productions, for research purpose only. After several steps, the modified cryoprecipitate is applied to a chromatographic column which can separate VWF fractions according to molecular weight (Figure 4). These VWF fractions can be characterized separately by testing all the FVIII/VWF activities, such as VWF antigen (VWF:Ag), VWF ristocetin cofactor (VWF:RCo), VWF collagen binding activity (VWF:CB) and factor VIII procoagulant (FVIII:C). The different FVIII/VWF activities can also be correlated with each other by measuring their ratios with the total amount of VWF protein tested as VWF:Ag, i.e. VWF:RCo/Ag, VWF:CB/Ag and FVIII/VWF:Ag. The results of the different VWF fractions, as well as of VWF pool obtained by mixing all these fractions, are reported in Table 3. The VWF fractions characterized by relatively higher molecular weight forms show VWF:RCo and VWF:CB ratios  $> 1.0$  while those with relatively lower molecular weight multimers always show ratios  $< 1.0$ : VWF:CB seems to be more sensitive than VWF:RCo to the presence of the higher molecular weight multimers. On the other hand, the FVIII/VWF:Ag ratio does not change significantly according to the different VWF fractions, suggesting that the FVIII/VWF complex is equally formed also in relatively lower molecular weight multimers (Table 3): the activities of differ-

**Table 3. Mean ratios of different FVIII/VWF activities of VWF purified fractions from cryoprecipitate 0 (range).**

VWF fraction	No. of experiments	VWF:RCo/Ag*	VWF:CB/Ag*	FVIII/VWF:Ag*
Fraction 152	5	1.24 (1.12-1.32)	1.27 (1.20-1.36)	0.18 (0.15-0.20)
Fraction 155	4	1.12 (1.08-1.19)	0.80 (0.69-1.12)	0.19 (0.16-0.20)
Fraction 170	6	0.93 (0.79-1.03)	0.51 (0.43-0.61)	0.18 (0.17-0.19)
Fraction 178	4	0.81 (0.71-0.90)	0.42 (0.37-0.56)	0.17 (0.14-0.18)
Pool of fractions 152-178	3	1.15 (0.80-1.19)	0.79 (0.60-0.85)	0.18 (0.16-0.19)
Normal plasma	100	1.09 (0.78-1.21)	0.82 (0.67-0.91)	n.c.
Cryoprecipitate	5	1.18 (0.90-1.25)	0.96 (0.81-1.16)	n.c.

\*VWF:RCo was tested by aggregometric in-house-made formalin-fixed platelets; VWF:CB was tested by ELISA using a mix of collagen type I (95%) and III (5%); VWF:Ag was tested by ELISA using in-house-made monoclonal antibodies; FVIII activity was tested by procoagulant assay.

ent VWF fractions purified from cryoprecipitate were investigated in detail using biochemical methods to test their binding to platelet receptors, namely glycoprotein Ib and IIb/IIIa complex in the presence of ristocetin or thrombin.<sup>20</sup> The data obtained showed that the affinity for the two receptors in relatively low molecular weight multimers is 5-8 times lower than that observed in higher molecular weight fractions: these data suggest that the same platelet interaction can be obtained by increasing VWF concentration 5-8 fold. The practical information to be derived from these *in vitro* studies is that relatively higher concentrations of FVIII/VWF concentrates should be used in patients when the concentrates show an *in vitro* significant loss of high molecular weight multimers. More recently, a purified VWF contained in a high purity FVIII concentrate has been compared with VWF purified by cryoprecipitate in tests

designed to measure the binding of VWF to platelet glycoproteins and VWF-dependent platelet adhesion to subendothelium under flow conditions: the results of these *in vitro* studies showed that platelet binding affinities and platelet adhesion to subendothelium were similar in both VWF preparations.<sup>21</sup> All these *in vitro* observations should be taken into consideration when a FVIII/VWF concentrate is prepared to be used in patients lacking FVIII/VWF activities. The characteristics of the plasma-derived concentrates containing FVIII/VWF which are commercially available in Italy, as derived from the Guidelines approved by the *Italian Association of Hemophilia Centers*,<sup>22</sup> are listed in Table 4.

#### **FVIII/VWF concentrates in VWD**

FVIII/VWF concentrates are the treatment of choice in VWD patients who are unresponsive to DDAVP.<sup>23,24</sup> FVIII/VWF concentrates have been pro-

**Table 4. FVIII/VWF concentrates commercially available in Italy (as from the Guidelines for the diagnosis and treatment of von Willebrand's disease approved by the Italian Association of Hemophilia Centers).**

Products (Manufacturers)	Purification	Viral inactivation	Specific activity* (U/mg prot.)	VWF:RCo/Ag° (Ratio)	VWF:RCo/FVIII° (Ratio)	Other proteins
Emoclot D.I. (Kedron)	Ion exchange chromatography	Solv./Det.+ 30 min. a 100°C	≥ 80	0.61	1.16	Albumin -
Fanhdi (Grifols)	Affinity chromatography (Heparin)	Solv./Det. + 72 hrs at 80°C	>100	0.83	1.48	Albumin +
Haemate P (Aventis Behring)	Multiple precipitation	Pasteurization 10 hrs at 60°C	40±6	0.96	2.54	Albumin +
Immunate (Baxter)	Ion exchange Chromatography	Det. + vapor heat 10 h at 60° 1h at 80°	100±50	0.47	1.10	Albumin +

\*Specific activity measured as FVIII before adding albumin as stabilizer. °VWF:RCo values are not available in the technical description of all concentrates: therefore only the mean values calculated by Producers on different concentrate stocks could be reported.

duced and tested by many authors but there is only one report describing four virus-inactivated FVIII/VWF concentrates evaluated in a cross-over randomized trial, after *in vitro* evaluation of the FVIII/VWF activities of these concentrates.<sup>25</sup> In that study, not one of the four FVIII/VWF concentrates tested showed a VWF protein with an intact multimeric structure similar to that of normal plasma or of cryoprecipitate, as detected by the correct analytical system to search for VWF high molecular weight multimers (i.e. low resolution agarose gels). The reasons for the loss of the large multimers in virus-inactivated FVIII/VWF concentrates has been further explored by Mannucci *et al.* who demonstrated that proteolysis was related to enzymes contained in leukocyte lysates contaminating plasma preparations.<sup>26</sup> Despite the abnormal multimeric structure of the VWF contained in these commercial products, all four FVIII/VWF concentrates were equally effective in increasing FVIII/VWF activities in type 3 VWD cases, as proven by pharmacokinetic studies. All were effective in obtaining normal and sustained levels of FVIII post-infusion. The VWF antigen (VWF:Ag) and VWF ristocetin cofactor (VWF:RCo) activity were also normalized in all FVIII/VWF concentrates. However no FVIII/VWF concentrate normalized the bleeding time (BT) in a sustained fashion, indicating once more that BT is not coincident with VWF:RCo. These experimental observations have been confirmed by clinical studies. A large multicenter clinical trial was started in 1993 with a high-purity plasma-derived FVIII/VWF concentrate treated by two methods of viral inactivation (solvent-detergent and heat treatment) and results of the pharmacokinetic and efficacy studies have been recently published.<sup>27</sup> This FVIII/VWF concentrate has been used effectively to treat more than 50 bleeding episodes and in surgical prophylaxis for 27 different operations. From these data and from the current clinical experience of physicians working in Hemophilia Centers, it is well known that clinical hemostasis following FVIII/VWF concentrates can be achieved in all types of VWD regardless of whether the BT is corrected. This is particularly true in the case of VWD patients during surgery. Some precautions should be taken when FVIII/VWF concentrates are used for several days to prevent bleeding after surgery because of the delayed response of FVIII:C, as originally observed following cryoprecipitate: in these cases, the daily dosage should be decided according to plasma FVIII levels. Mucosal bleeding can occur very frequently in VWD and may last for a long time. In the case of gastrointestinal bleeding, VWD patients can stay in the hospital for a very long time and require sometimes months of treatment. We have had several patients with this situation and most of them were managed with daily or every other day

infusions of FVIII/VWF concentrates. When bleeding persists despite replacement therapy, other additional options are available. DDAVP, given after cryoprecipitate, further shortened or normalized the BT in patients with type 3 VWD in whom cryoprecipitate failed to correct the BT.<sup>28</sup> Platelet concentrates (given before or after cryoprecipitate, at doses of  $4-5 \times 10^{11}$  platelets) achieved similar effects, both in terms of BT correction and bleeding control, in patients unresponsive to cryoprecipitate alone.<sup>29</sup> These data emphasize the important role of platelet VWF in establishing and maintaining primary hemostasis.

#### **FVIII/VWF concentrates in HA**

*Guidelines for Hemophilia Management* recommend that all newly diagnosed and previously untreated patients (PUP) with HA should be treated with recombinant FVIII products.<sup>30</sup> However, these recombinant FVIII products may not be sufficient to cover all the requests and a selection should be made. Moreover, recombinant products are still very expensive and developing countries cannot afford their high costs. So, plasma-derived FVIII concentrates are still widely used in HA patient care. Since HA patients, by definition, are missing only FVIII and show usually normal VWF levels, in the past guidelines have suggested using *high purity* plasma-derived FVIII concentrate devoid of or with only trace amounts of VWF. More recently the presence of the physiologic FVIII/VWF complex has been considered to play an important role in replacement therapy of patients with HA. There are several experimental observations and preliminary clinical data suggesting the use of FVIII/VWF concentrates in HA, as described above.<sup>13-15</sup> Pre-infusion levels of VWF:Ag are strongly associated with the half-life of transfused recombinant factor VIII concentrate in severe HA.<sup>13</sup> These data have encouraged the use of plasma-derived FVIII/VWF concentrates in situations where endogenous VWF may be limiting, such as during the course of massive FVIII infusion for major surgery and in severe liver cirrhosis. In severe cirrhosis endogenous VWF can be less effective because of enhanced VWF degradation due to actions of proteolytic enzymes not neutralized by the impaired liver function.<sup>31</sup> Moreover, in HA patients, there is no evidence that the use of highly purified concentrates can prolong life or the period between seroconversion and the emergence of symptomatic AIDS: since some patients with advanced HIV infection also have serious liver dysfunction and may respond less to concentrates without VWF, FVIII/VWF concentrates can be used in these patients. The use of FVIII/VWF concentrates has recently been proposed also in the treatment of HA patients with anti-FVIII inhibitors. The rationale for the use of plasma-derived FVIII con-

concentrates containing VWF resides in several experimental observations, as reported above.<sup>16-18</sup> Therefore, the use of therapeutic FVIII concentrate containing FVIII/VWF complex might be considered instead of preparations of FVIII containing little or no VWF in those patients who have FVIII inhibitors with C2 domain epitope specificity.<sup>16</sup> Some authors suggested testing inhibitor patients against a panel of concentrates when treatment is considered because they demonstrated that concentrates manifesting a low level of reactivity with the inhibitor have a better hemostatic effect *in vivo*.<sup>32</sup> These data have recently been confirmed and expanded by Gensana *et al*.<sup>33</sup> The presence of VWF may be a mechanism for prolonged antigen presentation to the immune system and thus may have a positive impact on the outcome of immune tolerance therapy.

Several reports have been published about the epidemiology of inhibitors in HA patients treated with plasma-derived FVIII concentrates<sup>34-40</sup> and some authors reported zero incidence of inhibitors in previously treated HA patients after exposure to single plasma-derived intermediate or high purity FVIII concentrates containing VWF.<sup>38-40</sup> Large-scale trials have been designed to confirm these preliminary observations because these data should be reproduced in a large cohort of previously untreated patients.

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## Variation in factor VIII inhibitor reactivity with different commercial factor VIII preparations: is it of clinical importance?

ERIK BERTORP

Factor VIII inhibitors may interfere with several important functional binding sites on the factor VIII molecule including that of von Willebrand factor, which binds to the C2 domain. There are *in vitro* and *in vivo* observations that inhibitors reacting against the factor VIII light-chain C2 domain may display a lower inhibitor titer against von Willebrand factor containing concentrates as compared to pure products. In low titer inhibitor patients it can be anticipated that von Willebrand factor containing concentrates give a better hemostatic effect. The role in immune tolerance induction is of great interest as an increased success rate and decreased treatment time may have a substantial impact on the cost of treatment. Case reports and treatment experience from some centers indicate a better success rate with von Willebrand factor containing concentrates. Even if formal, well-controlled studies are needed, it can already be recommended that inhibitor plasma should be tested against a panel of concentrates in order to select the less neutralized concentrate for use in inhibitor patients.

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**Key words:** hemophilia, inhibitor, PUP, immune tolerance induction, FVIII concentrates.

### Introduction

In recent years there has been a lot of discussion as to whether there is a difference in the incidence of inhibitor development, depending on the concentrate used, i.e. human plasma-derived of lower purity or highly purified recombinant factor VIII. The question has also been raised as to whether there is a difference in success, depending on the concentrate used for immune tolerance (IT) induction. Several authors have reported the different reactivity of inhibitors, both from congenital and acquired hemophilia, with commercial factor VIII preparations, and suggested a role for the von Willebrand factor (VWF) in this reactivity. Although these findings have not been clearly shown to play a clinical role, some hypothetical advantages can be put forward for concentrates containing VWF complexed with factor VIII.

### Inhibitor reactivity and type of concentrate

There are several functional binding sites that are targets for factor VIII inhibitory antibodies<sup>1,2</sup> (Figure 1). Thus, factor VIII inhibitory antibodies may interfere with the binding sites of factor X, IX, the VWF and phospholipid. Most hemophilic inhibitors have multiple specificities in their reactivity with factor VIII and react predominantly against A2, C2 and A3 domains. Hemophilia patients treated with plasma-derived factor VIII have a predominance of anti-light chain (anti-C2) reactivity, whereas anti-A2 antibodies are as common as anti-light chain activity in patients treated with recombinant factor VIII.<sup>3</sup> These *in vitro* results were of considerable interest in connection with a case that was treated at our center and reported in 1996.<sup>4</sup> This case, a 22-year-old patient with severe hemophilia A and a high titer inhibitor, was referred to our center because of a severe gastrointestinal bleed, and an unsatisfactory response to treatment with by-passing agents. On admission, the inhibitor titer was 50 Bethesda Units (BU). Extracorporeal adsorption of the patient's plasma was immediately started and 10 liters of plasma were absorbed. The inhibitor titer fell to below 5 BU and after a night's rest it had increased again to more than 40 BU. After adsorption of another 10 litres, 5000 units of Koate HP (Bayer) were infused and the factor VIII level rose to 50 units/dL. The treatment then continued with 5000 units three hours apart. After two infusions with Koate HP we faced a temporary shortage of product and Monoclate P (Aventis Behring) was given at the same dose. It was then observed that the post-infusion level decreased from around 35 units/dL to 14

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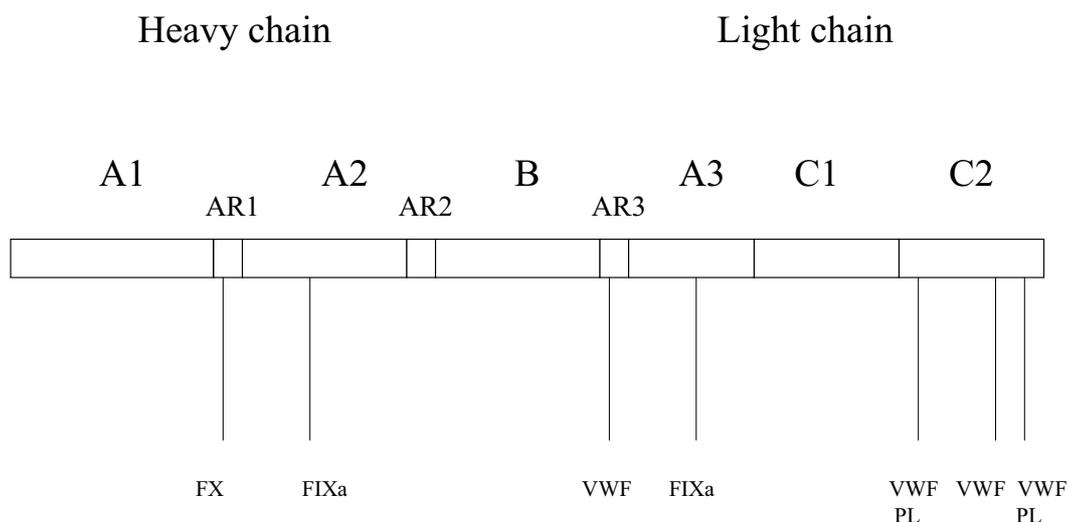


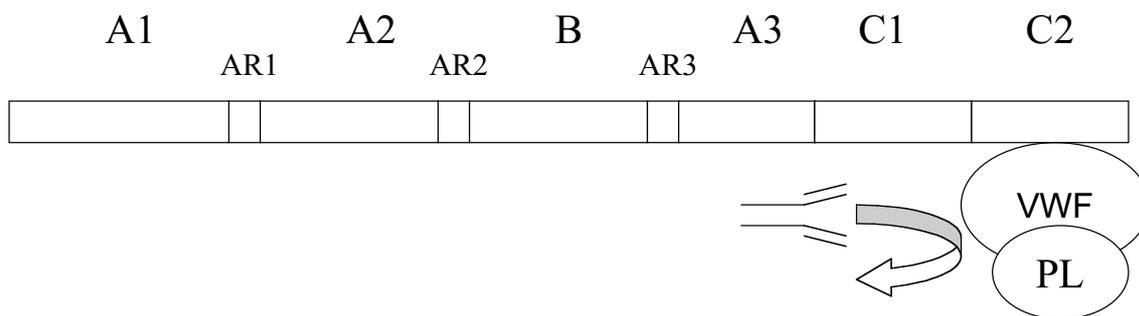
Figure 1. Factor VIII. Functional binding sites that are targets for factor VIII inhibitory antibodies.

units/dL. After another three hours Koate HP was again given and the post-infusion level increased to 30 units/dL. The bleeding stopped and the patient could be discharged to his home hospital after another couple of days.

It was obvious during this treatment that the patient's recovery was better with Koate HP than with Monoclate P. The former product is a VWF-containing intermediate purity plasma-derived product whereas Monoclate P is a monoclonal-antibody-purified product containing only trace amounts of VWF. This observation prompted us to perform *in vitro* experiments. In these we tested inhibitors from seven hemophilia patients (IgG fraction from one patient, plasmas from six patients). The patient's inhibitor samples were mixed with a panel of factor VIII concentrates. After incubation the inhibitor titer was analyzed according to the Malmö method (one Malmö inhibitor unit corresponds roughly to three BU). In five of the patients there was a clear pattern that the measured inhibitor titer was less when the assay system included the VWF-containing concentrates Koate HP and Haemate (Aventis Behring) than when it included the monoclonal-antibody-purified plasma-derived concentrates Monoclate P and Octonativ-M (Biovitrum), and the recombinant factor VIII concentrates Kogenate (Bayer) and Recombinate (Baxter). In two patients this pattern was especially pronounced. Thus the Malmö inhibitor titer in patient #1 (the above reported case) was 44 for Koate HP, 36 for Haemate, 60 for Monoclate P and 65 for Recombinate. In patient number six, the Koate HP titer was 5, Haemate 2, Monoclate P 14 and Recombinate 17.

**VWF content in factor VIII concentrates may interfere with inhibitor binding**

The reason why VWF-containing concentrates may give a better factor VIII recovery than concentrates devoid of VWF or only containing traces of amounts of VWF can be explained theoretically. In our reported case in whom *in vivo* recovery was measured, as well as in case #6 (*in vitro* study), the inhibitors react with the light-chain epitopes (region C2, AR-A3-C1) as shown by Prescott *et al.*<sup>3</sup> It has also been shown that the C2 region binds VWF as well as phospholipid.<sup>5,6</sup> Thus, one might hypothesize that the VWF contained in factor VIII concentrates may block the inhibitor reactivity, which is directed to the same region which binds VWF. This has been supported by *in vitro* studies demonstrating that factor VIII antibodies inhibit factor VIII binding to VWF and phospholipid<sup>7</sup> and are less inhibitory to factor VIII combined with VWF.<sup>8</sup> Schematically, this mechanism can be illustrated as in Figure 2. Several studies have corroborated that VWF blocks anti-light chain antibody reactivity with factor VIII and this has been shown both for allo-antibodies in congenital haemophilia<sup>9,10</sup> and for auto-antibodies.<sup>11,12</sup> However, this is not a constant finding and was not seen for every concentrate and inhibitor in our original study.<sup>4</sup> These aspects are being further explored in our center (*to be published*). The fact that the measured inhibitor titer does not always follow epitope reactivity for a specific plasma sample may indicate that mechanisms other than inhibitor blocking by VWF may be involved.



**Figure 2. Role of von Willebrand factor content in factor VIII concentrates. Factor VIII antibodies inhibit factor VIII binding to von Willebrand factor (and to phospholipids) and are less inhibitory to factor VIII combined with von Willebrand factor.**

### **Issues of clinical importance**

The main issues of a variation in inhibitor reactivity from a clinical point of view are:

- i) variation in reactivity may be of importance for factor VIII recovery in low-titer inhibitor patients;
- ii) immune tolerance induction success rate.

### **Recovery**

As shown by our inhibitor case receiving Koate HP and Monoclate P, there is, at least in certain cases, a possibility that VWF-containing concentrates, for example, give a better hemostatic effect if the inhibitor reactivity is less in such a product. The consequence of this would be that inhibitor plasma should be tested against a panel of factor VIII concentrates in order to identify a possible optimized treatment. Apart from giving better hemostasis, there is also a potential to use less of the product, i.e. the cost of the treatment can be reduced. However, it is important to stress that this has not generally been shown and is merely a tentative conclusion that can be drawn from our single case.

### **Immune tolerance induction success**

Several issues can be discussed here, such as inhibitor reactivity with factor VIII concentrates, clinical studies and theoretical considerations. If one takes the example that anti-C2 inhibitors are partially blocked by VWF, then they display a lower titer against VWF-containing concentrates. There are strong indications from both the international immune tolerance registry,<sup>13</sup> and the American immune tolerance registry<sup>14</sup> and from Bonn<sup>15</sup> that a low inhibitor titer gives a better chance of successful ITI. If the patients are easier to treat with VWF-containing concentrates, the total amount of prod-

uct used will decrease, i.e. cost efficacy will improve by choosing the *best* concentrate.

Clinical experience has indicated that a change from high to intermediate purity factor VIII concentrate may be beneficial for the success of immune tolerance induction. Kreuz *et al.*<sup>16</sup> reported that a switch from a pure FVIII concentrate in four patients, who showed an unsatisfactory response to treatment, rendered the patients amenable to successful ITI. The ITI success rate has decreased in the large German centers of Bonn and Frankfurt since the introduction of pure factor VIII.<sup>17-19</sup> However, this is not supported by the results from the North-American immune tolerance registry<sup>14</sup> and additional studies<sup>20,21</sup> have shown the feasibility of using recombinant factor VIII for ITI, given that the success rate did not correlate to the type of concentrate used in the treatment. The cited studies are usually small and definitely not designed to compare ITI success rate for different types of concentrates. Therefore, the clinical importance of VWF-containing concentrates to the outcome of ITI still remains to be elucidated. Other theoretical considerations may be put forward to support the clinical importance of variation in factor VIII inhibitor reactivity. Some hypothetical mechanisms could be that the VWF and/or phospholipids contained in concentrates may hide the epitopes and provide a more restricted antigenic challenge. Another consideration could be that the VWF prevents factor VIII degradation, thereby prolonging antigen presentation to the inhibitor-producing cells. Furthermore, the modulating effect on immune function could be transferred by the concentrates if they contain important components involved in the immune response such as anti-idiotypic antibodies, anti-CD antibodies and cytokines.

### Conclusions

Inhibitors may react differently with different types of factor concentrates and several mechanisms may explain this. Studies predominantly performed *in vitro* indicate a protective role of VWF against the inhibitors directed against the C2 region of factor VIII. Therefore, the VWF-content of factor VIII concentrates could be of clinical importance and have a substantial health economic impact. The role of VWF-containing concentrates regarding enhanced hemostatic effect and immune tolerance success rate merits further exploration as there are studies and clinical experience indicating the possibility of such beneficial effects. When starting treatment in an inhibitor patient with factor VIII concentrates, either at low inhibitor titers in order to achieve hemostasis or when implementing immune tolerance induction in patients with high titer antibodies, a recommendation could be that inhibitor plasma should be tested against a panel of concentrates in order to select the least neutralized concentrate.

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## Epidemiology of inhibitors and current treatment strategies

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The development of inhibitors is currently one of the most serious complications in the treatment of hemophilic children. Prospective studies of previously untreated patients (PUP) showed that up to 52% of patients with severe hemophilia A developed inhibitors during the first 50 exposure days (ED) (>100 for outliers). Inhibitor development is influenced by the type of hemophilia, the severity and the type of mutation. No significant differences in inhibitor incidence were found in prospective studies conducted with plasma-derived or recombinant products. However, no comparative study has been finished yet. A still ongoing prospective, multi-center PUP-study initiated by the German, Austrian and the Swiss Society of Thrombosis and Hemostasis Research (GTH) foresees the direct comparison of different types of concentrates with regard to inhibitor development. Preliminary results (update February 2002) show a slightly higher inhibitor development ( $p=0.08$ ) in severely affected hemophilia A patients treated with recombinant factor (F) VIII concentrates. However, the groups are very small and statistically reliable statements cannot be made at the moment. In case of inhibitor development rapid inhibitor elimination and immune tolerance induction (ITI) is the preferred way to reduce the high risk of bleeding episodes. In this respect, various therapeutic regimens, such as the administration of high doses of FVIII twice daily (Bonn protocol), or lower doses three times weekly (van Creveld protocol), have been attempted. Elimination of inhibitors from plasma by immune adsorption followed by immune suppression (Malmö protocol) has also been used. The influence of the type of concentrate used for ITI has never been investigated comparatively. 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 von Willebrand factor (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.

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**Key words:** hemophilia, inhibitor, PUP, immune tolerance induction, F VIII concentrates.

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The development of inhibitors currently remains the main problem in the treatment of hemophilic children and occurs in between 22 and 52% of patients with severe hemophilia A.<sup>1</sup> In particular high responding inhibitor patients (HR: > 5 Bethesda Units – BU) have a severe bleeding tendency. Furthermore, these bleeding episodes are difficult to control, even with FVIII bypassing agents (activated prothrombin complex, rFVIIa). Two questions arise for the treating physician: does VWF have an impact on the development of inhibitors and does VWF alter the course of ITI?

### **Epidemiology of inhibitor development**

The development of neutralizing antibodies against FVIII or FIX still represents the most serious complication of repeated FVIII or FIX replacement therapy. Prospective PUP-studies repeatedly showed that severe hemophilia A patients are commonly affected by this serious complication (22–52%). Moderate or mild hemophiliacs, as well as patients with hemophilia B, show this complication less frequently. However, the reported results range widely. This can be influenced by a number of variables, in particular by different study designs (observation period, exposure status to FVIII or FIX concentrates in non-inhibitor patients, the proportion of severely and moderately affected hemophiliacs particularly if discrepant definitions of severity are used, the type of mutation and race). However, the wide range of results can be caused by other variables, which may also play a role and which are still under investigation: the treatment regimen (prophylaxis, on-demand therapy, continuous infusion), the reason for the initial treatment (bleeding, surgical interventions, prophylaxis), concomitant diseases or interventions during the initial treatment phase with FVIII or FIX, in which the patients are at highest risk of developing inhibitors (inflammatory states, surgical interventions, vaccinations) and the impact of breast feeding on inhibitor development. The most intensive discussion focuses on the influence of the type of concentrate on inhibitor formation. Since the introduction of different purification and virus inactivation steps as well as recombinant manufacturing processes a variety of FVIII and FIX concentrates with different purity have become available. Comparing different recombinant and plasma-derived products the results of prospective PUP-studies show no significant difference in inhibitor incidence (Table 1). However, direct comparison of the results is impossible because of remarkable differences in study design and cohorts. In conclusion prospective PUP-

**Table 1. Inhibitor incidence in patients with severe and moderate hemophilia A treated with plasma-derived or recombinant products.**

Study	Inhibitor incidence	
	FVIII <2%	FVIII <5%
<b>Plasma-derived products</b>		
Ehrenforth <i>et al.</i> , 1992 <sup>1</sup>	52% (FVIII<1%)	33%
Addiego <i>et al.</i> , 1993 <sup>14</sup>	28%	
Ljung <i>et al.</i> , 1992 <sup>20</sup>	21%	
De Biasi <i>et al.</i> , 1994 <sup>15</sup>	22.9%	
<b>Recombinant products</b>		
Lusher <i>et al.</i> , 1997, (Kogenate®) <sup>12</sup>	30%	21%
Gruppo <i>et al.</i> , 1997 (Recombinate®) <sup>13</sup>	31%	
Lusher <i>et al.</i> , 2001 (Refacto®) <sup>11</sup>	30%	

studies are needed in order to investigate the influence of the type of concentrate.

**Prospective, multi-center PUP-study on inhibitor development initiated by the German, Austrian and Swiss Society of Thrombosis and Hemostasis Research (GTH)\***

In order to investigate different variables which may have an impact on inhibitor development in PUPs with hemophilia A and B a prospective, multi-center study was started in 1993 by the pediatric committee of the *German, Austrian and Swiss Society of Thrombosis and Hemostasis Research* (GTH).

PUPs with hemophilia A and B are included independently of their residual activity. The treatment with FVIII or FIX concentrates is carefully documented (reason, time, amount, batch number) and correlated to the development of inhibitors. In parallel, the patients are rigorously tested for inhibitor development particularly during the initial treatment phase: prior to the first exposure, every 3<sup>rd</sup> to 4<sup>th</sup> exposure day (ED) for the first 20 ED, every 10<sup>th</sup> ED until the 200<sup>th</sup> ED, every 3 months after the 200<sup>th</sup> ED and additionally if there is any suspicion of inhibitor development. Inhibitor assays are performed at the *home* laboratory and at a *central* laboratory using a modified Bethesda method (*Prof. Budde, Hamburg*). Inhibitor development is correlated with the age at first exposure to factor concentrate, mutation type causing hemophilia, HLA-type, the therapy regimen and the type of concentrate.

Up to February 2002 (study period 8.6 years), 216 patients had been enrolled. One-hundred and fifty-six have been exposed at least once to FVIII or FIX concentrate. Out of 128 hemophilia A patients 54 patients received plasma-derived FVIII concentrates (n=29 FVIII concentrates containing high amounts of VWF, n=25 with FVIII concentrates containing no

**Table 2. Inhibitor development in hemophilia A according to severity and type of concentrate – GTH-PUP study on inhibitor development in PUPs with hemophilia A and B (Update February 2002).**

Severity	pd-FVIII concentrates	r-FVIII concentrates
Severe (≤1%)	11/40 (28%)	16/40 (40%)
Moderate (>1-5%)	0/9	4/23
FVIII < 5%	11/49 (22%)	20/63 (32%)

or only traces of VWF) and 74 patients recombinant FVIII concentrates (61 full-size molecule, 13 B-domain deleted). All 28 hemophilia B patients, except one received plasma-derived FIX concentrate.

Out of 128 hemophilia A patients 32 developed inhibitors (15 high titer >5 BU, 15 low titer >0.6-5 BU, 2 transient inhibitors) after a median of 12 EDs (range 0-56) at the age of 0.9 years (median, range 0.3-10.8). The development of inhibitors was mostly observed in severe hemophilia A patients (33.8%). A slightly higher incidence, albeit non-significantly different ( $p=0.08$ ; Fisher's exact test) was found between the groups treated with recombinant and the plasma derived concentrates (Table 2). The exposure status of the non-inhibitor patients was not substantially different in both groups. So far no further evaluation regarding the type of recombinant or plasma-derived concentrate has been made because the subgroups are still too small. Out of 28 (15 severe) hemophilia B patients 2 developed inhibitors (1 high titer, 1 low titer).

To confirm the data and to make further evaluations in subgroups, more PUPs must be included and followed up. In conclusion no reliable statements can be given regarding the choice of concentrate on inhibitor development.<sup>19</sup>

**Immune tolerance induction: current treatment strategies**

Inhibitor patients usually present a very severe bleeding tendency. The most adequate management of inhibitor patients is, therefore, a rapid immune tolerance induction (ITI). Various therapeutic regimens have been attempted to achieve this.

A) The Bonn protocol foresees the administration of 50-100 IU FVIII/kg bw iv daily or every second day for low responders (0.6-5 BU) and 100-150 IU FVIII/kg bw every 12 hours for high responding patients (>5 BU). Additionally, high responders receive FEIBA® (Baxter, Glendale, USA) 50-100 IU/kg bw twice daily according to their bleeding tendency until the inhibitor titer falls below 2 BU.<sup>2-4</sup>

**Table 3. Comparison of three different ITI-protocols.**

	<i>Bonn Protocol (Frankfurt data)<sup>2</sup></i>	<i>Van-Creveld Protocol<sup>5</sup></i>	<i>Malmö Protocol<sup>6</sup></i>
No. of patients	21 (16HR, 5LR)	24	16 HR
Age [yrs]	0.4-6	1-43	1.6-53
Definition of success	<ul style="list-style-type: none"> <li>• no detectable inhibitor</li> <li>• normal recovery and half-life</li> <li>• no reappearance under prophylaxis</li> </ul>	<ul style="list-style-type: none"> <li>• Inhibitor titer &lt;2BU</li> <li>• Recovery &gt;50% of normal</li> <li>• Half-life &gt;6 h</li> <li>• No anamnestic response</li> </ul>	<ul style="list-style-type: none"> <li>• Normal recovery and half-life</li> <li>• Tolerance to further replacement therapy</li> </ul>
Success rate	LR 100% HR 87.5%	21 out of 24 (87%)	10 out of 16 (62.5%)
Duration of treatment	LR 1.5 mo (0.5-3) HR 4 mo (0.5-42)	<40 BU 6 mo (1-9) >40 BU 19 mo (12-27)	9-37 days

HR: high-responders; LR: low-responders.

- B) The van Creveld protocol (*low-dose regimen*) provides the administration of FVIII 25 IU/kg bw every other day and is decreased each time the absolute FVIII recovery is > 30% until a prophylactic dose (10-15 IU/kg bw) is reached.<sup>5</sup>
- C) The Malmö protocol includes the elimination of inhibitory antibodies from plasma by extracorporeal immune adsorption with protein-A-columns on two consecutive days (usually) followed by immune suppression with cyclophosphamide and intravenous gammaglobulins combined with a high dose FVIII protocol (Bonn protocol).<sup>6</sup>

Success rates between 60 and 100% are reported using the different treatment strategies. However, a direct comparison of the outcome is impossible, not least because of different definitions of success and different cohorts of patients (children, adults) (Table 3). A prospective study comparing high and low dose regimens is initiated.<sup>18</sup>

#### **Impact of choice of concentrate on success of ITI**

With the introduction of purer FVIII concentrates, containing small amounts or no von Willebrand factor (VWF), and their use in ITI, the question was raised of whether the type of product, in particular the content of VWF has an impact on success. This hypothesis was corroborated since two German hemophilia centers reported independently about a significantly decreased success rate. Except for the type of concentrate no parameter was changed in the treatment regimen.<sup>9,16</sup>

From 1979-1993, ITI was performed at the Frankfurt hemophilia center using FVIII concentrates with high content of VWF (FVIII/VWF). The dosage and frequency of FVIII administration was performed

according to the Bonn-protocol. A total of 21 patients were treated accordingly and the overall success rate was 91%. The median therapy duration was 4 months in high responders and 1.5 months in low responders.<sup>2</sup>

Since 1993, ITI has been initiated using recombinant and plasma-derived FVIII concentrates without VWF in 14 out of 16 patients (15 high responders, 1 low responder). After a median therapy duration of 3 months, ITI was achieved or inhibitor titer declined continuously in only 37.5% (6 out of 16 patients). The remaining 10 high responders showed no decline or even a rise in inhibitor titer. These patients were switched to plasma-derived concentrates with high amounts of VWF (Humate P<sup>®</sup>, Aventis/Behring, Marburg, Germany; Immunate<sup>®</sup>, Baxter, Glendale, USA; Haemoctin SDH<sup>®</sup>, Biotest, Dreieich, Germany; Pro-filate<sup>®</sup>, Grifols, Barcelona, Spain).

The inhibitor titer declined rapidly in 9 out of 10 patients after changing to a VWF-containing concentrate. However, complete ITI was achieved in 8 out of 10 patients after a median of 17 months (range 5-36). In comparison to formerly described treatment courses, the elimination time increased significantly. Remarkably the inhibitor reappeared in two patients after switching them to the pure FVIII concentrates and disappeared after returning them to a FVIII/VWF concentrate.<sup>16,17</sup>

#### **Discussion**

The development and elimination of inhibitors are currently the most striking issues in hemophilia treatment. Prospective PUP-studies revealed that the type and severity of hemophilia as well as the mutation type are strong risk factors for inhibitor

development. However, there are still more factors which may predispose a patient to develop an inhibitor or not: the onset of therapy, the therapy regimen, severe bleeding or surgical interventions, severe infections, vaccinations and the type of concentrate are all under discussion. Prospective PUP-studies, such as the GTH-PUP study are investigating these parameters. However, the preliminary data do not show significant results, particularly comparing plasma-derived and recombinant concentrates. More patients have to be included and followed up.

In contrast, there seems to be evidence that FVIII concentrates containing high amounts of VWF are more successful in achieving ITI. There seems to be particular benefit in patients with inhibitors directed against the C2 domain: VWF protects FVIII against degradation by proteinases by steric inhibition. In conclusion, FVIII can be presented to the immune system as the antigen, which may have an impact on tolerization. This hypothesis was confirmed in several *in vivo* and *in vitro* studies: Berntorp *et al.*<sup>8</sup> reported a higher *in vivo* recovery in C2 domain inhibitor patients after administration of FVIII/VWF concentrates. Additionally the plasmas tested against different types of concentrates showed a lower inhibitory activity against FVIII/VWF concentrates. This observation was confirmed by Suzuki *et al.*<sup>7</sup> FVIII inhibitors with C2 domain specificity were less inhibitory to FVIII complexed with VWF, whereas no difference was seen in A2 domain inhibitor plasma samples tested against pure FVIII and FVIII/VWF concentrates.

These reports and our observations during 23 years of ITT underline the importance of the choice of concentrate. The use of FVIII/VWF concentrates seems to have a substantial impact on the chance of success of ITI, in particular in patients with inhibitors directed against the C2 domain (light chain).

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## The role of plasma-derived factor VIII/von Willebrand factor concentrates in the treatment of hemophilia A patients

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Besides preventing bleeding episodes, common goals of the treatment of hemophilia include integrating of patients into a normal social life and optimizing their quality of life. Sufficient amounts of factor VIII (FVIII) concentrates, whether recombinant or plasma-derived, are continuously needed. Guidelines for quality assurance of treatment will be a cornerstone to maintain optimal clinical management of patients especially considering financial aspects. Advances in manufacturing technologies have made possible general availability of modern concentrates for the management of hemophilia A patients. Safety, cost and continuous supply of concentrates must be considered when deciding on a product for replacement therapy. As today's products have reached an excellent margin of safety with regard to virus transmission, the development and treatment of inhibitors is currently the main concern for physicians and patients. The incidence of inhibitors is influenced by various patient-related factors such as mutation type or severity of the disease. Plasma-derived FVIII concentrates containing von Willebrand factor (VWF) may have clinical advantages over pure FVIII concentrates with regard to inhibitor development and inhibitor eradication. Clinical trials comparing FVIII/VWF concentrates with pure FVIII concentrates are lacking, thus a lower inhibitor incidence has not yet been proven. Data from Germany on immune tolerance induction with FVIII/VWF concentrates indicate higher success rates with these than with pure FVIII concentrates. In addition FVIII/VWF concentrates are the therapy of choice when immune tolerance therapy with pure FVIII products is not successful.

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**Key words:** hemophilia A, aims of treatment, F VIII/VWF concentrates, inhibitor development, treatment of inhibitors.

Only a few decades ago treatment of hemophilia was limited to the infusion of blood or later frozen plasma. In the early sixties it was discovered that factor VIII (FVIII) activity can be enriched by cryoprecipitation.<sup>1</sup> Further development led to the introduction of intermediate purity and high purity FVI-II products manufactured from pooled human plasma. Genetically engineered FVIII concentrates have now replaced plasma-derived products to a certain extent or are favored for the treatment of specific groups of patients.<sup>2</sup> The availability of sufficient amounts of FVI-II concentrates has changed the clinical management of hemophilia dramatically by preventing patients from developing the sequelae of untreated bleeding episodes. Nowadays approximately 67% of hemophilia patients in Europe receive on-demand treatment, while the remaining 33% are on prophylactic factor substitution therapy.<sup>3</sup> As virus infections of patients through FVIII concentrates seems now to be only a very low remaining risk, the major clinical problem in hemophilia treatment today is the development and management of inhibitors. In this context the role of von Willebrand factor (VWF) in plasma-derived FVIII concentrates has recently been subject of increasing scientific interest.<sup>4,5</sup>

### **Aims of hemophilia treatment**

Hemophilic patients suffer from the occurrence and consequences of hemorrhages, particularly in joints and muscles. The most commonly affected joints are the knees, elbows and ankles. Joint hemorrhages cause a vicious circle of inflammation leading to synovitis. Synovitis itself is a trigger for more bleeding.<sup>6</sup> Therefore untreated joint hemorrhages will ultimately cause permanent disability. The correlation of FVIII consumption *pro capite* with the main disabilities of hemophilia is given in Table 1. Thus increasing availability of FVIII concentrates for replacement therapy is a prerequisite for defining treatment strategies to achieve common goals for modern hemophilia treatment. Such goals have been defined, for example, in Germany based on an agreement among hemophilia treaters<sup>7</sup> and include: i) prevention of bleeding episodes, ii) treatment of bleeding episodes and their complications, iii) conservation of joint functions, iv) integration into a normal social life and v) optimizing the quality of life of hemophilia patients. As approximately 80% of hemophilia patients worldwide do not receive sufficient treatment or indeed received no treatment at all there is a clear need for increased production capacity of recombinant FVIII concentrates as well as plasma-

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Table 1. Treatment when required.

	<i>No therapy</i>	<i>&lt; 1 IU/ inhabitant</i>	<i>2 IU/ inhabitant</i>	<i>4 IU/ inhabitant</i>	<i>6 IU/ inhabitant</i>
Bleeding to death	+	+	(+)	–	–
Crippling	+	+	+	–	–
Missing social integration	+	+	(+)	–	–
Dependent on governmental money	+	+	+	–	–
Bleeding episodes	+	+	+	(–)	(–)

derived FVIII products.

Besides increasing availability of clotting factor concentrates to treat and prevent life-threatening bleeds improved clinical management can be provided by specialized hemophilia treatment centers. A surveillance study in the US revealed a reduced mortality in hemophilia patients treated in hemophilia treatment centers.<sup>8</sup> Defining guidelines on quality assurance for hemophilia treatment is assumed to optimize the clinical outcome and the cost-benefit relation of treatment.<sup>9</sup> A description of a model for quality management and quality assurance has recently been published.<sup>10</sup>

#### **FVIII concentrates**

A huge variety of different FVIII concentrates is offered today for replacement therapy. From a pharmaceutical point of view plasma-derived products can be classified with respect to purification, stabilization with or without human albumin and content or not of VWF. With regard to recombinant FVIII products, first generation products are stabilized using human albumin, second generation recombinant FVIII avoids the addition of human albumin in the final container and third generation products currently in clinical trials are manufactured and formulated without human or animal proteins.

When selecting the appropriate concentrate for a given patient several issues must be taken into consideration. These include aspects such as viral safety, inhibitor development and financial concerns as well as sufficient availability of the chosen concentrate.

While hoping that the introduction of recombinant FVIII products would enable an unlimited supply, history has taught us that we are still faced with product shortages of both types of concentrates. For example, recall of batches recognized to show deviations from product quality specifications have been reported. In addition more detailed

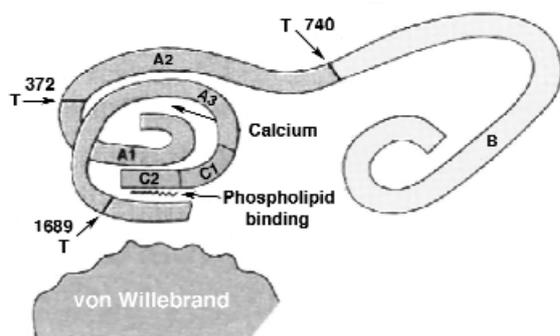
inspections of manufacturing facilities by regulatory authorities may lead to periodic termination of production because of GMP failures. Therefore no manufacturer can guarantee an unlimited supply and it seems important to maintain production of both recombinant and plasma-derived FVIII concentrates.

Increasing financial problems of several European health care systems will require cost-benefit analysis of the *status quo* of treatment for hemophilia. A continuing use of plasma-derived FVIII-concentrates may help to secure financing for hemophilia therapy in the future.

During the last ten years many measures have been taken to increase the margin of safety of plasma-derived FVIII products. Establishing quality standards for the procurement of source plasma in the US, for example, has led to rigorous donor screening methods excluding high-risk donors. In addition to serologic screening of single plasma donations for human immunodeficiency virus and hepatitis B and C viruses, genome amplification techniques to test source plasma in minipools have been introduced recently. With this method it is possible to further reduce the risk of using *window period* donations for fractionation. Together with the highly efficient virus inactivation steps incorporated in the manufacturing process plasma-derived FVIII concentrates nowadays provide an excellent margin of safety.<sup>11</sup> Although recombinant FVIII products are perceived to have a higher viral safety than plasma-derived products, they obviously cannot be considered to have no risk at all.<sup>12</sup>

#### **Inhibitor development and treatment**

Development of inhibitors remains the major problem with hemophilia treatment. The incidence of inhibitors in previously untreated children has been addressed in several prospective clinical trials. Incidences reported range between 10 and 52% of hemophilia A patients.<sup>13</sup> Genetic risk factors such



Hoyer, 1994

**Figure 1. Structural model of FVIII: domains and thrombin degradation sites.**

as mutation type, severity of hemophilia, race and ethnicity as well as therapy-related factors such as quantity and time of product exposure can influence inhibitor development.<sup>14</sup> In contrast, the impact of the type of product for replacement therapy in previously untreated patients remained unclear. It has been speculated whether VWF may play a role in inhibitor induction or inhibitor eradication. New experiments in a hemophilia A mouse model provide evidence that VWF can modulate the immunogenicity of FVIII.<sup>5</sup> The content of VWF in FVIII products was correlated with the immunogenicity of the product as assessed by the induced inhibitor titer.

Inhibitory antibodies mainly recognize epitopes on the A2, A3 and C2 domains of FVIII.<sup>15</sup> Binding sites for VWF are located on the amino terminal region of the light chain corresponding to the A3 domain and at the carboxy terminus within the C2 domain<sup>16</sup> (Figure 1). Therefore it has been suggested that VWF masks antibody epitopes on the light chain of the FVIII molecule which may be of importance for inhibitor development. In inhibitor patients VWF may theoretically protect FVIII from neutralizing antibodies thus permitting higher FVIII activities after replacement with VWF-containing FVIII concentrates.

There is a lack of clinical studies to prove a lower incidence of inhibitors in previously untreated patients using VWF-containing FVIII concentrates compared to high purity plasma-derived or recombinant FVIII concentrates. A prospective study of a high purity VWF-containing FVIII product reported, in an interim analysis, an inhibitor incidence of 8% in severe hemophilia A patients.<sup>17</sup> Epidemiological data on inhibitors in previously untreated patients in the hemophilia centers in Bonn and Bremen record a lower rate of inhibitor development in

**Table 2. Inhibitor Epidemiology (PUP). Hemophilia Centers of Bonn and Bremen, 1990–7/2001.**

Product	Total N = 139	Inhibitors
Recombinant F VIII	45	14 (31.1%)
Plasma-derived F VIII	94	20 (21.3%)

**Table 3. Change from high purity to plasma-derived VWF containing FVIII concentrates. Patients (1993–2001).**

	No. of patients changed concentrates (n=13)	
	Median	Range
Age at first ED (years)	1.0	0.1-50
Number of EDs until inhibitor (n)	13	4-100
Maximum inhibitor titer (BU)	97	8-287
Time until change of concentrate (months)	3.3	1.4-20
All patients initially treated with high purity concentrates (recombinant and plasma-derived). ED=exposure days		

patients treated with plasma-derived FVIII concentrates, as shown in Table 2. An ongoing prospective multicenter study also reported a trend towards a lower incidence for plasma-derived products.<sup>18</sup> However, it has not been analyzed whether the lower incidences are truly related to the use of VWF-containing FVIII products alone.

Several *in vitro* studies testing different FVIII concentrates against plasma samples from inhibitor patients showed that inhibitor antibodies with A2 plus light chain reactivity are less inhibitory to VWF-containing FVIII products.<sup>4,19</sup> In addition anti-C2 antibodies can inhibit factor VIII binding to VWF<sup>20</sup> and have been shown to be less inhibitory to VWF-containing FVIII concentrates.<sup>21</sup> Inhibitor patients have a higher risk of severe bleeds that are more difficult to treat with normal FVIII replacement therapy. High responder inhibitors cannot be treated with normal FVIII substitution. In order to prevent life-threatening bleeding episodes, complete and sustained elimination of inhibitory antibodies is needed. Immune tolerance induction is currently the preferred treatment strategy.<sup>22</sup> Several therapeutic regimens have been used with overall success rates between 60 and 80%.<sup>23</sup> However, the success rate for administration of high doses of FVIII twice daily (*Bonn protocol*) has been reported to be as high as 87%. Different variables

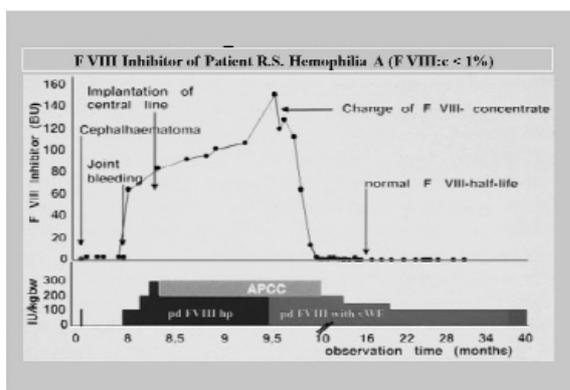


Figure 2. Change of ITT from hp pdF VIII to pdF VIII with high content of VWF.

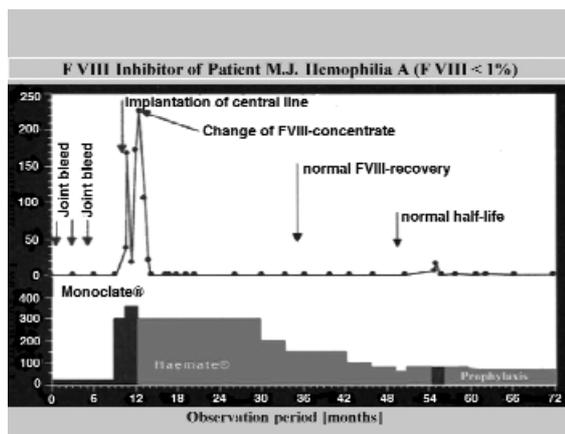


Figure 3. Change of ITT from hp pdF VIII to pdF VIII with high content of VWF.

such as inhibitor peak titer, uninterrupted treatment schedules and inflammatory events may contribute to the success of immune tolerance therapy.<sup>24</sup>

The German experience in immune tolerance induction with high dose FVIII substitution twice daily shows that the choice of concentrate also affects the success rate. With the use of an intermediate purity concentrate containing high amounts of VWF inhibitor, elimination has been achieved in 90% of patients after a median time of 4 months. In contrast, when using FVIII products without VWF the success rate dropped significantly.<sup>25</sup> We now can report on a total of 13 patients who did not respond within 3 months to immune tolerance induction therapy with high purity FVIII concentrates. The characteristics of these patients are listed in Table 3. After changing to VWF-containing FVIII products complete immune tolerance was achieved in 10 patients (77%) although a significantly longer period of treatment was necessary (median 17 months, range 5 – 36 months).

Figure 2 shows the course of an inhibitor patient who developed a high titer inhibitor after several bleeding episodes. Immune tolerance induction therapy was started using a high purity FVIII concentrate devoid of VWF, with increasing doses from 100 to 300 IU / kg bw. As the inhibitor did not decline after boosting, a VWF-containing concentrate was chosen for treatment. Subsequently the inhibitor declined rapidly, achieving complete tolerance after 6.5 months of treatment. Switching back to high purity FVIII products without VWF after successful immune tolerance induction may lead to the reappearance of the inhibitor, as occurred in 2 of our patients. Figure 3 shows that measurable inhibitor titers appeared during prophylaxis after terminating immune tolerance therapy.

The data compilation of immune tolerance

Table 4. Success rates of immune tolerance induction therapy. Hemophilia Centers Bonn and Bremen.

	< 1990 n = 51 Plasma-derived FVIII	≥ 1990-7/2001 n = 42 Recombinant FVIII (n = 14)	Plasma-derived FVIII (n = 28)
Success rate	87%	43%	82%
Success rate			
High responder	86%	54%	78%
Success rate			
Low responder	93%	72%	91%

induction therapy in the hemophilia centers in Bonn and Bremen confirms a higher success rate when using plasma-derived FVIII concentrates with a relevant VWF content. Before 1990 immune tolerance treatment was successful in 44 of 51 patients (86% success rate) using exclusively an intermediate purity FVIII/VWF concentrate. The success rate dropped significantly to 55% between 1990 and June 1999 when high purity FVIII concentrates without VWF were mainly used. Since July 1999 more patients have been treated again with concentrates containing VWF and the success rate increased again to 71%. Altogether 14 patients have been treated with recombinant FVIII concentrates and 28 patients with plasma-derived FVIII/VWF concentrates. The success rates achieved are 43% (6/14) for the recombinant group and 82% (23/28) for the plasma-derived group. The success

rates with respect to low and high titer inhibitors are shown in Table 4.

### Conclusions

Plasma-derived FVIII concentrates are still used to a certain extent for the treatment of hemophilia A patients although recombinant FVIII has gained an increasing market share over the last ten years. The question arises now whether physicians can afford to rely completely on recombinant FVIII. The content of VWF currently differentiates some of the available plasma-derived FVIII products from products manufactured by genetic engineering. While pure plasma FVIII concentrates can be replaced by recombinant FVIII without consequences, FVIII/VWF complex concentrates may serve better for some groups of patients. Prospective clinical trials are necessary to confirm a possible lower inhibitor incidence in previously untreated patients. High success rates in immune tolerance induction therapies have been reported for FVIII/VWF complex concentrates. Based on our experience in Germany these concentrates are the treatment of choice for inhibitor patients, when immune tolerance induction therapy remains unsuccessful with high purity FVIII products. In times of increasing financial problems in the health care environment the cost of replacement therapy is attracting more and more attention. Plasma-derived FVIII products are generally cheaper than recombinant FVIII products and provide an excellent margin of safety. Therefore FVIII/VWF complex concentrates should be considered more valuable than merely an alternative therapy option.

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## PANEL DISCUSSION

*Question: Just a quick question to the last three speakers: given this data, if a patient walks into your clinic today, what do you tell the family? What do you tell the patient? What are their options for them?*

**Dr. Auerswald:** I think you can show the patient the possibility of concentrates that are available in your country or in your center and at the moment I would not give a concentrate that seems to be of a higher risk of inhibitor development. So, it would be a plasma-derived FVIII concentrate, usually of high purity or a recombinant concentrate that has a low inhibitor development rate.

*Question: So would you recommend that you test any patient that you start in the laboratory first to see what their chances are? As a clinician, I wanted to know exactly what we should do. Should we test them in the lab first? Should we start them on a recombinant or a plasma-derived product or start them on a plasma-derived product and wait until they develop an inhibitor?*

**Dr. Berntorp:** I can say our strategy is to start patients on recombinant products, I am talking about non-inhibitor patients now. And we, of course, do not say that the risk of inhibitor development is higher with recombinant products than with plasma-derived products because I do not think there is data supporting that for the time being. If the patient develops an inhibitor, we continue immune tolerance induction with the same product. If the patient is very resistant to immune tolerance induction then we take up the discussion of switching product to a plasma-derived product of lower purity. I think today, plasma-derived products are so safe that the benefit of the possibility of inducing tolerance by a switch of concentrates is far extending the risks of giving a plasma-derived concentrate, because having an inhibitor is really the worst thing you can have in a hemophilia patient if we do not talk about virus diseases. But I mean you should really put effort to induce tolerance and do not look too much at the type of concentrate because they are safe today. That is, at least, our policy.

**Dr. Kreuz:** What I did not mention is that if we have a patient with inhibitors and we want to use an immune tolerance regimen, we first test various FVIII concentrates in the lab, and that I think is your question, and

various lots also. Maybe we test 12 to 15 concentrates that are available on the market in big amounts. Then we can reserve the best one for this child and then we get, I think, the best result and the fastest immune tolerance.

*Question: For Dr. Kreuz and Dr. Auerswald: the patients who failed immune tolerance on high purity and then responded on low purity, what products were they being treated with when they developed the inhibitor, was they recombinant, and if so did you do epitope mapping of those inhibitor antibodies?*

**Dr. Auerswald:** The problem is that in these patients we had inhibitor treatments with high purity plasma-derived concentrates as well as recombinants who failed. We normally used a concentrate with a high VWF which was usually Humate P.

*Question: I think it was Dr. Berntorp who said that most of the inhibitors developed in patients with high purity reacted in the heavy chain. In these patients who responded when you switched, do you know what the inhibitor reactivity was? Do you know which epitope they reacted to?*

**Dr. Berntorp:** No, not in our case. We only had one such case, and I think it was a general comment to this panel testing. I think you must consider that the inhibitor reactivity, if you follow a patient longitudinally, can differ in reactivity. I mean, at one moment the inhibitor reactivity can be higher against one epitope and other times it can be higher at another epitope. I think it is a very tricky question, at least to really test against panels and extrapolate that to the clinical situation. But still, if you want to do something, you should test them and we also do that, but we do not have very much study material.

**Dr. Auerswald:** I can tell only of one patient who failed and his epitope was on the A3 domain. I do not know about the others.

**Dr. Federici:** When I was presenting and preparing this talk and I was thinking about the possibility of raising antibodies in hemophilia patients, it came into my mind the possibility of whether the type of mutations of these

patients can help us to organize a study to get the best therapeutic approach for them? Do you think it is important to know where the mutation of a patient is located in terms of introducing this in practical therapy for these patients? I know this is a very controversial issue, but I would like to have your ideas about this.

**Dr. Auerswald:** I think it is a multifactorial problem and mutation is only one factor of it. We have no proven study that shows us that, for example, for intron 22 inversion, one concentrate is better than another one. So, I think this is an interesting point to study. For example, we know that in the United States in African-American patients, we have an inhibitor rate of about 50% and they have different mutations. I think you cannot choose a concentrate only from the mutation type.

**Dr. Berntorp:** In a study comprising more than 500 families we are considering such questions but we have no data today to really draw any conclusions about your questions, but I think data will emerge in a couple of years. We will know more and also more about the practical therapeutic approach of inhibitor treatment.

**Dr. Kreuz:** I think if you look at the studies which are done we cannot say yes or no, but I think there are some results. If you look at the Kogenate FS study, there are patients who have a high risk to develop inhibitors and until now, after 3 and a half years there is a low incidence of inhibitor develop-

ment. So I think until now we have no data but it is a very good question. I want to add to your question that you ask for the VWF-containing concentrates which were used for the change. We have had the most experience with Humate P in the past. When there was a shortage of Humate P, we also had very good success with the product of Grifols, of Biotest, and preparations which contained enough VWF.

**Dr. Berntorp:** I have a question to Dr. Kreuz and Dr. Auerswald. You have fantastic study material. When you have switched concentrates and achieved success, you must save a lot of money for society and also from the humanitarian point of view you must do a lot of good. Have you done any health economic analysis on how much you saved by your increased success rate by switching concentrates?

**Dr. Federici:** How much money do you save by using plasma-derived concentrates instead of recombinant material?

**Dr. Kreuz:** As we showed in our experience, the elimination period of the inhibitor was four times longer if we used the high purity FVIII concentrates. I think if we start immediately with a concentrate that has the best recovery in the lab test - I told you we do it in the lab - there are big differences if we compare the concentrates and their lots with the patient's plasma.