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**COMMITTEE FOR PROPRIETARY MEDICINAL PRODUCTS  
(CPMP)**

**NOTE FOR GUIDANCE ON THE CLINICAL INVESTIGATION  
OF PLASMA DERIVED ANTITHROMBIN<sup>1</sup> PRODUCTS**

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<sup>1</sup> Previously known as Antithrombin III

# CLINICAL INVESTIGATION OF PLASMA DERIVED ANTITHROMBIN PRODUCTS

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## 1. INTRODUCTION

### 1.1 Physiology

Antithrombin is a serine proteinase inhibitor of major importance in the control of blood coagulation. The latter is a very efficient and fast-reacting system with the capacity to produce clots within minutes in order to stop bleeding from any lesion to blood vessels. However, clotting at the wrong site, or an overshooting activation of coagulation, may lead to serious problems. A clot is formed after a cascade-like proteolytic activation of clotting factors. The key enzyme thrombin converts fibrinogen to fibrin and thus initiates clotting, and activates further important mediators: the coagulation factors V and VIII, which are non-enzymatic accelerating cofactors; platelets and several other cell types, which carry thrombin receptors on their surface; and factor XIII, which is responsible for clot stabilisation.

Antithrombin forms with thrombin a complex, which is rapidly cleared from the circulation by internalisation into liver cells. The formation of this complex is strongly accelerated in the presence of heparin, or heparan sulphate on the vessel wall; antithrombin has also been referred to as a heparin cofactor. Not only the procoagulant actions of the key enzyme thrombin, but also the further thrombin generation is inhibited, since antithrombin inhibits also the “upstream” coagulation proteases including the starter moiety, the tissue factor-factor VIIa complex.

In addition to its role in haemostasis, thrombin generation and fibrin deposition are intimately connected with inflammatory reactions. Particularly in severe sepsis, strong activation of coagulation occurs as part of the so-called systemic inflammatory reaction syndrome (SIRS). This activation may lead to disseminated intravascular coagulation (DIC), causing disturbances of organ perfusion and progressive consumption of coagulation factors, resulting in exhaustion of the haemostatic system and bleeding. Antithrombin appears to be involved in the regulation of inflammatory reactions, but the mechanisms involved are still incompletely understood.

### 1.2 Clinical use

A total (homozygous) deficiency of antithrombin is thought to be not compatible with life. A partial (heterozygous) deficiency with a biologic activity around 50% of normal levels may be associated with familial thrombophilia, predisposing up to 70% of affected family members to suffer from thrombosis before they reach an age of 40 years. Congenital antithrombin deficiency has a prevalence of about 1 in 3,000 to 5,000 with distinct types of underlying mutations, which are of different clinical significance<sup>1</sup>. Regular prophylactic substitution of antithrombin in patients with congenital deficiency is not necessary. However, substitution with antithrombin in congenitally deficient individuals is considered for prophylaxis of thrombosis in certain risk situations such as the peri-partum period or major surgery<sup>2</sup>, or for treatment of thrombosis.

While the above mentioned use of antithrombin in individuals with congenital deficiency is widely accepted, the situation is far less clear for an acquired reduction of antithrombin levels, which may occur frequently in various clinical situations<sup>3; 4</sup>. Generally, a lowered antithrombin level alone, e.g. due to impaired synthesis, should not be a reason for substitution, when the patient is in a stable clinical condition. From the symptoms encountered in congenital deficiency, it might be deduced that antithrombin replacement could contribute to the prevention of thromboembolic complications in patients with acquired deficiency, particularly if the measurable response to anticoagulation with heparin is impaired. However, there are only few clinical studies available in this indication<sup>5,6</sup>. There are many experimental data, case reports and clinical pilot studies<sup>7</sup>, pointing to a favourable influence of antithrombin substitution on laboratory signs and symptoms of DIC, particularly

in septic shock. A statistically significant proof of clinical benefit for endpoints such as mortality in prospective controlled studies is hard to obtain in the very heterogeneous population of sepsis patients, receiving multimodal intensive care. There are only two published controlled trials available. The earlier trial<sup>8</sup> showed a positive trend in terms of reduction of mortality, but failing to yield a statistically significant result due to the low number of patients included. The later trial<sup>9</sup> found no effect of high-dose antithrombin therapy on 28-day all-cause mortality in adult patients with severe sepsis and septic shock when administered within 6 hours after the onset. High-dose antithrombin was associated with an increased risk of haemorrhage when administered with heparin. There was some evidence to suggest a treatment benefit of antithrombin in the subgroup not receiving concomitant heparin.

This Note for Guidance describes the information to be documented to demonstrate efficacy and safety when an application for a marketing authorisation for antithrombin is made, including viral safety and clinical trials. These data are required for:

1. products for which an application for a marketing authorisation is to be submitted, referred to as ‘new products’ in the text and
2. authorised products where a significant change in the manufacturing process has been made (e.g. additional viral inactivation/removal steps or purification procedures), referred to as ‘modified products’ in the text.

The clinical trials described in this Note for Guidance should be performed according to the ICH Note for Guidance on Good Clinical Practice” (CPMP/ICH/135/95) as well as the Note for Guidance on Clinical Investigation of Medicinal Products for the Treatment of Venous Thromboembolic Disease (CPMP/EWP/563/98) and the Points to Consider on Clinical Investigation of Medicinal Products for Prophylaxis of Intra- and Post-Operative Venous Thromboembolic Risk (CPMP/EWP/707/98).

It may be preferable for patients entered into clinical trials to be vaccinated against hepatitis A and B, although there is no evidence of transmission of these viruses by antithrombin concentrates. In patients who are vaccinated, evidence of immunisation should be present at baseline.

### **1.3 Efficacy**

The same principles for demonstrating efficacy of antithrombin apply independently of its source or manufacturing process (e.g. plasma derived or recombinant antithrombin).

#### **1.3.1 Congenital antithrombin deficiency**

In clinically evaluating antithrombin concentrates for substitution in patients with congenital deficiency, the initial trial may be one that examines the pharmacokinetics of antithrombin. Appropriate pharmacokinetic data (incremental recovery, half-life, area under the curve (AUC), mean residence time (MRT) and clearance) are important information to establish the appropriate dose regimen of a new antithrombin product. However, it has to be taken into account that in patients with congenital antithrombin deficiency residual activity of about half the normal plasma level is present.

In patients with congenital antithrombin deficiency and a personal or family history of severe thrombotic events efficacy would be demonstrated if it can be shown

- a) that these patients remain free of such events when antithrombin substitution is given in high risk situations,

- b) that under antithrombin substitution commenced following diagnosis of deep vein thrombosis or thromboembolism, neither extension, nor recurrence of thrombosis, nor new episodes of embolism occur.

Pregnant women around labour or lactating women with congenital antithrombin deficiency comprise a group of patients with increased risk of thrombosis who might benefit from antithrombin substitution. Although there are no formal embryo/foetal toxicity studies conducted with antithrombin these patients may be included in clinical efficacy trials.

### **1.3.2 Acquired antithrombin deficiency**

In patients with acquired deficiency, the pharmacokinetics of antithrombin may be significantly altered due to enhanced turnover because of the underlying diseases. Furthermore, the risk of thrombosis or DIC is also strongly determined by factors other than the antithrombin level itself. Therefore, if an indication for treatment of patients with acquired deficiency is claimed, data from studies with relevant clinical endpoints should be provided.

## **1.4 Safety**

Safety aspects of antithrombin products include viral safety, heparin induced thrombocytopenia with preparations containing heparin, and other adverse events.

Depending on the manufacturing process, immunogenicity may be an issue.

### **1.4.1 Adverse events**

All adverse events occurring in relationship with any use of the new product should be recorded and reported.

Safety data from trials in indications not claimed in the application can be used as supportive data.

Since antithrombin preparations may contain heparin, the risk of heparin induced thrombocytopenia (HIT, Type I and Type II) should be considered. Type I HIT represents a transient decrease of platelet counts in the early treatment phase, which is of minor clinical importance. Type II HIT is characterised by a significant drop of platelet count to less than 100,000/ $\mu$ l and/or a decrease by more than 50% in comparison with baseline values, on two consecutive measurements and severe or even life-threatening thromboembolic complications. However, so far no cases of type II HIT associated with antithrombin administration have been reported. Moreover, since most of the patients receiving antithrombin replacement are treated concomitantly with heparin, it may be difficult to attribute HIT type II as an adverse event to antithrombin administration in these patients.

### **1.4.2 Viral safety**

Manufacturers of plasma products such as antithrombin are obliged to optimise viral safety by three principal complementary approaches:

- rigorous selection of donors and screening of donations, including testing for HBsAg, antibodies to hepatitis C virus (HCV) and HIV 1+2,
- screening plasma pools for HBsAg, antibodies to HCV and HIV 1+2, and HCV RNA by nucleic acid amplification technology (CPMP/390/97, March 1998),
- the use of appropriate viral inactivation/removal methods according to the recommendations in the “Note for Guidance on virus validation studies: the design, contribution and interpretation of studies validating the inactivation and removal of viruses” (CPMP/BWP/268/95, February 1996) and the current edition of the “Note for Guidance on plasma-derived medicinal products” (CPMP/BWP/269/95).

The above-mentioned procedures are now considered to be highly effective and demonstrative of the viral safety of the product with respect to enveloped viruses. Therefore it is no longer considered appropriate to use clinical trials to investigate viral safety with regard to enveloped viruses.

These procedures may be of limited value against non-enveloped viruses, such as hepatitis A virus and Parvovirus B19. The safety of the products with respect to non-enveloped viruses cannot currently be adequately evaluated in clinical studies.

The applicant is still required to provide all available data gathered on patients treated with the product in clinical trials. Investigators should continue using their normal clinical practice of monitoring patients. The applicant should demonstrate that there are systems in place to collect information on patients treated with the product and to respond rapidly to any reports of infection with a full investigation.

For products with an entirely novel manufacturing process other principles may apply. These applications should be discussed with the Regulatory Authorities prior to submission.

## **2. PRODUCTS FOR WHICH AN APPLICATION FOR A MARKETING AUTHORISATION IS TO BE SUBMITTED: 'NEW PRODUCTS'**

### **2.1 Efficacy**

#### **2.1.1 Congenital antithrombin deficiency**

A pharmacokinetic trial should be performed in at least 12 subjects with congenital antithrombin deficiency (antithrombin activity  $\leq 60\%$ ). The study should record incremental recovery, *in vivo* half-life, area under the curve (AUC), clearance and mean residence time (MRT) in patients in a stable condition without signs of acute thromboembolic events, preferably patients foreseen to undergo elective surgery. Patients should be at least 18 years of age and should not have received an infusion of antithrombin for at least 14 days.

Samples for antithrombin activity determination should be taken before injection of 50 IU/kg of the new antithrombin product and 10-15, 30 and 45 minutes, 1, 3, 6, 24, 48, 72, 96, 120, 144, and 168 hours after the infusion. At least 3 different lots should be employed in the trial. Incremental recovery is determined as the peak level recorded within the first hour after infusion and reported as [IU/ml]/[IU/kg].

It is anticipated that some deviation from the recommendation may occur in clinical practice. For this reason, it is very important to record the exact time post-infusion at which the actual samples were taken and to use these precise values in the analysis.

If the biological activity and pharmacokinetics are consistent with published data on plasma-derived antithrombin, any available information at the time of the marketing authorisation application on the clinical efficacy of the product in patients with congenital antithrombin deficiency should be provided (prophylaxis of thromboembolism in high risk situations and prevention of progression or recurrence of thrombosis).

Within the first five years of marketing, data on the clinical response of patients with congenital antithrombin deficiency should be provided together with the PSURs.

The occurrence of deep vein thrombosis should be actively assessed by objective measures, such as doppler sonography. In case of any symptoms suggestive of pulmonary embolism, full clinical evaluation should be performed using an appropriate validated method, e.g. ventilation/perfusion lung scan, spiral computed tomography and/or pulmonary angiography.

If the biological activity and pharmacokinetics are not consistent with published data on plasma-derived antithrombin, a formal clinical trial will be required prior to authorisation.

### **2.1.2 Acquired antithrombin deficiency.**

An acquired reduction of plasma antithrombin activity may occur in various diseases and clinical situations, e.g. chronic liver disease, postoperative period, shock, severe infections, particularly in DIC of any cause. The reduction may be due to different mechanisms, e.g. impaired synthesis, blood loss or dilution, and antithrombin consumption. In patients with acute thrombosis, particularly in those receiving heparin therapy, there may be an increased turnover of antithrombin due to enhanced utilisation.

A reduction of the antithrombin plasma activity alone is not a reason for the administration of antithrombin concentrate. The indication will be determined by the intended clinical benefit to the treated patient. While the mechanisms of the clot-limiting action of antithrombin are quite clear, the interactions with mediators of inflammation are incompletely understood. Thus, the design of clinical trials and the number of patients needed to support the application will greatly depend on the particulars of the claimed indication(s) in acquired antithrombin deficiency. Indications are mainly centred around the prophylaxis or treatment of either severe consumptive thrombohaemorrhagic disorders such as DIC of several causes, or thromboembolic events:

- a) Thrombohaemorrhagic disorders: Statistically significant proof of clinical benefit on endpoints like mortality in prospective controlled studies prove difficult to obtain in this very heterogeneous population of patients with serious conditions complicated by DIC and receiving multimodal intensive care. Nevertheless, data on such clinical endpoints, including mortality, should be provided if an indication for treatment in these patients is claimed.
- b) Thromboembolic events: To show efficacy in the prevention of thrombosis in patients with acquired antithrombin deficiency, the substitution of antithrombin should be given in a suitable, defined clinical setting in addition to standard prophylaxis as accepted for the underlying risk situation. The efficacy in treatment of thrombosis should be studied in patients with freshly diagnosed thromboembolism receiving accepted standard treatment for that condition. The clinical outcome as assessed by defined endpoints should be compared to a control group with a similar condition of acquired antithrombin deficiency receiving standard prophylaxis or therapy without antithrombin substitution. Guidance on study design and assessment for thromboembolic events is given in the documents CPMP/EWP/707/98 and CPMP/EWP/563/98.

## **2.2 Safety**

Clinical safety will be assessed in all patients receiving the antithrombin product:

- in patients included in the pharmacokinetic trial: blood pressure, heart rate, temperature, respiratory rate and adverse events
- in all patients participating in clinical trials: adverse events.

All adverse events should be recorded and reported in accordance with ICH guideline “structure and content of clinical study reports” (CPMP/ICH/137/95E3)

The effect of antithrombin is greatly enhanced by heparin. The half-life of antithrombin may be considerably decreased with concomitant heparin treatment due to accelerated antithrombin turnover. Therefore, the concurrent administration of heparin and antithrombin to a patient with an increased risk of bleeding must be carefully monitored clinically and biologically. Special attention should be paid to ensure all bleeding events are reported.

### Viral safety

Compliance with CPMP recommendations with regard to viral safety under 1.4.2 is necessary for all plasma derived products and is verified by information supplied in Part II of the dossier.

A pre-treatment serum sample from each patient included in the clinical trials should be stored at  $-70^{\circ}\text{C}$  for possible future testing.

### **2.3 Treatment of children**

In congenital antithrombin deficiency, the first thrombotic events usually occur in young adults. However, in some cases, thrombotic complications or DIC may be also encountered in children and neonates. Since children may respond differently to plasma products compared to adults, experience in children under the age of six years should be reported if the product is intended for use in children. The incremental antithrombin recovery should be monitored, as well as clinical endpoints.

The number of children treated should be reflected in the SPC. Until experience in children has been gained, the SPC should include a statement that there are insufficient data to recommend the use of the product in children less than 6 years of age.

## **3. CHANGE IN THE MANUFACTURING PROCESS OF AUTHORISED PRODUCTS: 'MODIFIED PRODUCTS'**

### **3.1 Introduction**

The currently available antithrombin preparations differ with respect to purity and method of viral inactivation/removal. Changes in the manufacturing procedures may lead to significant changes in the product and may thereby alter the structure of antithrombin and its activity. The effects of changes in the manufacturing process (e.g. viral inactivation steps or purification procedures) on the biological characteristics and activity of the product should be investigated. If significant impact on the activity of the antithrombin cannot be excluded, data on pharmacokinetics, efficacy and safety should also be provided with the application.

### **3.2 Efficacy**

Evidence should be provided to demonstrate that the change in the manufacturing process has not affected the pharmacokinetics of the product.

A comparative pharmacokinetic trial should be performed in at least 12 subjects with congenital antithrombin deficiency (antithrombin activity  $\leq 60\%$ ). The study should record incremental recovery, *in vivo* half-life, area under the curve (AUC), clearance and mean residence time (MRT) in patients in a stable condition without signs of acute thromboembolic events. Patients should be at least 18 years of age and should not have received an infusion of antithrombin for at least 14 days. Samples for antithrombin activity determination should be taken before injection of 50 IU/kg of the new antithrombin product and 10-15, 30 and 45 minutes, 1, 3, 6, 24, 48, 72, 96, 120, 144 and 168 hours after the infusion. At least 3 different lots should be employed in the trial. Incremental recovery is determined as the peak level recorded within the first hour after infusion and reported as [IU/ml]/[IU/kg].

It is anticipated that some deviation from the recommendation may occur in clinical practice. For this reason, it is very important to record the exact time post-infusion at which the samples were taken and to use these precise values in the analysis.

If an indication in acquired antithrombin deficiency is claimed, additional clinical data may be required to support the application, depending on the particulars of the claimed indication(s).



### 3.3 Safety

Please refer to requirements for new antithrombin products (See 2.2)

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