Antithrombin – Early prophecies and present challenges

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The focus of this review is on findings that have a bearing on clinical issues and our understanding of the biology of antithrombin. I appreciate the offer from the Editor-in-chief to give the review a personal touch, and have described my first purification and activity studies performed in the years 1965–1970 in more detail. I have also included details of the enjoyable cooperative projects that I took part in during those pioneer years.

Introduction

The early prophecies

The concept of antithrombin stems from P. Morawitz in 1905 who considered it responsible for the gradual loss of thrombin activity after blood had clotted (1). Gasser in 1916 presented a modern looking thrombin generation curve (Fig 1). Rapid generation of thrombin was followed by a slow exponential decay. He attributed the decay to the formation of a complex with antithrombin (2), and he measured the level of the complex by liberating thrombin using alkali-acid treatment. The formation of complexed thrombin gradually increased as thrombin activity declined (Fig 1). It took a visionary mind to deduce this reaction mechanism so correctly from these simple experiments.

Heparin cofactor

J. McLean prepared a crude hepatic tissue extract that markedly retarded the coagulation of blood (3). The active component, later called heparin, did not affect the coagulation of fibrinogen by thrombin. Heparin obviously cooperated with a component of the blood which was called heparin cofactor (4). A. J. Quick suggested that heparin accelerated the normal antithrombin of the blood (5). The suggested identity between the progressive antithrombin which slowly inactivates thrombin, and the heparin cofactor was supported by some authors, but other authors interpreted the findings as suggesting that the two activities resided in different proteins. Purification studies were hampered by denaturation and loss of activity. W. Seegers reported that thrombin was adsorbed on fibrin (6), classified as antithrombin I (see M. Mosesson [7], see page 105 in this issue), and heparin cofactor as antithrombin II. Antithrombin III was a more convenient term than progressive antithrombin, and the only survived name of this strange classification system (6).

In the following the term antithrombin (abbreviated AT) is used synonymous with antithrombin III or progressive antithrombin, in agreement with prevailing use.

Assay of AT in a patient material

In 1963 A. Hensen and E. A. Loeliger developed the first standardized assay for AT used in plasma samples from clinical material (8). Subnormal levels of AT were found in hepatic cirrhosis, but essentially normal levels in groups of patients with acute venous thromboembolism (VTE) or myocardial infarction. The authors concluded that the assay was not useful in the diagnosis of thrombotic disease. Discussing the possible role of AT *in vivo*, it was wisely commented that our ignorance is based on the fact that no patient with an isolated AT deficiency has so far been found, and that no purified AT has so far been studied (8). Observations during the subsequent five years changed the scene completely.

The break-through

Hereditary AT deficiency causing thrombophilia

In the classical report from 1965, Olav Egeberg (Fig. 2) reported low AT and low heparin cofactor activity in about half the members of the "Mi family"(9). Several of these family members had suffered from VTE. The proneness to thrombosis had long been known in this family. The reliable assay of Hensen and Loeliger was what Egeberg needed, and the measured AT activity was about half normal in all affected members, whereas heparin cofactor was subnormal in the same individuals. This confirmed the hypothesis that heparin accelerated the activity of the progressive AT (9). The genetic disposition was identified as inherited with an autosomal, dominant trait. Trauma, operation, infection and in particular, pregnancy was often complicated by VTE. With Egeberg's publication (9) venous thrombophilia was established as an inheritable disorder.

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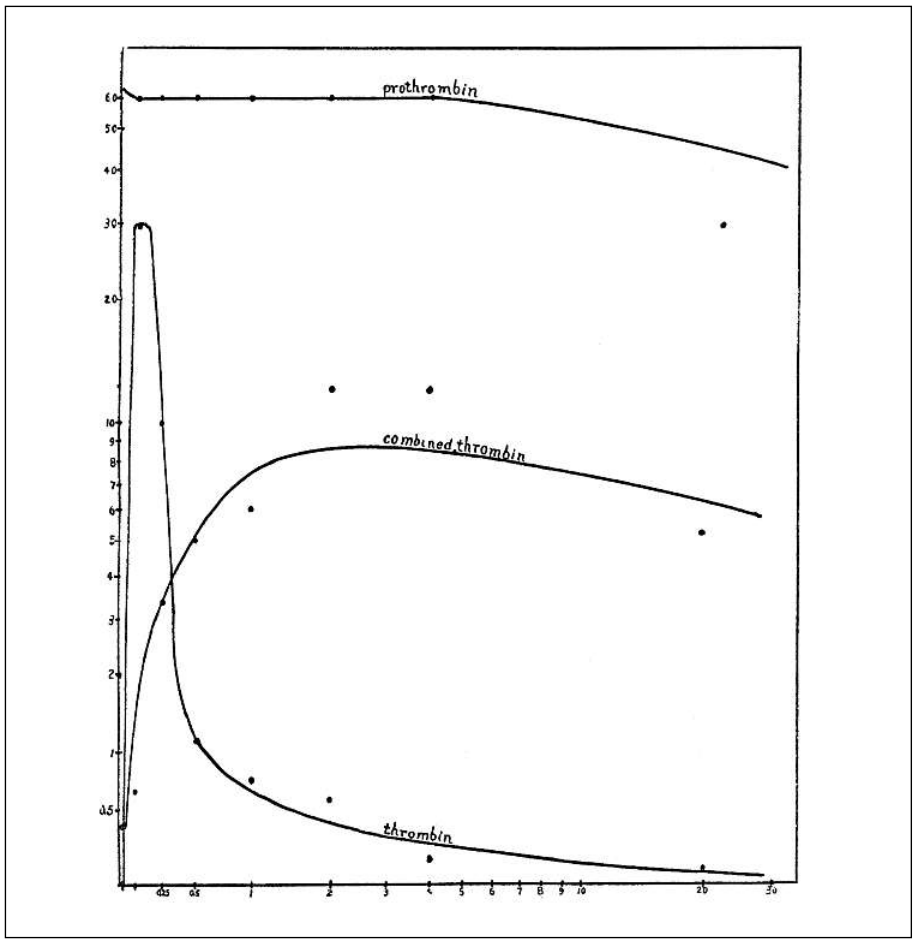

Figure 1: Thrombin generation and the formation of thrombin-antithrombin complex during incubation of human serum with a small amount of cephalin. From Gasser (ref. [2]). Prothrombin (upper curve), thrombin (peaked curve) and complexed thrombin (slowly rising curve).

A homogenous AT preparation with heparin cofactor activity

My start in AT research arose from observations in plasma incubated with low concentrations of thrombin. In order to verify a gelation test for soluble fibrin (10), I performed in-vitro model studies. Fibrin formation was monitored by quantitative N-terminal amino acid analysis, and was progressively retarded during the first few minutes (11). This observation was for me the impetus to purify the thrombin inhibitor(s) responsible for inhibition of thrombin in plasma. I had started the purification study when Egeberg's publication appeared in 1965.

We both worked in Oslo. Egeberg was an established scientist in Paul A. Owren's group. My mentor, Hans Christian Godal had departed from that group a few years earlier, and established a small research group at Ullevål University Hospital (Fig. 3). Hans Christian Godal was primarily interested in fibrinogen. The relations between the two groups were friendly, but distant.

Reading the reports of authors who had attempted purifying AT, I was struck by the great loss of activity that resulted from the procedures used. I decided to accept only those procedures that did not reduce the recovered activity. Using lenient chromatographic procedures, I separated two thrombin inhibitors from plasma: α_2-macroglobulin which accounted for about 25% of the thrombin inhibition in starting plasma, and AT which accounted for about 75% (12). In a mixture of fibrinogen and AT mimicking plasma concentrations, incubated with low concentration of thrombin, fibrin formed at a similar rate as had been observed in citrated plasma. Fibrinogen counteracted the inhibition by AT. Crude estimates indicated that with a physiological AT concentration, thrombin had a half-life of about 40 seconds under these conditions (13). Addition of 0.1 IU/ml heparin accelerated this inhibition 20-fold (14).

Trace amounts of contaminating proteins were removed by preparative discontinuous electrophoresis. A manuscript describing the purification of homogenous AT with heparin cofactor activity was accepted in 1967 and published the next year (15). I later learnt that at a conference held in early 1967, N. Heimburger had reported the purification of AT with heparin cofactor activity (16). In immunoelectrophoresis, heparin accelerated the migration of the protein. Chemical analysis showed that the inhibitor is a glycoprotein. The preparation contained higher molecular weight and inactive aggregates, but inhibited thrombin, plasmin and trypsin. From the specific activity reported for the preparation (16) it may be calculated that about 70% of the preparation was inactive protein.

When AT was incubated with highly purified thrombin in equimolar concentrations, a 1:1 complex was formed, as demonstrated by gel filtration (17). The inactivation of thrombin proceeded as a second order reaction. This AT preparation also inhibited plasmin and trypsin (15), inhibited platelet aggregation induced by thrombin (17) or factor Xa (18).

Cooperation with Olav Egeberg

When I first contacted Olav Egeberg in 1967 and suggested a cooperative study, he seemed a bit reluctant. Paul Owren later wrote that Olav Egeberg was "one of the few original scientists, an individualist who has pursued his own course, without teamwork, and who succeeded exceptionally well". I emphasized that I would indeed appreciate a cooperation with him and we agreed to join forces. Egeberg provided blinded plasma samples from members of the "Mi family" and normal individuals. Activity assays after gel filtration revealed normal activity in the α_2-macroglobulin peak in all samples. In plasma from affected members of the "Mi family", the AT peak had low activity (19).

AT prevents DIC in chicken embryos

Cooperation with Morten Simonsen's group in Copenhagen showed me that science could also be fun. For me it all started when he one morning in 1968 came to my office and said "I am Morten Simonsen, have you read my letter? – Oh no? Well, that is really not so strange. I posted it in Copenhagen last night". Things had to go fast with him. I neither knew his name, nor was aware that he was a big name in immunology, who, as a young man, had described the "graft-versus-host reaction" in patients after transplantation surgery. He sat down and told me about his new experimental model, the chicken embryo, which I understood was a fertilized egg.

His intention was to use the chicken embryo model to understand how the body tolerated break-down products of its own tissues. At that stage, the experiments consisted of injecting lipoproteins derived from chicken allantoic fluid into a vein of the chicken embryo. The result was a generalized haemorrhagic syndrome. The embryo usually died within four hours. The syndrome could also be provoked by injection of thrombin. It was prevented by the injection of plasma or serum from chicken, or from various mammal organisms. Purification of guinea pig or human plasma by chromatography resulted in a protective protein which he assumed was identical to the highly purified AT that I had recently reported (15). Immediately, I accepted to participate in a cooperative study. Jens Jensenius, who was Simonsen's student coworker, came to Oslo where we planned the experiments. I prepared human and bovine AT and brought them to Copenhagen. Jens Jensenius and Lewis Mann had prepared a huge number of eggs with 13-day embryos. The whole set of experiments – injections of lipoprotein and candidates for prevention, blood smears for semiquantitation of thrombocytes – was performed during one long day. In the evening Jens Jensenius and his partner took me to the theatre. The next day was busy with measuring end points in the chicken embryos by transillumination of the eggs in order to quantitate the prevention of haemorrhagic syndrome. On that evening, Morten Simonsen gave an informal party in his home. That was the last event of my Copenhagen visit.

The analysis showed that 22 μg human AT prevented the fatal reaction in 50 –100% of the eggs. Bovine and guinea pig AT were also protective, as was 1 IU of heparin, but α_2-macroglobulin and some other protein inhibitors were not protective. Our manuscript submitted to the journal Science was returned, saying that in its rambling and over-detailed form the authors might be happier sending it to a thrombosis journal. "On the other hand, there is a story here for Science readers if it could be untangled and presented succinctly." By that time Lewis Mann had left Copenhagen. Morten Simonsen was busy with new projects and asked me to revise the manuscript. I condensed the text, and chose a dramatic title. The low blood volume of the embryos had prevented coagulation studies. Accordingly, we did not use the expression "Disseminated Intravascular Coagulation" (DIC), but concluded that the syndrome "may have features in common with the severe haemorrhagic reactions seen after major surgery, pathological deliveries, and in malignancy. If this is correct, treatment of the disorders with antithrombin might be possible". The paper was quickly accepted and appeared in Science in June 1969 (20).

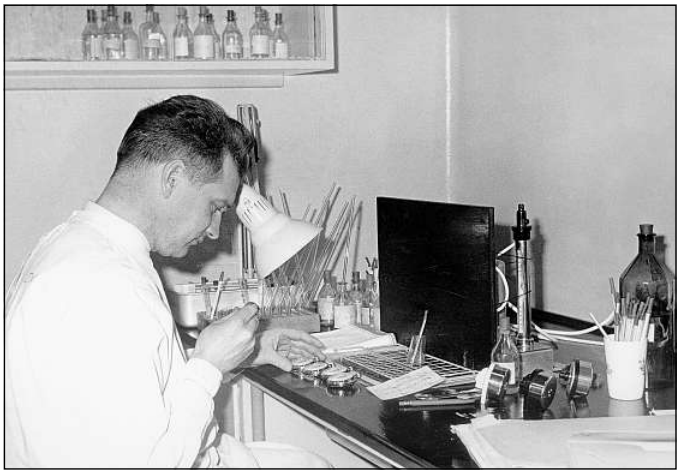

Figure 2: Olav Egeberg determining clotting times at the Coagulation Laboratory, Rikshospitalet, Oslo (1960).

Studies with a specific rabbit antiserum

My friend Magne Fagerhol (Fig. 4) prepared a monospecific antiserum in rabbits against human AT. We showed that human serum contained less AT antigen than did plasma (21), confirming the earlier findings of N. Heimburger (16). The antiserum blocked 70% of the progressive AT activity in normal human plasma, and abolished the heparin cofactor activity (21). The single radial immunodiffusion method was used in subsequent studies. Ö. Grimmer and M. Fagerhol later developed a rapid electroimmunoassay and by inclusion of heparin in the buffer, AT migrated in front of all other serum proteins (22).

O. Egeberg cooperated in a study that compared thrombin inhibition and the concentration of protease inhibitors in plasma samples from members of the "Mi family", of patients with DIC, severe VTE, and liver cirrhosis. In all these patient groups, AT activity was very low. The activity averaged 47% of the mean of control subjects, and was lower than AT antigen which was 58%, suggesting inactive AT molecules in these patients. This discrepancy was not observed in the group of unselected control subjects (23).

It was stimulating to cooperate with O. Egeberg. Besides his research in AT deficiency, his main research activity was in haemophilia. It was a great loss to our scientific community when he suddenly died in 1977.

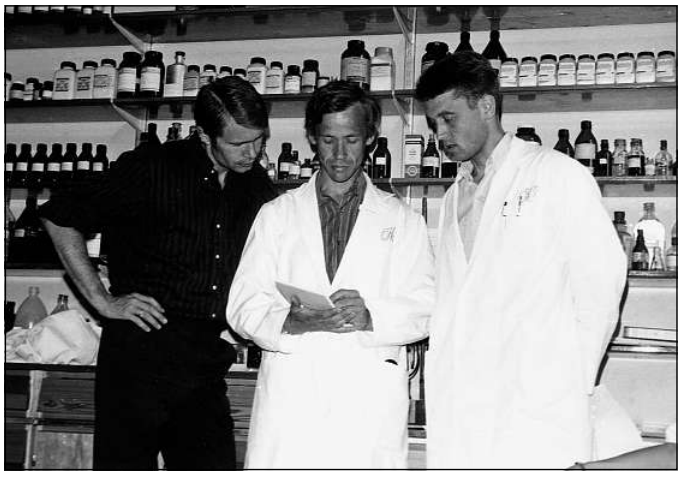

Figure 3: The author between his colleagues Claes Eika (left) and Harald Arnesen at the Haematological Research Laboratory, Ullevål University Hospital, Oslo (1968).

AT and hormones

It took a relatively short time for Magne Fagerhol to study the influence of age or sex on AT antigen in sera from about 2,000 healthy blood donors. I had argued that we should rather measure AT in plasma, and said "studying serum is like studying the battlefield after the fight is over". For practical reasons, obtaining 2,000 plasma samples was unrealistic. Our results indicated that below 45 years of age, AT antigen was lower in women than in males. Oral contraceptives (OC) appeared to lower the AT antigen level by about 15% (21). At that time the risk of VTE in women using OC was hotly debated. Our results indicated a substantial effect of estrogen on the serum AT level. Later studies have in fact shown that estrogen effects are somewhat less dramatic in plasma. Enhanced clotting may have contributed to the low serum AT levels. Our paper was much quoted and later qualified as a citation classic in Current Contents.

In two out of the about 2000 serum samples examined, AT was below 60% of mean for that age and sex group (21). This could mean that the two blood donors had an inherited AT deficiency, but for ethical reasons this track was not pursued.

As we had observed a relation between the lower AT levels in women using OC and their tendency to thrombosis, we later determined AT in about 25,000 serum samples from females using combined OC. In five of these women, the AT level was in the same low range as found in the "Mi family". Based on these findings, we suggested that the association of AT deficiency with thrombophilia might occur in about 1 in 5,000 in the Norwegian population (24). Altogether, 40 of the OC users were reported to us as developing VTE, but only in two of them a low AT level was found. Determination of AT prior to prescription of OC was not recommended.

AT becomes a major research issue

During the 1970s, screening for AT deficiency was performed in many clinical centres. Sophisticated studies on the AT molecule and its interactions resulted in new insights. I moved to Aker University Hospital in 1970 to work as a clinician in the department of medicine. Although I became responsible for a clinical coagulation laboratory, my commitment in basic AT research terminated here. As a clinician, care for patients affected by AT deficiency was a primary concern.

The following part of the present review concerns main findings of AT research obtained from 1970 and onwards. A detailed review of AT research in the very active 1970–1980 period was published in 1981 (24).

AT is a polyvalent serine protease inhibitor

AT also inhibits factor Xa (18, 25, 26), plasmin and trypsin (15, 16). Rosenberg et al. scaled up purification of AT and studied the inhibitory reactions in more detail. Heparin accelerated the inactivation of thrombin but did not change the 1:1 stochiometry. The thrombin-AT complex could not be dissociated with denaturating or reducing agents, while chemical modification of lysine residues eliminated heparin cofactor activity. It was postulated that heparin binding causes a conformational change exposing the arginine reactive site that reacts with thrombin (27). Further studies showed that AT in fact inhibited all the five serine proteases factors IIa, Xa, IXa, XIa, XIIa and that heparin accelerated the reactions (27, 28, 29). Moreover, AT with heparin inhibits factor VIIa, but AT alone is ineffective (30). Boyer et al. further demonstrated that generation of thrombin was accelerated in AT-deficient plasma (31). In solution, inactivation of human thrombin was more rapid than that of factor Xa, irrespective of whether heparin was present or not (32). E. Marciniak demonstrated that factor Xa bound to the factor V-phospholipd complex was relatively protected from inhibition (33).

Reaction rates of the inhibitory reactions were determined in purified systems and in plasma (34): Björk et al. showed in 1982 that following the initial binding of enzyme to AT, inactive, proteolytically cleaved inhibitor and free enzyme could be dissociated by treatment with ammonia or hydroxylamine. The enzyme had cleaved a single Arg-Ser bond in the carboxy-terminal end of the AT molecule (35), and the formation of cleaved, inactive AT is promoted by heparin (35, 36). A very slow release of thrombin from the thrombin-AT complex had been observed by W. Seegers already in 1964 (37). The reaction producing inactive inhibitor apparently competes with the formation of a stable enzyme-inhibitor complex (35). One may wonder whether the release of thrombin observed in these studies might relate to the surprising observations of H. Gasser in 1916 (2) regarding release of thrombin from "combined thrombin".

Interaction with glycosaminoglycans

Fractionations of AT cofactors according to affinity and size

In order to prepare sufficient AT for clinical use, the heparin affinity column was essential in plasma fractionation (38). Heparin is a heterogenous mixture of sulphated glycosaminoglycans (Lindahl [39], see page 109 in this issue). A comprehensive review of the separation of heparin into fractions with varying activity has been presented by T. Barrowcliffe (40). Main results may be summarized as follows: The AT affinity column separated heparin into fractions with high and low affinity to AT. About one third of unfractioned heparin was retained on an AT

affinity column, and these molecules contained the pentasaccharide which is the specific AT binding sequence in heparin.

Acceleration of the thrombin-AT reaction requires heparin molecules with 18 sugar units. Below that size, only factor Xa inhibition is accelerated. Animal studies suggested that low-molecular-weight heparin (LMWH) provoked less bleeding than unfractionated heparin. Clinical studies have at most shown a trend in favour of LMWH in this regard. Main advantages of LMWH are its longer duration in the circulation and a more predictable plasma concentration.

LMWH and AT deficiency

E. Marciniak had observed that during treatment with unfractionated heparin the AT level dropped by about 30%. In a patient with hereditary AT deficiency treated with unfractionated heparin, very low AT levels resulted (39). The AT level remained normal during thromboprophylaxis with LMWH in pregnant women (40). In hereditary AT deficiency, the AT level is not decreased by use of LMWH (U. Abildgaard, unpublished observations).

Heparan sulfate and vitronectin

In vivo, AT distributes between plasma, a non-circulating intravascular pool, and an extravascular pool. The stationary intravascular AT is probably attached to and activated by heparan sulfate (HS) of the endothelium (43). HS is known to accelerate AT action (44), and this stationary AT pool may be subnormal in individuals with hereditary AT deficiency (45). HS-bound AT may rapidly inactivate thrombin by forming the thrombin-AT (TAT) complex. The TAT complex may subsequently bind vitronectin forming a ternary vitronectin-TAT complex (46). Vitronectin serves, among other functions, as a regulatory adhesive protein at the blood-vessel wall interphase. Vitronectin-TAT complexes are rapidly cleared from the circulation (46) as they are taken up by hepatic HS proteoglycans which mediate their internalization and degradation (47).

The AT-enzyme interaction

AT is a member of the serpin superfamily of protease inhibitors that have broad specificity and share much of their protein structure as well as their inhibitory mechanisms. Comparative structural studies by crystallography and functional consequences of the various mutations have provided an understanding of the molecular interactions of AT with target enzymes (48). The molecule has an ellipsoid form, with a mobile peptide loop containing the reactive site extending from the molecule. A hinge region of the reactive loop makes it flexible, allowing for structural changes. Heparin bound to the heparin binding site of AT renders the reactive loop more accessible to the target enzymes. The enzyme first binds reversibly to the active loop of AT and in a second reaction, the enzyme splits the Arg-Ser bond of the reactive loop and is then drawn into the central part of the AT molecule, forming a covalent and stable enzyme-AT complex. Alternatively, the active enzyme may be released leaving behind an inactive inhibitor. Excess thrombin or the presence of heparin may favour the latter reaction (35, 37). Inactivated AT may explain lower activity in comparison to antigen levels in patients with hypercoagulation (23).

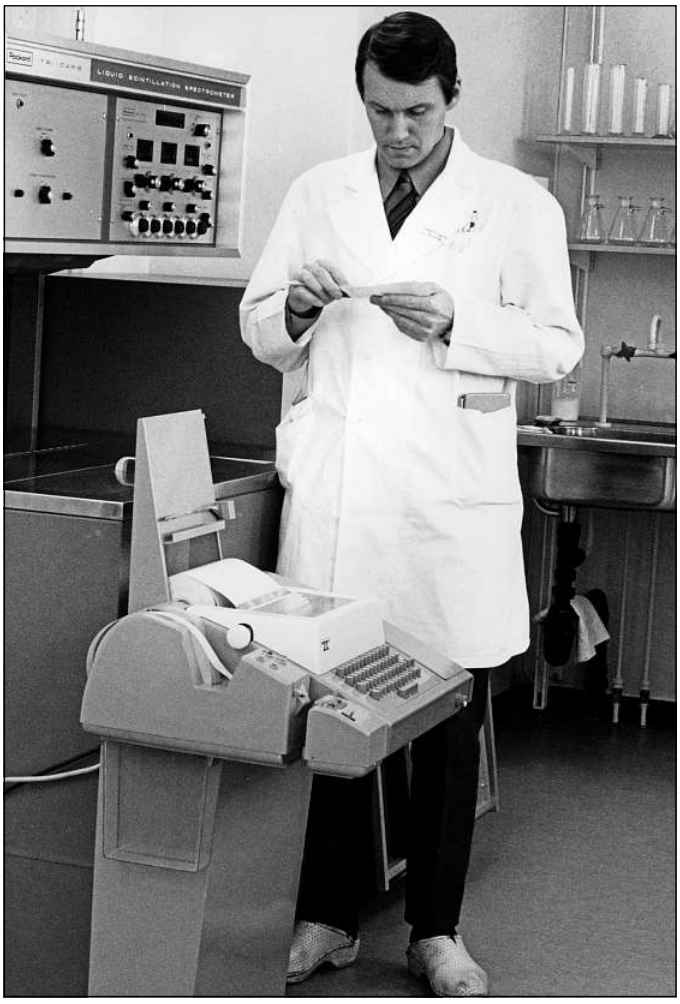

Figure 4: Magne K. Fagerhol in the laboratory of the Department of Immunology and Transfusion, Ullevål University Hospital (1980).

Clinical aspects

Hereditary AT deficiency: different types

Familial AT deficiency with the same characteristics as reported by O.Egeberg (9) was reported from 1973 on in many countries. Affected family members of this classical form of AT deficiency are heterozygotes, with half-normal AT concentration and half-normal functional activity. This is the classical type I AT deficiency.

An abnormal AT, "AT Budapest" as the cause of familial AT deficiency and thrombophilia was reported by Sas et al (49). AT antigen concentration was normal, but functional activities were low in affected family members. This qualitative type of deficiency is classified as type II (50). Type II deficiency is heterogenous, and the thrombotic tendency varies considerably. In some affected families, crossed immuno-electrophoresis may disclose loss of heparin binding in the abnormal AT fraction (49).

A clinically silent AT deficiency with normal antigen and progressive AT activity, but low heparin cofactor, was reported by Penner et al. (51) and was also identified in other families.

Heterozygotes have probably no increased risk of thrombosis, but homozygotes suffer from arterial and venous thrombosis.

A study in 9,669 blood donors revealed type I AT deficiency in two individuals, and type II AT deficiency in sixteen persons (49). None of these type II deficiencies appeared to confer a high thrombotic risk to the affected individuals, despite defective heparin binding of the AT molecule in only three subjects (52).

Functional assay of AT is usually performed with heparin in chromogenic substrate assays. If a low AT activity and the clinical situation suggest congenital AT deficiency, AT antigen assay should be performed to disclose a possible type II deficiency. If antigen concentration is normal, the progressive AT activity in absence heparin should be performed.

Mutations of the AT molecule

Human AT is a single chain glycoprotein of 432 amino acids, while the reactive site that is recognized and cleaved by target enzymes is located at Arg393-Ser394. Cloning of human AT was achieved in 1982 (51). The AT mutation database (52) presently covers about 130 different AT mutations. Type I mutations disrupt protein production from the affected allele. Type II mutations give rise to abnormal AT molecules which usually constitute 50% of the total AT in plasma of affected subjects. The mutations may be separated into three categories. The abnormal AT functions may be the result of effects on the reactive site, the heparin binding site, or to pleiotropic changes affecting the conformation of the molecule. Studies of the mutations have greatly contributed to elucidate the structure and functions of the AT molecule (48).

Combined thrombophilias

Thrombophilia caused by inherited deficiency of protein C or protein S has similar clinical consequences as those for AT deficiency, but coexistence of these deficiencies with AT deficiency is very rare. Mild thrombophilias due to the Leiden mutation of factor V or the prothrombin G20210A mutation are present in about 2–10 % of the European populations. These mutations are not rare in patients with AT deficiency. In combined thrombophilia, VTE often occurs in young age.

AT substitution in hereditary deficiency

Therapeutic AT concentrates were used in compassionate studies from the late 1970s, and approved by registration authorities in 1981–1982 (53). Conventional treatment with heparin of acute VTE in patients with AT deficiency may often be effective. If an additional thrombogenic factor such as pregnancy coexists, symptoms may progress despite adequate heparin treatment. In such situations, AT concentrate usually has a convincing beneficial effect (56, 57).

Perioperative substitution by AT (55) and prophylactic administration peripartum (58) to AT-deficient individuals have become adopted regimens. Recombinant human AT (rhAT) has been administered perioperatively to patients with AT deficiency with the desired effect. Owing to the rapid turnover of rAT, the total doses used were several times higher than routinely used with plasma-derived AT concentrate (59).

Acquired deficiencies of AT

Reduced synthesis or increased elimination

Low levels of AT may be caused by defective synthesis in liver disease, urinary loss and in L-asparginase treatment (24). Extremely low levels of AT have been reported in acute fatty liver of pregnancy (60). As already mentioned, treatment with unfractionated heparin reduces the AT concentration, probably caused by thrombin proteolysis (37). In patients admitted with massive thrombosis, AT levels may be subnormal in blood samples drawn before treatment was started, probably reflecting consumption of the inhibitor (24).

Consumption of AT in the DIC syndrome

The overt DIC syndrome occurs in patients with severe sepsis, obstetric complications and multiple trauma. Its clinical manifestations are bleeding and organ failure. Subnormal AT levels in experimental DIC were early recognized as caused by consumption coagulopathy (61). A striking drop in AT levels occurs in patients with the overt DIC syndrome (23, 62, 63, 64) and consumption has been confirmed by very rapid turnover of supplemented AT (62). Additional mechanisms that may suppress the AT level are proteolysis by elastase (64), reduced synthesis, and leakage from the vascular bed.

A low AT level in patients with DIC syndrome has serious prognostic significance. The AT level is an independent predictor of the clinical outcome in sepsis patients (65). In patients with a DIC-associated diagnosis treated in intensive care units, their lowest AT value was an independent predictor of 28 day mortality (66), as in patients with severe burns (67).

AT supplementation in DIC

In an experimental study in rats, DIC induced by infusion of *E. coli* was attenuated and mortality reduced when AT was supplemented to the infusion (68), while in some other animal studies, results were less clear. Taylor et al. reported that high doses of AT maintaining a level of 4 U/ml during infusion of endotoxin were required to prevent a fatal outcome in baboons (69). In a subsequent study, rhAT improved survival and attenuated inflammatory responses in baboons lethally charged with *E. coli*. High AT doses were used, and plasma levels were far above physiological levels. A detailed study of the marked effect on coagulation and inflammatory parameters suggested that the interaction of AT with the endothelium may be the key event to promote AT-dependent survival (70). AT treatment appeared to down-regulate the inflammatory syndrom in polytraumatized patients, with reduced levels of elastase and inflammatory interleukins (64).

Clinical trials of AT treatment in DIC

In the first clinical study, B. Blauhut and H. Vinazzer randomised 51 patients with shock and DIC to infusion of either AT, heparin, or both AT and heparin. Recovery of AT was higher by immunoassay than by activity assay. AT treatment was associated with significantly shorter duration of symptoms. In patients given AT and heparin, blood loss was increased, while mortality was not significantly affected. Substitution with AT resulted in a more rapid normalization of coagulation parameters, whereas addition of heparin showed no advantages and increased bleeding (62).

Additional randomised studies arrived at essentially similar results. AT supplementation was associated with significant more rapid recovery. Meta-analyses of several smaller randomised clinical trials with AT suggested significant reduction in mortality from severe DIC (71).

The large KyberCept trial included patients who additionally had received heparin as prophylaxis against VTE. The mortality was nearly identical in the AT treatment and the placebo group, interpreted as there was no indication for the use of AT treatment in this patient population (72). Members of the KyberCept trial group have performed post-hoc analyses of the results of the trial, and question the validity of the negative conclusion. Concomitant heparin administration was associated with excess bleeding which often was serious. The early warning about the ill effects of heparin combined with AT (60) had apparently been overlooked in the design of the trial. Five hundred thirty-six patients that did not receive heparin and DIC was confirmed through their laboratory parameters. In these patients, the 28-day mortality following AT treatment was 22.2% versus 40.6% in controls ($p < 0.01$) (73). Any bleeding was associated with a very high risk of fatal outcome in all patient groups at 90 days, while VTE during the initial four days was very rare (74).

Patients with the DIC syndrome require expensive intensive care unit attention, often for prolonged periods. Although the efficacy in terms of reduced all-cause 28-day mortality has not been proven, treatment with AT concentrate is still administered to many of these patients. Lacking a clear result regarding mortality, the documented improvement of DIC parameters, organ dysfunction, and length of stay might justify the treatment with AT. But these are too soft endpoints and cannot stand alone.

Present challenges

Improving the care for individuals with AT deficiency is the main clinical challenge today. However, variable practice reflects relative lack of solid knowledge. A new, carefully designed and strictly monitored randomised clinical trial appears to be required for a rationally based use of AT supplementation in sepsis and DIC. The design of such a trial is in itself a challenge (75). In the meantime, reporting to what extent this therapy actually is used in various centres and what the indications are could be hypothesis-generating. The therapy is expensive but may reduce total costs in the long run. Studies with a health economic approach are warranted.

In patients with type I AT deficiency, randomised trials may also be the best way of defining optimal strategies for prophylaxis of VTE. Even restricted to type I AT deficiency, stratification according to presence of additional risk factors such as the factor V Leiden mutation would be necessary. Therapeutic options after a first episode of VTE could be oral anticoagulation for indefinite time, or LMWH only in risk situations. For the multiple type II AT deficiencies, an international registry might be an approach to obtain systematic information.

There is a high thrombotic risk related to pregnancy for AT-deficient women. The risk obviously varies with the type of deficiency and the presence of other thrombogenic factors, but precise knowledge is scant. A large registry of the effects of prophylaxis against VTE used in women with defined type of AT deficiency is operative in France (J. Conard, personal communication) and will in time provide valuable data.

We have witnessed great achievements in AT research. Technological advances have been a main development factor. New developments create new possibilities, also for thrombophilia research and therapy (76). The physician who meets the patients usually can no longer perform the sophisticated experiments, but can communicate with the scientist. Ideas, as indispensable as the technology, grow from cooperation.

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