

This Week's Citation Classic®

Fagerhol M K & Abildgaard U. Immunological studies on human antithrombin III.

Influence of age, sex and use of oral contraceptives on serum concentration.

Scand. J. Haematol. 7:10-7, 1970.

[Natl. Blood Group Reference Lab., Natl. Inst. Public Health; Red Cross Blood Transfusion Ctr.; and Haematological Research Lab., Dept. IX, Ullevaal Hosp., Oslo, Norway]

Purification of antithrombin-III (At-III), production of antiserum in rabbits, and its use in functional and immunological assays are described. At-III is the dominant thrombin inhibitor and heparin cofactor. Variations in At-III levels related to age, sex, and use of oestrogen pills are described. [The *SCI*® indicates that this paper has been cited in more than 220 publications.]

The Dominant Thrombin Inhibitor

Magne K. Fagerhol

Blood Bank and Department of Immunology

Ullevaal Hospital

0407 Oslo 4

and

Ulrich Abildgaard

Medical Department A

Aker Hospital

0514 Oslo 5

Norway*

We had chosen one major serine protease inhibitor each as subject for our doctoral theses: α_1 -antitrypsin¹ and antithrombin-III (At-III).² We had realized the potential of immunological methods for studies on clotting factors, their inhibitors, and interactions between them. One of us (UA) had purified At-III, and the other (MKF) had spent a Council of Europe scholarship in the laboratory of Carl-Bertil Laurell Malmö, Sweden, to learn new immunological methods. We were thus

soon able to raise antibodies against At-III and complete the studies reported.

Among the obstacles was the need to avoid immunization against trace contaminants in the At-III preparations. This was achieved soon after submission of the manuscript in two alternative ways: (1) dissection of At-III immunoprecipitates in immunoelectrophoretic gels and their injection into lymph nodes in rabbits; and (2) further purification by preparative electrophoresis in agarose gels containing heparin; under such conditions At-III migrates in the prealbumin region.

How closely related our two serine protease inhibitors really are became dramatically evident 13 years later when M.C. Owen *et al.*³ described the Pi (Pittsburgh) variant of α_1 -antitrypsin where a single amino acid substitution made it a potent thrombin inhibitor, causing fatal bleedings.

The quantitative methods we developed were used to show that At-III is the most important thrombin inhibitor in man.⁴ We also screened for At-III deficiency in more than 30,000 women using or planning to use oral contraceptives. We found that At-III levels dropped gradually during the three weeks on the pill and that oestrogens were responsible for the effect.

We believe that our report attracted attention because so many colleagues are interested in the prevention of thrombosis and because our methods allowed large-scale testing in search of the few who have inherited At-III deficiency.

1. Fagerhol M K. *Studies on the Pi system of human serum α_1 -antitrypsin*. Oslo, Norway: Universitetsforlaget, 1969. (Cited 5 times.)
2. Abildgaard U. *Studies on the fibrinogen-fibrin conversion in human plasma. Influence of antithrombins, colloids, and ions*. Oslo, Norway: Universitetsforlaget, 1969. (Cited 5 times.)
3. Owen M C, Brennan S O, Lewis J H & Carrell R W. Mutation of antitrypsin to antithrombin. α_1 -antitrypsin Pittsburgh (358 Met→Arg), a fatal bleeding disorder. *N. Engl. J. Med.* 309:694-8, 1983. (Cited 120 times.)
4. Abildgaard U. Antithrombin and related inhibitors of coagulation. (Poller L, ed.) *Recent advances in blood coagulation*. London: Churchill Livingstone, 1981. Vol. 3. p. 151-73. (Cited 35 times.)

*Received July 16, 1990