

BRIEF REPORT: FATAL HEMORRHAGE IN A PATIENT WITH AN ACQUIRED INHIBITOR OF HUMAN THROMBIN

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THROMBIN (factor IIa) is a serine protease that performs a number of functions in blood coagulation.¹ Among its most important actions is converting fibrinogen into fibrin monomers, which polymerize to form the fibrin clot. Thrombin participates in the activation of factors V, VIII, and XIII, as well as of platelets.² By binding to thrombomodulin on vascular endothelial cells, it forms a complex that activates protein C, thereby limiting the extent of an emerging clot.³ Cleavage of thrombin's inactive zymogen precursor, prothrombin (factor II), is required to generate functionally active thrombin.

Acquired inhibitors of certain coagulation factors are relatively common, but autoantibodies against thrombin occur infrequently.⁴ Most acquired inhibitors of thrombin appear in patients exposed to topical preparations of bovine thrombin or fibrin "glue" during major surgery; these antibodies react mainly with bovine coagulation proteins.⁵⁻⁸ Acquired inhibitors directed primarily against human thrombin are usually associated with predisposing conditions⁷ and are extremely rare in patients without an underlying disease.

In this report, we describe a patient with a fatal bleeding diathesis caused by a spontaneously acquired inhibitor of thrombin. Clinical evaluation, laboratory investigation, and a full autopsy failed to reveal an underlying disease. Certain features of the clinical presentation and laboratory results should permit the early recognition of antibody inhibitors of thrombin and allow the institution of therapy.

CASE REPORT

The patient, a 72-year-old woman, presented with rectal bleeding, ecchymoses on the chest and back, and a hematoma of four days' duration on the upper right arm, measuring 6 by 10 cm. The hemoglobin level was 6.8 g per deciliter (4.22 mmol per liter), and the hematocrit was 20 percent, with normal red-cell indexes. The platelet count and the white-cell count were also normal. Results of coagulation tests were as follows: prothrombin time, 17.1 seconds (international normalized ratio, 2.1), activated partial-thromboplastin time, 60 seconds (ratio, 2.0), and thrombin time, 118 seconds (normal range, 16 to 27 seconds). The fibrinogen level was 3.81 g per liter (11.2 μ mol per liter) (normal range, 1.5 to 4.0 g per liter [4.4 to 11.8 μ mol per liter]), and the D-dimer assay for detection of fibrin-degra-

ation products was not elevated. Levels of serum iron, folate, and B₁₂ were normal, and the reticulocyte index was 4.6 percent (normal range, 0.5 to 1.7 percent).

The patient was not taking any medications, including nonsteroidal antiinflammatory agents, and denied any history of bleeding or alcohol abuse. Except for an appendectomy at the age of 10, she had never undergone a major surgical procedure.

The patient received two units of packed red cells, which increased her hemoglobin level to 10.4 g per deciliter (6.45 mmol per liter). On the fourth day after admission, flexible sigmoidoscopy established that the source of the patient's rectal bleeding was an anal hemorrhoid. Further studies of her coagulopathy demonstrated an IgG antibody directed against thrombin. Her hemoglobin levels remained stable while additional studies, including chest radiography, mammography, serum and urine protein electrophoresis with immunofixation, antinuclear-antibody screening with an autoantibody panel, and a bone marrow biopsy, were performed to rule out an occult cancer or autoimmune disorder. The results of all these studies were within normal limits. Treatment with 100 mg of prednisone per day was begun. On the 12th day after admission, she received another two units of packed red cells and was discharged, only to be readmitted later that same evening because of pain in the right hip.

On her readmission, physical examination was unremarkable and the hemoglobin level was 10.2 g per deciliter (6.33 mmol per liter). The patient's course was uneventful until the fourth day after readmission, when she was found to have increasing abdominal distention, large bilateral flank hematomas, and a hemoglobin level of 6.1 g per deciliter (3.79 mmol per liter). Despite plasma exchange and immunosuppressive therapy, she continued to hemorrhage and required intubation for ventilatory support. She had no response to further therapy and died 22 days after her original admission.

A postmortem examination revealed a large left retroperitoneal hematoma containing 3.5 liters of blood and a massive left hemothorax containing 4 liters of blood. It was concluded that the cause of death was respiratory failure, resulting from the hemothorax.

METHODS

Enzyme Immunoassays

Human prothrombin (Enzyme Research Laboratories), human thrombin (Sigma), bovine thrombin (Sigma), and bovine factor Va (Enzyme Research Laboratories) were diluted to 1 μ g per milliliter in phosphate-buffered saline and dialyzed against 0.05 M sodium carbonate (pH 9.6) at 4°C. Ninety-six-well plates were coated with 0.2 μ g of protein per well and incubated overnight at 4°C. Serial plasma dilutions (1:10 to 1:1,000,000) in phosphate-buffered saline were incubated for two hours at room temperature. After these samples were washed four times with Stago Wash (Stago Diagnostics), peroxidase-conjugated goat antihuman IgG (Sigma) was added at a dilution of 1:1000 in Stago dilution buffer (Stago Diagnostics) and incubated for two hours at room temperature. We added 200 μ l of peroxidase substrate (Sigma) and quantitated the development of color by measuring absorbance at 490 nm (V_{max} Kinetic Microplate Reader, Molecular Devices).

Immunoblotting

Human prothrombin, human thrombin, bovine thrombin, and bovine factor Va were diluted to 1 μ g per microliter in phosphate-buffered saline. From each sample, 2.5 μ g was electrophoresed in 12 percent polyacrylamide gel and then transferred to nitrocellulose membranes (Schleicher and Schuell) on a miniapparatus (BioRad). The filters were pretreated overnight at room temperature in blocking buffer (5 percent bovine serum albumin, 0.2 percent polysorbate 20 [Tween 20], 0.01 percent sodium azide) and then incubated with plasma from the patient diluted to 1:500 in blocking buffer for three hours at 25°C. After the filters were washed four times with 0.1 percent Tween 20 in 1 \times phosphate-buffered saline, peroxidase-conjugated goat antihuman IgG (Boehringer-Mannheim) at a dilution of 1:50,000 was added for one hour at 25°C. After the filters were

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Dr. Skålhegg is supported by a grant from the Norwegian Research Council.

washed six times as described above, enhanced chemiluminescence (ECL, Amersham) was performed and all filters were exposed to Hyperfilm-ECL (Amersham) for two minutes.

RESULTS

The dilute-Russell's-viper-venom assay for the lupus inhibitor and an anticardiolipin-antibody assay were negative. Fibrinogen levels were normal when measured with kinetic and nephelometric methods, thereby ruling out a dysfibrinogenemia. The thrombin time was remeasured with human thrombin in place of bovine thrombin and continued to be markedly prolonged (111 seconds). Incubation of the patient's plasma with normal plasma at a 1:1 ratio for 1 hour did not affect the thrombin time, which remained elevated at 99 seconds. These results suggested the presence of an inhibitor of human thrombin.

Assays for factors II, V, VIII, IX, and X (Table 1) showed decreased activity for all factors except factor V. In each case, a 1:1 mixture of the patient's plasma with normal plasma did not appreciably change the factor-activity level, whereas increasing dilution of the patient's plasma resulted in increased factor activities. Since the final step of each factor-activity assay requires that thrombin cleave fibrinogen to initiate clot formation, these activity assays also suggested the presence of an inhibitor of thrombin. After passing the patient's plasma over a protein A-Sepharose column (Sigma) to remove the IgG fraction, we obtained normal factor-activity levels. The effluent from the column gave normal prothrombin, activated-partial-thromboplastin, and thrombin times. We therefore concluded that the inhibitor was an IgG antibody.

The specificity of the patient's autoantibody inhibitor of thrombin was confirmed by immunoblotting (Fig. 1). Although blotted human and bovine thrombin incubated with the patient's plasma yielded bands corresponding to bound IgG, no binding was seen for human prothrombin or bovine factor Va. By contrast, plasma from a patient exposed to bovine coagulation factors (fibrin glue) during major surgery was intensely reactive with bovine factor Va, bovine thrombin, human thrombin, and even human prothrombin, indicating the presence of antibodies against various coagulation factors. No bands were seen when plasma from a healthy person was used (data not shown). These immunoblot results show that our patient's IgG inhibitor was directed against human thrombin and a cross-reactive site on bovine thrombin.

To measure the level of inhibitor, we performed direct enzyme immunoassays (Table 2). The patient's plasma bound to human thrombin to a titer of 1:10,000 but to bovine thrombin to a titer of only 1:100. Plasma from the patient exposed to fibrin glue gave the opposite result: binding to bovine thrombin was detectable to a titer of at least 1:1,000,000, whereas binding to human thrombin was not seen beyond dilutions of 1:100. Furthermore, undiluted plasma from our patient failed

Table 1. Results of Assays of Coagulation-Factor Activity.

FACTOR	ACTIVITY	1:1 MIX*	DILUTED PLASMA†	AFTER COLUMN‡
	%		%	%
II	25	NC	46	80
V	71	NC	128	ND
VIII	5	NC	80	138
IX	8	NC	16	76
X	42	NC	104	ND

*Denotes a 1:1 mixture of the patient's plasma with normal plasma. NC denotes no change.

†Plasma was diluted 1:40 in veronal buffer (Sigma).

‡Plasma was passed over a protein A-Sepharose column (Sigma). ND denotes not done.

to bind to bovine factor Va, although plasma from the patient exposed to bovine coagulation factors was reactive at a dilution of 1:1,000,000.

DISCUSSION

Our patient, a previously healthy elderly woman without any personal or family history of bleeding, presented to the emergency room with an acute hemorrhage. Coagulation tests revealed a prolonged prothrombin time, a prolonged activated-partial-thromboplastin time, and a markedly prolonged thrombin time. Factor-activity levels were markedly decreased, and mixing studies suggested the presence of an inhibitor of thrombin. We found that the inhibitor was an IgG antibody against a site on thrombin exposed or created after its conversion from prothrombin. The inhibitor's ability to interfere with cleavage of fibrinogen by both human and bovine thrombin suggests that it is directed against one or more conserved epitopes at the active site of thrombin, the fibrinogen-binding site, or both. Such binding characteristics imply a similarity to hirudin, a potent thrombin inhibitor known to occupy various functional sites on the thrombin molecule.² Further studies of our patient's inhibitor may determine its exact binding specificity.

Most examples of acquired thrombin inhibitors occur in patients exposed to bovine topical thrombin or fibrin-glue preparations during major surgery.⁷ Prospective studies have shown that one third to one half of such patients become immunized to bovine thrombin and bovine factor Va and have abnormal clotting-based coagulation.^{8,11} Most of these patients are asymptomatic, but some with thrombin inhibitors that cross-react strongly with human thrombin have severe hemostatic complications and may even bleed to death.¹¹

Acquired inhibitors directed specifically against human thrombin are rare, and they almost always occur in patients with an underlying disease, especially of the autoimmune type.⁷ Indeed, the first characterized inhibitor of thrombin was found in a patient with rheumatoid arthritis in 1957.¹² In 1964, Hawiger et al.¹³ isolated an inhibitor of thrombin from a patient with

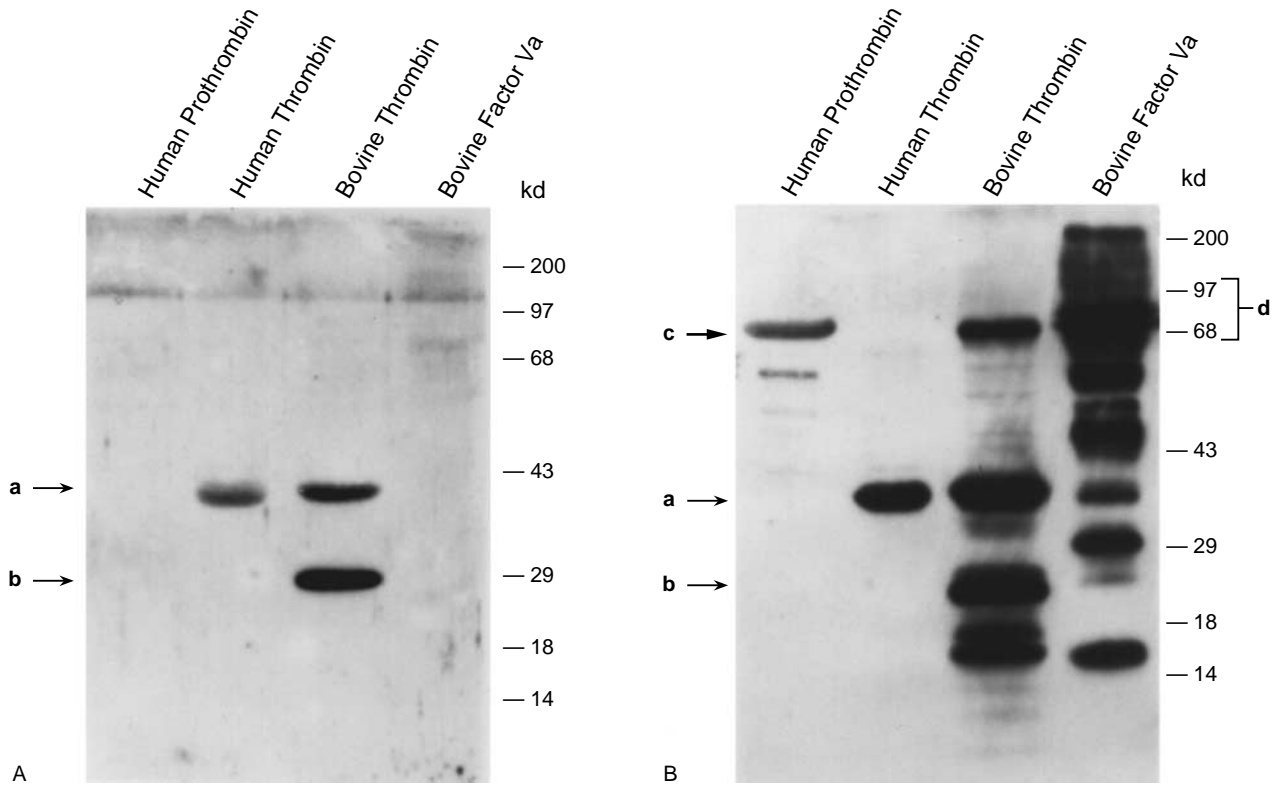


Figure 1. Detection of the Acquired Inhibitor of Thrombin by Immunoblotting.

Human prothrombin (factor II), human thrombin (factor IIa), bovine thrombin (factor IIa), and bovine factor Va were transferred to nitrocellulose membranes and incubated with 1:500 dilutions of plasma from the patient (Panel A). The blots were then incubated with a peroxidase-conjugated goat antibody against human IgG and subjected to enhanced chemiluminescence to detect immunoreactive proteins. In Panel A, arrow **a** points to α -thrombin (molecular weight, 38,000) and arrow **b** points to a form of bovine β -thrombin generated by additional cleavages in this species.^{2,9} Panel B shows that IgG in plasma from a patient exposed to bovine coagulation factors during surgery reacts with α -thrombin (arrow **a**), bovine β -thrombin (arrow **b**), human prothrombin (molecular weight, 72,000) (arrow **c**), and various cleavage products of bovine factor Va (bracket **d**).^{2,9,10} Comparison of Panels A and B shows that plasma from the patient exposed to bovine coagulation factors reacts with a number of coagulation factors, whereas the immunoreactivity of our patient's plasma is limited to thrombin.

systemic lupus erythematosus. An acquired inhibitor of thrombin has also been described in a patient in whom a lupus-like syndrome developed during procainamide therapy.¹⁴ Antibody inhibitors of thrombin differ from the more common antiprothrombin antibodies found in some patients with lupus inhibitors.¹⁵ These antiprothrombin antibodies are not neutralizing inhibitors, but

by binding prothrombin they accelerate its clearance from the plasma.¹⁶ In some cases (about 10 percent),¹⁷ the rapid elimination of prothrombin can cause profound hypoprothrombinemia and abnormal bleeding. In patients with acquired thrombin inhibitors, by contrast, the thrombin time is more prolonged than the prothrombin time, and a bleeding diathesis, which may be severe, is typical. In certain cases the acquired thrombin inhibitor binds to a site involved in the molecule's anticoagulant function and produces thrombotic disease.¹⁸ Some patients with either monoclonal or polyclonal gammopathies occasionally have inhibitors of thrombin.¹⁹⁻²²

Table 2. IgG Antibody Titers against Human and Bovine Coagulation Factors, as Measured by Enzyme Immunoassay.

SUBJECT	ANTIBODY TITER			
	HUMAN PROTHROMBIN (FACTOR II)	HUMAN THROMBIN (FACTOR IIa)	BOVINE THROMBIN (FACTOR IIa)	BOVINE FACTOR Va
Our patient	Negative	1:10 ⁴	1:10 ²	Negative
Normal control (no history of exposure)	Negative	Negative	Negative	Negative
Patient exposed to bovine thrombin	1:10	1:10 ²	>1:10 ⁶	>1:10 ⁶

Four reports²³⁻²⁶ have described thrombin inhibitors in patients without a diagnosed predisposing illness. One patient had evidence of underlying autoimmune disease²³; one probably had a lupus inhibitor²⁴; a third had recently undergone major surgery,²⁵ raising the possibility of exposure to bovine thrombin; and the fourth had a history of recurrent thrombosis.²⁶ Despite

an extensive workup for occult cancer and a complete autopsy, no evidence of systemic disease was uncovered in our patient. We therefore conclude that the patient's inhibitor occurred spontaneously. Despite therapy begun only one week after presentation, the patient had a massive hemorrhage and bled to death. Given her outcome and that of others like her,^{11,23} we recommend searching for an inhibitor of thrombin in patients with bleeding or thrombosis who present with prolongations in prothrombin time, activated-partial-thromboplastin time, and thrombin time but who have normal platelet counts and fibrinogen levels. Immediate and aggressive treatment of such thrombin inhibitors may reduce morbidity and mortality in these patients.

REFERENCES

- Colman RW, Marder VJ, Salzman EW, Hirsh J. Overview of hemostasis. In: Colman RW, Hirsh J, Marder VJ, Salzman EW, eds. Hemostasis and thrombosis: basic principles and clinical practice. 3rd ed. Philadelphia: J.B. Lippincott, 1994:3-18.
- Mann KG. Prothrombin and thrombin. In: Colman RW, Hirsh J, Marder VJ, Salzman EW, eds. Hemostasis and thrombosis: basic principles and clinical practice. 3rd ed. Philadelphia: J.B. Lippincott, 1994:184-99.
- Esmon CT, Owen WG. Identification of an endothelial cell cofactor for thrombin-catalyzed activation of protein C. *Proc Natl Acad Sci U S A* 1981; 78:2249-52.
- Feinstein DI. Acquired inhibitors of blood coagulation. In: Hoffman R, Benz EJ Jr, Shattil SJ, Furie B, Cohen HJ, eds. Hematology: basic principles and practice. New York: Churchill Livingstone, 1991:1380-94.
- Stricker RB, Lane PK, Leffert JD, Rodgers GM, Shuman MA, Corash L. Development of antithrombin antibodies following surgery in patients with prosthetic cardiac valves. *Blood* 1988;72:1375-80.
- Flaherty MJ, Henderson R, Wener MH. Iatrogenic immunization with bovine thrombin: a mechanism for prolonged thrombin times after surgery. *Ann Intern Med* 1989;111:631-4.
- Lawson JH, Pennell BJ, Olson JD, Mann KG. Isolation and characterization of an acquired antithrombin antibody. *Blood* 1990;76:2249-57.
- Banninger H, Hardegger T, Tobler A, et al. Fibrin glue in surgery: frequent development of inhibitors of bovine thrombin and human factor V. *Br J Haematol* 1993;85:528-32.
- Furie B, Furie BC. The molecular basis of blood coagulation. In: Hoffman R, Benz EJ Jr, Shattil SJ, Furie B, Cohen HJ, eds. Hematology: basic principles and practice. New York: Churchill Livingstone, 1991:1213-31.
- Hoyer LW, Wyshock EG, Colman RW. Coagulation cofactors: factors V and VIII. In: Colman RW, Hirsh J, Marder VJ, Salzman EW, eds. Hemostasis and thrombosis: basic principles and clinical practice. 3rd ed. Philadelphia: J.B. Lippincott, 1994:109-33.
- Nichols WL, Daniels TM, Fisher PK, Owen WG, Pineda AA, Mann KG. Antibodies to bovine thrombin and coagulation factor V associated with surgical use of topical bovine thrombin or fibrin "glue": a frequent finding. *Blood* 1993;82:Suppl 1:59a. abstract.
- Loeliger A, Hers JFP. Chronic antithrombinaemia (antithrombin V) with haemorrhagic diathesis in a case of rheumatoid arthritis with hypergamma-globulinaemia. *Thromb Diath Haemorrh* 1957;1:499-528.
- Hawiger J, Hanicki Z, Struzik T. On the immunologic nature of antithrombin in the course of lupus erythematosus disseminatus. *Acta Med Pol* 1964;5:53-60.
- Galanakis DK, Newman J, Summers D. Circulating thrombin time anticoagulant in a procainamide-induced syndrome. *JAMA* 1978;239:1873-4.
- Rapaport SI, Ames SB, Duvall BJ. A plasma coagulation defect in systemic lupus erythematosus arising from hypoprothrombinemia combined with antiprothrombinase activity. *Blood* 1960;15:212-27.
- Bajaj SP, Rapaport SI, Barclay S, Herbst KD. Acquired hypoprothrombinemia due to non-neutralizing antibodies to prothrombin: mechanism and management. *Blood* 1985;65:1538-43.
- Fleck RA, Rapaport SI, Rao LV. Anti-prothrombin antibodies and the lupus anticoagulant. *Blood* 1988;72:512-9.
- Arnaud E, Lafay M, Gaussem P, et al. An autoantibody directed against human thrombin anion-binding exosite in a patient with arterial thrombosis: effects on platelets, endothelial cells, and protein C activation. *Blood* 1994;84:1843-50.
- Craddock CG Jr, Adams WS, Figueroa WG. Interference with fibrin formation in multiple myeloma by an unusual protein found in blood and urine. *J Lab Clin Med* 1953;42:847-59.
- Frick PG. Inhibition of conversion of fibrinogen to fibrin by abnormal proteins in multiple myeloma. *Am J Clin Pathol* 1955;25:1263-73.
- Perkins HA, MacKenzie MR, Fudenberg HH. Hemostatic defects in dysproteinemias. *Blood* 1970;35:695-707.
- Barthels M, Heimburger N. Acquired thrombin inhibitor in a patient with liver cirrhosis. *Haemostasis* 1985;15:395-401.
- Sie P, Bezeaud A, Dupouy D, et al. An acquired antithrombin autoantibody directed toward the catalytic center of the enzyme. *J Clin Invest* 1991;88:290-6.
- Scully MF, Ellis V, Kakkar VV, Savidge GF, Williams YF, Sterndale H. An acquired coagulation inhibitor to factor II. *Br J Haematol* 1982;50:655-64.
- Gabriel DA, Carr ME, Cook L, Roberts HR. Spontaneous antithrombin in a patient with benign paraprotein. *Am J Hematol* 1987;25:85-93.
- Costa JM, Fiessinger JN, Capron L, Aiach M. Partial characterization of an autoantibody recognizing the secondary binding site(s) of thrombin in a patient with recurrent spontaneous arterial thrombosis. *Thromb Haemost* 1992; 67:193-9.

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