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ARTICLE *in* CANCER · FEBRUARY 2001

Impact Factor: 4.89 · DOI: 10.1002/1097-0142(20010215)91:4<636::AID-CNCR1046>3.0.CO;2-V

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Acquired Bleeding Disorder in a Patient with Malignant Lymphoma

Antibody-Mediated Prothrombin Deficiency

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Supported in part by a gift from the Pick Family Foundation.

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Received June 1, 2000; revision received October 25, 2000; accepted October 30, 2000.

BACKGROUND. Bleeding manifestations secondary to acquired hemostatic abnormalities in cancer patients have been well described. Bleeding due to the development of hemostatic inhibitors is observed less frequently. In this report, the authors describe a patient with a low grade lymphoma who presented with an acquired bleeding disorder and abnormal hemostatic screening tests.

METHODS. Patient plasma samples were collected initially and during the course of treatment. Mixing studies and specific coagulation factor assays were performed to detect and confirm any deficiencies. Patient immunoglobulin G was isolated from plasma, and binding to prothrombin was demonstrated by immunoblot method and enzyme-linked immunosorbent assay (ELISA) techniques.

RESULTS. Initial prolongations in the prothombin time and the activated partial thromboplastin time suggested a factor deficiency in the common pathway of coagulation. Factor assays confirmed that the coagulation abnormality in this patient was the result of an acquired prothrombin (factor II) deficiency. This was confirmed by an immunoassay for prothrombin antigen. Further studies demonstrated the presence of a noninhibitory antibody to prothrombin that interacted with a calcium dependent epitope.

CONCLUSIONS. Successful treatment of the lymphoma resulted in clearance of the antibody and complete correction of all hemostatic abnormalities and manifestations. An acquired prothrombin deficiency has not been reported previously in association with a malignancy, and this patient represents the first such documented case. ***Cancer* 2001;91:636–41.** *© 2001 American Cancer Society.*

KEYWORDS: hypoprothrombinemia, lymphoma, antiprothrombin antibody, bleeding disorder.

Abnormalities in the coagulation system have been well described in patients with malignant disorders. Hemostatic complications constitute the second most common cause of mortality in cancer patients.[1] The hemostatic system often is activated in cancer patients, which can result in thrombotic events.[2–4] The expression of procoagulant activity by malignant cells and/or as a result of therapeutic interventions in patients with cancer contribute to a significant risk of thrombosis.[4,5] In its most extreme form, hemostatic activation can result in disseminated intravascular coagulation.[6]

Although they are less common than thromboembolism, hemorrhagic disorders also have been observed in cancer patients. Metastatic disease and treatment with radiation or chemotherapy can result in bone marrow and/or hepatic dysfunction, leading to deficiencies of platelets and coagulation factors. In one study, clinically significant hemostatic deficiencies were observed in 15% of cancer

TABLE 1
Results of Serial Coagulation Studies From Initial Presentation to Recovery

		Pretreatment				
Test	**Normal value**	**12/98**	**1/99**	**2/99**	**4/99**	**6/99 (posttreatment)**
PT	10–13 seconds	13.3[a]	14.4[a]	15.5[a]	—	9.9
50/50 PT[b]	—	—	11	—	—	—
aPTT	35–44 seconds	56[a]	44.9[a]	—	50.6[a]	35.1
50/50 aPTT[b]	—	—	34	—	—	—
Factor II	70–150%	—	42[a]	39[a]	—	111
Factor V	70–150%	—	93	—	—	—
Factor X	70–150%	—	89	—	—	—
Prothrombin antigen	94–119 μg/mL	—	49[a]	37[a]	—	124

PT: prothombin time; aPTT: activated partial thromboplastin time.

[a] Abnormal assay results.

[b] The assay was repeated after a 1:1 mix with normal plasma.

patients.[7] However, transient defects in many patients may go unrecognized.

Less frequently reported is the development of hemostatic inhibitors. Hemostatic inhibitors are usually antibodies that interfere with clotting factor function and often lead to bleeding. Hemostatic inhibitory antibodies directed against factor VIII or the von Willebrand-factor VIII complex are the most commonly reported inhibitors in cancer patients.[8,9] However, inhibitory antibodies against other coagulation factors also have been reported.[9,10]

In this report, we described a patient with a low grade lymphoma who presented with an acquired bleeding disorder. Characterization of the coagulation abnormality in this patient demonstrated an acquired prothrombin (factor II) deficiency resulting from a noninhibitory antibody. The antibody discovered in this patient apparently accelerated the clearance of the coagulation factor. Successful treatment of the lymphoma concurrently resulted in a complete correction of the hemostatic abnormalities and clearance of the antibody.

CASE REPORT

A woman age 60 years was referred because of a 2-month history of recurrent leg swelling and extensive bruising of her arm and leg without associated trauma. Review of systems was unremarkable, with the exception of an 8-year history of easy bruising with minimal trauma. She denied any past or present bleeding from gums, mouth, urinary tract, or gastrointestinal tract. The patient's surgical history included a tonsillectomy at age 18 years, normal spontaneous vaginal deliveries of her children, and a hysterectomy at age 34 years for uterine fibroids. The patient denied any complications. Her family history was negative for bleeding disorders.

On physical examination, the patient was a well developed and slightly obese female. A tender, swollen left calf with multiple, large ecchymoses of varying age were observed. The patient was found to have left inguinal adenopathy (2 $cm^2 \times 2$ cm^2) and right axillary adenopathy (1 $cm^2 \times 2$ cm^2) on examination. The rest of her physical examination was unremarkable.

The initial laboratory evaluation revealed moderate anemia, with a hemoglobin of 10.4 g/dL with normal indices, a white blood cell count of $8.6 \times 10^3/\mu L$ with normal differential count, and a platelet count of $356 \times 10^3/\mu L$. The patient's prothrombin time (PT) and activated partial thromboplastin time (aPTT) both were prolonged (Table 1). The thrombin clotting time (TT) and a bleeding time were normal (Table 1). Further coagulation studies by 50:50 mix failed to demonstrate the presence of an inhibitor. Coagulation factor assays demonstrated a severe deficiency of prothrombin (Table 1). Serologic tests to detect underlying autoimmune disease all were negative, including antinuclear antibodies, DNA binding antibodies, rheumatoid factor, and complement levels. Tests for anticardiolipin, antiphosphatidylserine antibodies (immunoglobulin G [IgG], IgA, and IgM), and the lupus anticoagulant all were negative. Serum protein electrophoresis and a urine electrophoresis with immunofixation found no evidence of a monoclonal gammopathy.

Biopsies of both the inguinal and the axillary lymph nodes were performed. The patient developed significant hematomas and ecchymoses from the biopsy sites. Histologic examination of the biopsy subsequently revealed a small, cleaved, follicular center

cell non-Hodgkin lymphoma. The patient underwent staging by bone marrow biopsy, computerized tomography (CT), and gallium scan. The bone marrow biopsy revealed extensive bone marrow involvement (70%). The CT scan demonstrated bilateral axillary and inguinal adenopathy, and the gallium scan was negative for uptake.

The patient was treated with six cycles of chemotherapy, consisting of mitoxantrone and fludarabine. Coagulation studies (see Table 1) were normalized. One year after diagnosis, the patient is asymptomatic without bleeding symptoms and with no clinical or laboratory evidence of an underlying disease.

MATERIALS AND METHODS

Coagulation assays were performed using plasma collected in 0.45% sodium citrate. Plasma that was not used immediately was aliquoted, frozen, and stored at −70 °C. Screening assays, including PT, aPTT, TT, and ristocetin cofactor activity, were performed by using standard laboratory methods. Quantitative fibrinogen, protamine sulfate assay for fibrin monomer, D-dimer assay, dilute whole-blood clot lysis assay, template bleeding time, and specific coagulation factor assays were performed in accordance with published techniques used in our laboratory.[11–13] Prothrombin and von Willebrand antigen levels in patient plasma were measured by enzyme-linked immunosorbent assay (ELISA; Enzyme Research, South Bend, IN). Purified human prothrombin, factor X, and factor IX were prepared as reported previously.[12,13]

Patient and control IgG was isolated from stored patient plasma (both pretreatment and posttreatment) and from control normal plasma by sequential ammonium sulfate precipitation followed by DEAE-sephadex chromatography. Protein concentrations of the isolated IgG were determined using a Coumassie Blue protein assay (BioRad Laboratories, Hercules, CA).

The binding of the patient's pretreatment IgG to purified human prothrombin, factor X, and factor IX was evaluated using the coagulation proteins immobilized on nitrocellulose paper. Proteins in four serial dilutions from 5 μg were applied using a dot-blot apparatus, and the nitrocellulose paper was then incubated for 1 hour in 3% bovine serum albumin (BSA) in 0.05 M Tris and 0.15 M NaCl, pH 7.4 (TBS). After the blot was washed four times in TBS with 0.05% Tween 20, it was incubated for 3 hours at 37 °C with 0.25 μM of pretreatment antibody in TBS, 0.05% Tween 20, and 0.3% BSA. After washing the blot four times, bound human IgG was detected with goat antihuman IgG alkaline phosphatase-conjugated antibody using chemiluminescent detection (Immune Star chemiluminescence assay; BioRad Laboratories).

The interaction of patient pretreatment antibody and posttreatment antibody with purified prothrombin was evaluated in a direct-binding ELISA. A 96-well microtiter plate (Immulon II; Dynatech) was coated with 20 μg/mL of prothrombin in 0.05 M sodium borate, pH 8.5, overnight at 4 °C. The wells were then washed with TBS and blocked with 3% BSA in TBS. Wells were then incubated for 2 hours with serial dilutions of pretreatment, posttreatment, and normal IgG in TBS, 0.05% Tween 20, 0.3% BSA, and 3 mM $CaCl_2$. The wells were then washed with TBS, 0.05% Tween 20, and 3 mM $CaCl_2$. Bound antibody was detected with horseradish peroxidase-conjugated rabbit antihuman IgG. The wells were developed with o-phenylenediamine dihydrochloride substrate, and antibody binding was assessed by monitoring absorbance at 490 nm. In duplicate experiments, the calcium dependency of antibody binding was evaluated by washing the wells with TBS, 0.05% Tween 20, and 5 mM ethylenediamine tetraacetic acid (EDTA) before adding the detecting antibody.

RESULTS

Initial hemostatic screening assays demonstrated prolongations in both the PT and the aPTT that corrected on a 50:50 mix with normal plasma (Table 1). These findings suggested either a single factor deficiency in the common pathway of coagulation or the possibility of multiple factor deficiencies. Screening by prolonged incubation of the 50:50 mix also yielded a corrected PT and aPTT, which excluded the possibility of a functional inhibitor of coagulation factors. A normal thrombin time excluded a quantitative or qualitative defect in fibrinogen or the presence of a heparin-like anticoagulant (Table 1).

Screening assays excluded disseminated intravascular coagulation, accelerated fibrinolysis, or acquired von Willebrand. Specific coagulation factor assays revealed that the patient had a significant functional deficiency of prothrombin (Table 1).

An ELISA for prothrombin antigen demonstrated deficiencies in prothrombin that correlated with the functional deficiencies. Normal levels of factor X activity excluded any obvious hepatic synthetic defect or vitamin K deficiency, suggesting that the patient's prothrombin deficiency resulted from increased clearance (Table 1).

Patient IgG was isolated from both pretreatment and posttreatment plasma samples. The binding of the patient IgG to prothrombin and other vitamin K dependent coagulation factors, factor X and factor IX, was evaluated using an immunoblot method. The dot

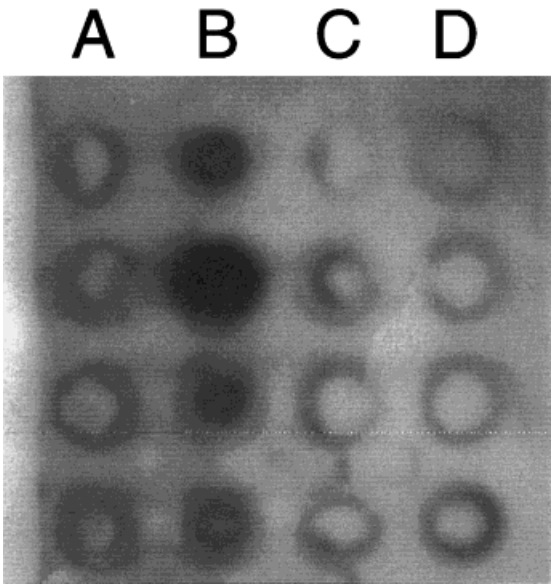

FIGURE 1. Dot blot showing the interaction of pretreatment antibodies with prothrombin, factor X, and factor IX. Lane A: buffer control; lane B: prothrombin; lane C: factor X; lane D: factor IX. Identical blots incubated with posttreatment and normal immunoglobulin G demonstrated no binding (data not shown).

blots demonstrated the presence of an antibody (IgG) that bound prothrombin, with no evidence of significant binding to factors IX or X (Fig. 1). Dot blots using posttreatment IgG failed to demonstrate binding with prothrombin, suggesting complete clearance of the antibody.

Purified patient pretreatment IgG and normal control IgG were mixed separately with normal plasma at a concentration of 100 μg/mL and were tested by coagulation assay (aPTT and PT) for inhibition. In this study, purified patient IgG did not prolong the coagulation assays over that observed with normal control IgG, supporting the previous studies and confirming that the antiprothrombin antibody did not inhibit prothrombin function (data not shown).

A direct-binding ELISA technique was used to quantify more carefully the interaction of the antiprothrombin IgG with prothrombin. The presence of bound antibody in pretreatment plasma was detected readily by a rabbit antihuman IgG conjugate. The posttreatment plasma demonstrated no such binding and paralleled the normal control plasma (Fig. 2). The assay was repeated in the presence of calcium and after washes with EDTA. This study demonstrated that the antibody detected in the pretreatment plasma samples was calcium dependent (Fig. 3). This would suggest that the antibody is directed against a prothrombin epitope that is metal dependent. This most likely would involve the amino-terminal γ-carboxyglutamic acid-containing domain of prothrombin.

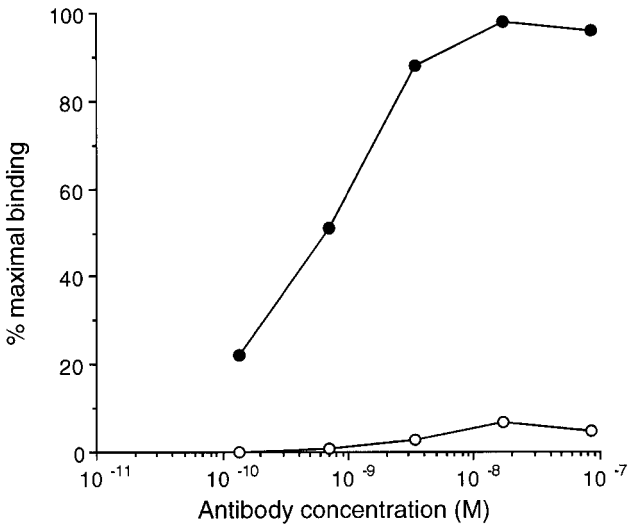

FIGURE 2. Direct binding enzyme-linked immunosorbent assay to evaluate the binding of pretreatment (solid circles) and posttreatment (open circles) patient immunoglobulin G to prothrombin.

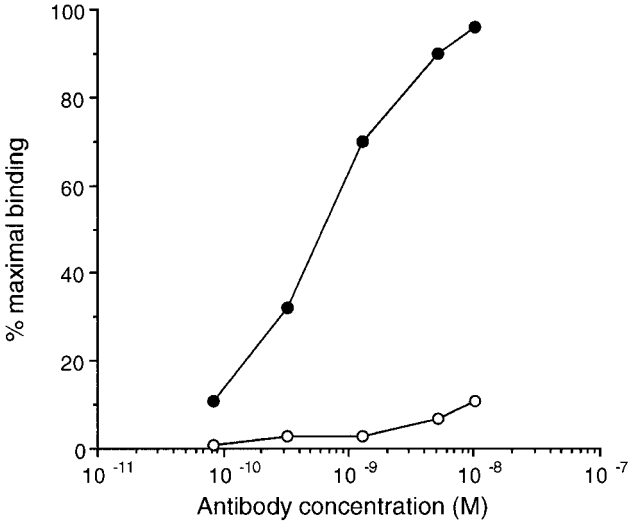

FIGURE 3. Binding of pretreatment immunoglobulin G to prothrombin in the presence of 3 mM $CaCl_2$ (solid circles) and 5 mM ethylenediamine tetraacetic acid (open circles).

DISCUSSION

In this report, we described a previously healthy woman who developed a hemorrhagic disorder associated with a low grade, non-Hodgkin lymphoma. Further studies demonstrated a deficiency of prothrombin due to a novel noninhibitory antiprothrombin antibody. This antibody bound to prothrombin in a calcium dependent manner, resulting in accelerated clearance of the protein. Although she manifested easy bruising for years, she did not seek medical advice until recurrent extremity swelling caused by her

lymphoma became evident. Clinically, treatment of her lymphoma resulted in a complete remission and correlated with clearance of the antibody with correction of all hemostatic abnormalities.

Hemostatic abnormalities observed with plasma cell and lymphoid neoplasms, such as multiple myeloma, plasma cell leukemia, Waldenstrom macroglobulinemia, lymphoma, and primary amyloidosis, are associated with paraprotein production.[14,15] Hemostatic abnormalities from such antibodies usually are not apparent clinically and are detected only by careful scrutiny of laboratory findings. Approximately 10% of patients will have purpura, epistaxis, or hematuria, and, although they are rare, severe and even fatal hemorrhages have been reported.[15] However, a serum protein electrophoresis in our patient failed to demonstrate the presence of a paraprotein.

The most common antiprothrombin antibodies described have been associated with the antiphospholipid syndrome.[16–18] Such antibodies are a heterogeneous family of immunoglobulins that, despite their name, do not bind to phospholipids but are directed at plasma proteins with affinity for anionic (phospholipid) surfaces.[18] Some of the antigenic targets of these antibodies include β2-glycoprotein I, prothrombin, high and low molecular weight kininogens, annexin V, (activated) protein C, and protein S.[19,20] Because most of the antigens are involved in blood coagulation, some antiphospholipid antibodies hamper the regulation of blood coagulation and provide an explanation for the prolongation of the aPTT, which is designated as the lupus anticoagulant.[18] However, the presence of these antibodies cannot readily explain the high rate of thrombosis in many patients with the lupus anticoagulant.

This patient demonstrated the presence of antiprothrombin antibody that binds to human prothrombin (factor II) and accelerated its clearance. In contrast to inhibitory antibodies associated with the antiphospholipid syndrome, the prothrombin antibody in our patient was not associated with lupus-like anticoagulant or antiphospholipid antibodies. The antibody in this patient was determined to be calcium dependent and did not inhibit prothrombin activity in vitro.

Antiprothrombin antibodies differ widely in their immunologic and functional properties and may vary by their affinity for prothrombin.[18] Except for the prothrombin antibody associated with the lupus anticoagulant, spontaneously acquired inhibitors to prothrombin are uncommon.[9] An antiprothrombin antibody that was not associated with any identifiable clinical entity was reported previously by Bajaj et al.[21] Those authors described a patient with an acquired prothrombin deficiency that was attributed to a depletion of plasma prothrombin antigen rather than functional impairment of prothrombin activity. Further studies demonstrated the presence of antibodies that bound to prothrombin in a calcium dependent manner and did not interfere with its coagulant activity, similar to our patient. Those authors postulated that the prothrombin deficiency stemmed from the rapid clearance from the circulation of prothrombin antigen-antibody complexes. This finding was supported clinically by the patient's response to corticosteroids, which are known to impair macrophage phagocytic activity and, thus, retard the clearance of these complexes.[17]

The patient described in this report represents another example of an acquired autoantibody associated with the presence of a lymphoproliferative disease. It is interesting to postulate that the patient's long history of easy bruising may have represented the first clinical manifestations of the onset of her low grade, small cleaved lymphoma, which was characterized on presentation by hypoprothrombinemia. However, an alternate explanation may be that the antiprothrombin antibody observed in this patient was a manifestation of an underlying autoimmune disorder that subsequently evolved into a lymphoproliferative process. Regardless of the mechanisms responsible for the development of the hemostatic disorder in this patient, we believe that this report represents the first description of an antiprothrombin antibody associated with a lymphoproliferative disorder.

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