Abnormal Fibrinogen in Thrombotic Thrombocytopenic Purpura

By Isaac Rivero and Norton D. Ritz

During the course of coagulation studies on blood of a patient with thrombotic thrombocytopenic purpura, an abnormal fibrinogen was identified by chemical and immunoelectrophoretic methods. The studies on this previously undescribed association form the basis of the present report.

Thrombotic thrombocytopenic purpura (TTP) is a frequently fatal disease characterized by thrombocytopenia, hemolytic anemia, shifting focal neurologic signs, fever, and renal involvement. Incomplete variants of the syndrome have been described. Pathologic confirmation requires the demonstration of widespread hyaline occlusions of terminal arterioles and capillaries. However, a typical clinical course without histologic confirmation may furnish enough for accepting this diagnosis.

The abnormal fibrinogen which was demonstrated in the patient’s plasma was antigenically similar to normal fibrinogen but migrated more rapidly toward the cathode.

Materials and Methods

Immunoelectrophoresis

Immunoelectrophoresis in agar was performed by the method of Grabar and Williams using barbital buffer (pH 8.6 and ionic strength 0.0375). To obtain immunoelectrophoretic patterns of all the proteins in serum or plasma, electrophoresis was performed for two hours. When plasma was studied specifically for fibrinogen, electrophoresis was performed for four hours.

Antisera

Antihuman fibrinogen antiserum was prepared by subcutaneous injection of Cohn’s fraction I* in rabbits. The antiserum so obtained were absorbed with normal human serum until a special antibody for fibrinogen was obtained. Antisera to human plasma, human serum, and human gamma globulins (Igα, Igδ, and Igγ) were also used.

From the Hematology Division, Department of Medicine, Maimonides Hospital, Brooklyn, New York, and the State University of New York, Downstate Medical Center, Brooklyn, New York.

These studies were supported by a grant from the Metropolitan Chapter of the National Hemophilia Foundation and the Murray Katchman Hemophilia Fund.

First submitted February 7, 1967; accepted for publication November 26, 1967.

Isaac Rivero, M.D.: Formerly Research Fellow in Hematology, Maimonides Hospital, Brooklyn, New York; Chief, Section of Hematology and Immunology, Internal Medicine II, Universidad Nacional de Cuyo Mendoza, Argentina. Norton D. Ritz, M.A., M.D., F.A.C.P.: Attending Hematologist and Chief of O.P.D., Hematology Clinic, Maimonides Medical Center, Brooklyn, New York; Clinical Assistant Professor of Medicine, State University of New York, Downstate Medical Center, Brooklyn, New York.

*Merck, Sharp and Dohme Co.
ABNORMAL FIBRINOGEN

Fibrogen Studies

1. Ammonium sulfate precipitation and thrombin methods. Levels of fibrinogen in plasma were determined by precipitation with ammonium sulfate and nephelometric measurement of the turbidity. In a second method, the fibrinogen was clotted by the addition of thrombin and the washed clot was quantitated by the Folin-Ciocalteu method.

2. Heat precipitation Method. Ten ml. of citrated plasma were incubated for 10 min. at 56 C. to precipitate fibrinogen. The precipitate was separated by centrifugation and carefully washed in cold saline. One ml. of 10 per cent sodium hydroxide was added and the tube was heated in boiling water for 10 min. to redissolve the precipitate. The solution was cooled and 9 ml. of saline added. The proteins were then determined by the Folin-Ciocalteu method.

3. Heparin cold precipitation method. Ten ml. of heparinized plasma (1 mg. of heparin per ml. of blood) were kept overnight in a refrigerator at 4 C. The protein content of the precipitate was assayed by a technic similar to that described for the heat precipitable material.

4. Cold-ethanol precipitation method (Cohn's fraction I). Two ml. of citrated plasma were treated with 1 ml. of 20 per cent ethanol in acetate buffer, pH 7.2 at minus 3 C. to obtain precipitation of fraction I. The precipitate was then redissolved in 0.9 per cent saline, and the electrophoretic characteristics of the fibrinogen contained in it were studied by immunoelectrophoresis. Fibrinolytic studies were performed using Astrup bovine fibrin plates, Chromium-labeled casein, and immune methods to detect degradation products of fibrinogen in the serum.

CASE REPORT

The patient was an eleven year old white boy who was in good health until May 1964, when he developed acute pharyngitis, for which he was treated with penicillin intramuscularly. Three weeks later he experienced severe headaches, fever, nausea, and abdominal pain. Upon admission to the hospital on June 25, 1964, he appeared pale and acutely ill. The temperature was 101 F., pulse 110 per min., respirations 20 per min., and the blood pressure 124mm./80mm. Numerous petechiae and ecchymoses were present on the extremities. The liver and spleen were each felt 2 cm. below the costal margins. The blood pressure ranged between 125mm./80mm. and 145mm./110mm. during the first week of his hospital stay; the temperature varied between 100 F. and 102 F.

The initial laboratory data were as follows: Hemoglobin 6.7 Gm. per cent, hematocrit 19 per cent, and reticulocytes 1.1 per cent. The red blood cells showed fragmentation, triangular cells, acanthocytes, and “helmet” shapes. Stippled and nucleated erythrocytes were also seen. White blood cells were 10,500/cu. mm. with 75 per cent segmented forms, 4 per cent bands, 10 per cent monocytes, and 11 per cent lymphocytes. Platelets were 4,000/cu. mm. A bone marrow examination showed an increased number of megakaryocytes and moderate erythroid hyperplasia. The megakaryocytes had smooth margins without apparent platelet formation. A tissue section of the bone marrow clot did not disclose any blood vessels. The Coombs antiglobulin test was negative. The coagulation studies revealed a coagulation time of 9 min. (Lee-White), bleeding time 6.5 min. (Duke), prothrombin time 12 sec. (Quick method), proaccelerin 80 per cent of normal, and partial thromboplastin time 43 sec. (normal 35 to 55 sec.). The thrombin time was 13 sec. (normal). Clot retraction was absent after 24 hours. No fibrinolysis was detected. The zone of lysis
Fig. 1.—Initial immunoelectrophoresis of patient's plasma. Immunoelectrophoresis for two hours in agar, barbital buffer. pH 8.6; ionic strength 0.0375. Upper well: normal plasma. Lower well: patient's plasma. Middle trough: anti-human plasma. The arrow points to the abnormal fraction of fibrinogen, not observed in normal plasma.

produced by the patient's plasma on an Astrup fibrin plate over 24 hours was 64 mm.², a normal value in this laboratory. The 51 chromium-tagged casein proteolysis assay of the patient's plasma was 4.98 per cent, (normal 2.3 to 4.5 per cent). The fibrinogen level determined by ammonium sulfate precipitation was 194 mg./100 ml. plasma. Blood urea nitrogen was 22 mg. per cent, and total bilirubin 1.2 mg. per cent. The serum complement level was 87 units (normal 60 to 150 units). Three LE cell preparations were negative. The urine showed 4+ proteinuria with large numbers of granular and red cell casts, abundant white blood cells, and a specific gravity of 1.025. The fasting blood sugar was 100 mg. per cent, creatinine 0.8 mg. per cent, and cholesterol 226 mg. per cent. The serum electrolytes were normal. The serum glutamic oxalotransaminase was 38 units, serum glutamic pyruvate transaminase 11 units, and the lactic dehydrogenase over 2,000 units. Stools were negative for blood on four occasions.

The patient was transfused with two units of packed red blood cells and given 40 mg. of prednisone daily.

Three days after admission, he developed slurred speech, photophobia, and confusion. He had four episodes of generalized and focal convulsions. The following day, he experienced a transitory hemiparesis and aphasia. The neurologic signs shifted and fluctuated in intensity during the next week. An electroencephalogram on July 24 showed a diffuse slowing of the alpha and delta waves. At this time, a diagnosis of thrombotic thrombocytopenic purpura was made on the basis of thrombocytopenia, hemolytic anemia, focal shifting neurologic signs, fever, and renal involvement.

Paper electrophoresis of the patient's plasma and serum was normal. Semi-quantitative determinations of gamma globulins and transferrin by agar diffusion technics were normal, but the haptoglobin was markedly diminished. Immunoelectrophoresis of the plasma compared with normal plasma or with
Fig. 2. — Immunoelectrophoresis of patient's plasma performed under the same conditions as in Figure 1 over four hours. Upper well: normal plasma. Lower well: patient's plasma. Middle trough: anti-human fibrinogen which detects fibrinogen but not IgM (to avoid overlapping of fibrinogen and IgM line). The patient's plasma contains a fraction antigenically identical to fibrinogen which migrates toward the cathode.

Fig. 3. — Immunoelectrophoresis of patient's plasma and serum for four hours. Upper well: patient's plasma. Lower well: patient's serum. Middle trough: anti-human fibrinogen. There are no degradation products of fibrinogen in the patient's serum, indicating no fibrinolysis.

the plasma of both parents revealed an abnormality which was present only in the patient's fibrinogen (Fig. 1). The abnormality consists of a fraction antigenically related to normal fibrinogen which migrated toward the negative pole as a second hump of the fibrinogen precipitation line. It appeared in an area where no precipitation lines had been previously observed in the plasma immunoelectrophoretic patterns of over one hundred normal persons and hospitalized patients. Immunoelectrophoresis of the plasma over a period of four hours using specific antihuman fibrinogen confirmed the abnormality (Fig. 2). However, the patient's serum did not contain degradation products of fibrinogen (Fig. 3), indicating that there was no fibrinolysis by methods then available. The collection of the patient's plasma with different anticoagulants including sodium citrate, sodium oxalate, heparin, or ethylene di-amine tetra-acetic acid (EDTA) did not affect the observed abnormality. The thrombin time of the patient's plasma was 13 sec., a normal value in this laboratory.

The assays of the patient's fibrinogen as performed by different technics are noted in Table 1. The fibrinogen was incompletely precipitated by ammonium sulfate but its clottability was preserved. Precipitation of the patient's fibrin-
Table 1.—Assay of Patient's fibrinogen by Various Methods

<table>
<thead>
<tr>
<th>Date</th>
<th>Ammonium Sulfate mg.%</th>
<th>Thrombin (Folin-ciocalteu) mg.%</th>
<th>Heat mg.%</th>
<th>Cold Heparin mg.%</th>
</tr>
</thead>
<tbody>
<tr>
<td>6/29/64</td>
<td>192</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>7/13/64</td>
<td>62</td>
<td>220</td>
<td>35</td>
<td>45</td>
</tr>
<tr>
<td>7/30/64</td>
<td>60</td>
<td>300</td>
<td>60</td>
<td>—</td>
</tr>
<tr>
<td>8/5/64</td>
<td>98</td>
<td>250</td>
<td>180</td>
<td>20</td>
</tr>
<tr>
<td>8/19/64</td>
<td>185</td>
<td>300</td>
<td>200</td>
<td>18</td>
</tr>
<tr>
<td>5/26/65</td>
<td>194</td>
<td>283</td>
<td>120</td>
<td>—</td>
</tr>
<tr>
<td>Normal Values</td>
<td>200-400</td>
<td>200-400</td>
<td>150-300</td>
<td>15-30</td>
</tr>
</tbody>
</table>

Fig. 4.—Patient's clinical course demonstrating initial favorable response of Hemoglobin, Reticulocytes and Platelets to 200 mg. of prednisone daily, his relapse after nine months, and his response again to 60 mg. of prednisone daily.

Fibrinogen by heat was also impaired. Cold heparin precipitated an increased amount of protein from the patient's plasma which was not well characterized electrophoretically, since it did not completely redissolve in normal saline. It was not certain that this material was fibrinogen. In summary, the abnormal fraction was probably not precipitable by ammonium sulfate or heat, but it was clottable by thrombin and precipitable by ethanol.

Two days after the appearance of neurologic symptoms, the patient's daily dose of prednisone was increased from 40 mg. to 200 mg. and maintained at this level for the next two weeks. The clinical and laboratory manifestations began to improve rapidly. The temperature returned to normal within two days. The neurologic symptoms cleared after four days. The proteinuria and urinary formed elements disappeared in two weeks. The blood urea nitrogen,
which had risen to 37 mg. per cent four days after admission, fell to a normal level two weeks later. The reticulocytes, hemoglobin, and platelets began to rise (Fig. 4). After twelve days, the liver and spleen were no longer palpable.

The patient’s fibrinogen was re-examined serially and showed reversion to normal characteristics and values 24 days after admission, as well as a disappearance of the abnormal fraction previously found in immuno-electrophoresis (Table 1 and Fig. 5). The patient was discharged much improved after five weeks. The dose of prednisone was gradually tapered during the next month and discontinued after another month. His clinical course is shown in Figure 4.

In May 1965, a year after the onset of his illness, the patient had a recurrence of symptoms following a sore throat and several days of fever. He received no penicillin or other antibiotics. The hemoglobin fell to 10 Gm. per cent, reticulocytes rose to 12.5 per cent, and platelets dropped to 25,000 (Fig. 4). Abnormal fragmented and contracted erythrocytes were again visible in the blood smear. Proteinuria recurred. Immuno-electrophoresis of his plasma again showed the abnormal fibrinogen found at the onset of the disease (Fig. 5). Prednisone in a dose of 60 mg. produced a prompt clinical remission...
within two weeks and a disappearance of the abnormal fibrinogen. After three
months, the prednisone was stopped. The blood and urine remained normal.
In July 1965, a kidney biopsy was performed which showed normal glomeruli
by light and electron microscopy. An immunofluorescent study with fluores-
ccein-labeled antihuman fibrinogen on formol fixed kidney tissue failed to
demonstrate deposition of material antigenically related to fibrinogen. An intravenous pyelogram was normal. The patient has continued to be clinically and
hematologically normal up to the present time, three years after the onset of
the illness.

**Discussion**

Although histologic confirmation was not obtained, it is felt that the typical
clinical and hematologic findings justified the diagnosis of thrombotic throm-
bocytopenic purpura in this patient. He is alive and well three years after the
original illness following the use of “large” doses of prednisone alone. A relapse of the condition one year after its onset was controlled by smaller
doses of prednisone.

Several cases of thrombotic thrombocytopenic purpura have been reported
in which the plasma fibrinogen was reduced, presumably on the basis of
intravascular clotting and defibrination. It is conceivable that in other patients
fibrinogen may be altered enzymatically without being measurably reduced.
The method of fibrinogen assay can also introduce discrepancies (Table 1),
as noted in this patient. The absence of an abnormal fibrinogen in the subject’s
parents excludes a congenital fibrinopathy.

Beck and Jackson have recently demonstrated that when fibrinogen was
partially degraded by progressively longer exposure to plasmin or trypsin, the
precipitability of fibrinogen by ammonium sulfate or by heat also diminished
progressively. The clottability of the degraded fibrinogen by thrombin was
preserved longer during plasmin incubation than with trypsin. With both
enzymes, breakdown products of fibrinogen were observed to migrate more
rapidly to the cathode. On immunoelectrophoresis, this produced lengthening
of the fibrinogen precipitin line toward the cathode. The diminished ammon-
ium sulfate and heat clottability along with preservation of thrombin clottabil-
ity of the altered fibrinogen and an abnormal immunoelectrophoretic pattern
produced by these workers experimentally correspond to the findings observed
in this patient. We have been able to experimentally reproduce a similar altera-
tion in human fibrinogen by incubating fresh plasma with papain. It would,
therefore, appear that the disease-state thrombotic thrombocytopenic purpura
may be associated with a pathologically altered, partially degraded, circulating
fibrinogen.

**Summary**

An abnormal fibrinogen was demonstrated in the plasma of an eleven year
old boy with thrombotic thrombocytopenic purpura during the acute disease
and during one clinical relapse nine months later. The altered fibrinogen was
antigenically similar to normal fibrinogen but migrated more rapidly toward
ABNORMAL FIBRINOGEN

the cathode. The abnormal fibrinogen was incompletely precipitated by ammonium sulfate or heat, but was clottable by thrombin.

Thrombotic thrombocytopenic purpura may be associated with incomplete in vivo defibrination and a circulating partially degraded fibrinogen. A patient with this syndrome is alive and well forty months after treatment with 200 mg. of prednisone daily.

SUMMARIO IN INTERLINGUA

Un fibrinogeno anormal esseva demonstrate in le plasma de un puero de dece-un annos de etate con thrombotic purpura thrombocytopenic durante le phase acute del morbo e durante un recidiva clinic novem menses plus tarde. Le alterate fibrinogeno esseva antigenicamente simile a fibrinogeno normal sed migrava plus rapidemente verso le cathodo. Illo esseva precipitate inconipletemente per sulfato de ammonium o per calor sed esseva coagulabile con thrombina.

Thrombotic purpura thrombocytopenic pote esser associate con incomplete disfibrination in vivo e un partialmente degradate fibrinogeno in le circulation. Un patiente con iste syndrome vive e se porta ben quaranta menses post le institution de un tractamento con prednisona a un dosage diurne de 200 mg.

REFERENCES

Abnormal Fibrinogen in Thrombotic Thrombocytopenic Purpura

ISAAC RIVERO and NORTON D. RITZ