Thrombotic Thrombocytopenic Purpura: Report of a Case With Disseminated Intravascular Platelet Aggregation

By P. B. Neame, J. Lechago, E. T. Ling, and A. Koval

The nature and etiology of the vascular occlusions encountered in thrombotic thrombocytopenic purpura (TTP) have been subject to controversy for a number of years. Disseminated platelet thrombosis has been suggested in the earlier literature, although later views have favored fibrin thrombi resulting from vascular damage or disseminated intravascular coagulation (DIC). The serial coagulation study and the light- and electron-microscopic findings in a case of TTP are described here. The multiple vascular occlusions were due to the presence of densely packed platelet aggregates in which a variable quantity of fibrin was present. Less commonly, loose platelet aggregates were noted. Fibrin under the endothelial lining was occasionally observed in relationship to the vascular occlusion and was thought to be secondary to the release of various substances from aggregating platelets. The serial coagulation study and the histologic examination of tissues showed no evidence of disseminated intravascular coagulation. This case shows that the occlusions observed in TTP can be due to disseminated intravascular platelet aggregation in the absence of DIC. Although TTP might be of variable etiology, it is felt that cases showing disseminated intravascular platelet aggregation should be distinguished from DIC in order to plan therapy on a rational basis.

Since Moschowitz' first described thrombotic thrombocytopenic purpura (TTP) there has been a lack of understanding of its etiology and controversy over the nature of the thrombi seen in the small vessels in this condition. The occlusions were initially considered to be agglutinated erythrocytes but were later thought to be due to platelet thrombi. Altschule considered the thrombotic lesion to consist of platelets but felt that capillary damage was the earliest manifestation of the disease. Other writers agreed that they were platelet thrombi, but the vascular origin of the occlusion was stressed when subendothelial hyaline material was noted infrequently in capillaries or arterioles. Orbison thought that marked vascular dilatation was a characteristic and significant lesion. Craig and Gitlin concluded, in 1957, that the hyaline thrombi were composed of a saline insoluble derivative of fibrinogen or fibrin and that immunohistochemically reactive platelet material was not present in the lesion. Subsequent electron-microscopic study by Feldman et al. showed subendothelial deposits of fibrin, and their ultrastructural and immunohistochemical observations suggested that TTP was a widespread vas-
cular disease in which fibrin precipitated within both the walls and the lumina of the vessels where it was accompanied by aggregated platelets. Umlas and Kaiser,\textsuperscript{11} when reviewing the subject in 1970, felt that TTP might be produced by primary vascular diseases, such as vasculitis and glomerulonephritis, or might be secondary to diseases causing disseminated intravascular coagulation (DIC). Antiplatelet agents have since been used in TTP and have been found to be occasionally followed by remission of the disease.\textsuperscript{12,13} This has led to the suggestion that intravascular platelet aggregation had taken place on damaged vessel walls.\textsuperscript{12}

We have recently seen an acute case of TTP showing marked thrombocytopenia, hemolytic anemia with numerous fragmented erythrocytes, including helmet cells, in the blood, fluctuating neurological signs, evidence of renal damage, and fever. In this paper we record the serial hemostatic survey as well as the light- and electron-microscopic findings observed in this case and provide evidence that, at least in some cases, TTP is a disturbance in which disseminated intravascular platelet aggregation is a prominent feature.

\textbf{CASE REPORT}

A 54-yr-old female was admitted to the Hotel Dieu Hospital on September 25, 1972 in a stuporous state. A week before admission malaise, aches, and pains developed, progressing to nausea, vomiting, increased fatigue, spontaneous bruising, and somnolence. Admission was precipitated by deterioration in the state of consciousness and development of expressive aphasia.

On admission physical examination revealed a moderately obese white female with pallor, icterus, expressive aphasia, petechiae on the trunk and lower limbs, and hemorrhages in the right fundus. Her temperature was 38.5°C, blood pressure 150/75, pulse rate 65/mm, and a respiratory rate of 20/min. Her neurological state varied showing a fluctuating right hemiplegia together with periods of restlessness associated with an expressive aphasia. There was no hepatosplenomegaly, lymphadenopathy, or arthritis.

\textit{Investigations}

The hematocrit was 21\% with a reticulocyte count of 10\%. Total white cell count was 8450/cu mm, with 86\% neutrophils, 1\% bands, 10\% lymphocytes, 2\% monocytes, and 1\% basophils. Platelets numbered 15,000/cu mm. A blood film showed marked anisocytosis and poikilocytosis with numerous fragmented and helmet forms (Fig. 1). Urinalysis showed 2+ proteinuria, a moderate number of epithelial cells, 4-6 granular casts, a few epithelial casts, 5-6 red cells and 2-5 white blood cells/hpf, a moderate amount of hemoglobin, and no glucose or acetone. Blood electrolytes and random sugar were normal. Serum bilirubin was 5.1 mg/100 ml (indirect 4.0 mg/100 ml), serum creatinine 2.1 mg/100 ml and blood urea nitrogen 19 mg/100 ml. Direct and indirect Coombs test and antinuclear factor were negative. Alkaline phosphatase was 2.1 Bodansky units and SGOT 145 units (normal 8-40). Three blood cultures were negative. Stool was positive for occult blood. Electrocardiogram showed possible inferolateral ischemia.

![Fig. 1. Peripheral blood smear showing numerous fragmented red cells including helmet forms, triangular cells and occasional microspherocyte. Note absence of platelets. Leishman stain. x 450.](attachment:fig1.png)
Table 1. Thrombotic Thrombocytopenic Purpura—Serial Hemostatic Survey

<table>
<thead>
<tr>
<th>Day of Hospitalization</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<th>9</th>
<th>10</th>
<th>11</th>
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<td>Platelet count (x 1000/cu mm) N 150-400,000/cu mm</td>
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<td>16</td>
<td>42</td>
<td>20</td>
<td>22</td>
<td>7</td>
<td>8</td>
<td>8</td>
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<tr>
<td>Thrombin time (N 14 sec)</td>
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<td>13</td>
<td>14</td>
<td>15</td>
<td>18</td>
<td>20</td>
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<td>17</td>
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<tr>
<td>Fibrinogen level (N 1/64-1/512)</td>
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<td>1/512</td>
<td>1/512</td>
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<td>Plasma fibrinogen (N 200-400 mg/100 ml)</td>
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<td>300</td>
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<td>Fibrinogen degradation products (N &lt; 10 µg/ml)</td>
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<td>Partial thromboplastin time (N 25-45 sec)</td>
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<td>28</td>
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<td>34</td>
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<td>46</td>
<td>33</td>
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<tr>
<td>One stage prothrombin time (N 12-14 sec)</td>
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<td>13</td>
<td>14</td>
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<td>14</td>
<td>20</td>
<td>30</td>
<td>36</td>
<td>14</td>
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</table>

* Splenectomy performed
† Vitamin K1 administered

Skull x-rays were normal. Echoencephalogram revealed no midline shift. Electroencephalogram showed changes compatible with brainstem dysfunction. Bone marrow aspiration showed normoblastic erythroid hyperplasia with slightly increased numbers of megakaryocytes. No blood vessels were seen on section of the marrow particles.

The diagnosis of TTP was considered on admission and treatment was initiated with intravenous hydrocortisone 1 g/day and dipyridamole 600 mg/day via nasogastric tube. Her condition deteriorated and she continued to have fluctuating neurological findings. Splenectomy was performed on the fifth hospital day with no beneficial effect. Rectal aspirin, 600 mg/day was substituted for the dipyridamole. She developed focal and generalized seizures terminally and expired on the twelfth hospital day.

Hemostatic Survey

Table 1 contains the results of the daily hemostatic survey. Thrombocytopenia is severe but the fibrinogen levels are within normal limits. There is evidence of mild fibrinolysis. Postoperatively vitamin K deficiency is also noted. This was corrected by vitamin K1 therapy.

Histologic Examination

Sections were obtained from a pre-mortem splenectomy specimen and from a full necropsy performed 11 h after death. Tissues for light microscopy were fixed immediately in 10% buffered formalin. Sections were stained with hematoxylin-phloxin-saffron, phosphotungstic-acid hematoxylin (PTAH), periodic acid Schiff (PAS), Alcian blue, Verhoeff’s elastic Van Gieson, Masson’s trichrome and silver impregnation for reticulin. For electron microscopy, tissues which had been primarily fixed in 10% formalin for 2-15 days were cut into small blocks and transferred for 2 h to 3% glutaraldehyde in 0.1 molar phosphate buffer, pH 7.2. These tissues were then processed for electron microscopy in the routine way. Electron micrographs were taken with a Hitachi HU 11C electron microscope.

Light Microscopy

The most striking lesion was the occlusion of capillaries and arterioles of multiple organs. These included the heart, brain (mainly cerebral cortex), pancreas, and kidneys and, to a lesser extent, adrenals, neurohypophysis, bone marrow, and lungs. Small artery occlusions were also occasionally observed in the heart, kidneys, and pancreas and were the prominent lesion in the spleen.

The capillary and arteriolar occlusions contained amorphous granular material; the latter form resembled aggregated platelets and could be more easily seen in the loosely aggregated portions (Fig. 2). Staining with PTAH showed either no fibrin or, more commonly, a few fibrin strands
surrounding and sometimes penetrating clumps of amorphous or granular material (Fig. 3). Endothelial proliferation was observed in some of the capillaries and arterioles and was prominent in the brain. The small artery occlusions showed two forms. In the less common type, loosely aggregated platelets were observed (Fig. 4). In the more frequent type, clumps of amorphous or granular material, reminiscent of densely packed platelets, were noted. Staining with PTAH
Fig. 4. Small splenic artery occluded by loose platelet aggregates. An occasional red and white blood cell is observed. (HPS).

showed no fibrin in association with loosely aggregated platelets, whereas variable quantities of fibrin formed a rim around amorphous or granular clumps (Fig. 5). Endothelial proliferation was seen in some of the small arteries with this type of thrombus.

Ischemic lesions were not frequent even when numerous capillaries were involved, but small infarcts were noted in the spleen, kidneys, myocardium, and pancreas, usually near occluded small arteries. In the brain marked endothelial proliferation of capillaries and small arterioles was associated with small cortical foci of ischemic necrosis and hemorrhage.

Electron Microscopy

Electron microscopy clearly detailed the structure of the occlusions and confirmed the impression gained by light-microscopic examination. The most common lesion was composed of tightly packed platelets showing varying degrees of cytoplasmic alteration and degranulation interspersed with variable quantities of fibrin (Figs. 6 and 7). Fibrin was also found in small amounts underneath the endothelial lining (Fig. 6) and, occasionally, among the muscle cells of

Fig. 5. A small splenic artery shows amorphous and granular clumps of material, reminiscent of platelets, surrounded by fibrin. Endothelial proliferation is present. (PTAH).
Fig. 6. Electron micrograph of an occluded small splenic artery. Tightly packed platelets (Plt), showing various degrees of alteration and with varying quantities of interspersed fibrin (Fib) can be seen. A small amount of subendothelial fibrin (Fib) is observed. Endothelial cell (End). X 6000.

Fig. 7. A higher magnification of area framed in Fig. 6 is shown. Altered platelets with interspersed fibrin can be seen. X 27,000.
larger vessels. The endothelial cells of small arteries first showed evidence of phagocytic activity and later proliferation. Some vessels showed prominent endothelial proliferation and, rarely, a polymorphonuclear leukocyte or fibroblast could be seen in the wall.

DISCUSSION

The problem encountered in the discussion of TTP is to define the clinical and pathological parameters characterizing this entity. In its typical acute form the major clinical manifestations are thrombocytopenia, hemolytic anemia with characteristically fragmented red blood cells, fluctuating neurological findings, fever, and renal abnormalities. The pathological diagnosis has been equated with a demonstration of hyaline thrombi in the microcirculation. These clinical and pathological findings, however, are found in other conditions, and some cases of TTP reported in the literature do not contain sufficient information to distinguish them from forms of disseminated intravascular coagulation or microangiopathic hemolytic anemia. This makes it difficult to relate the results obtained in the present study to some of those obtained in the past by others. The second problem is that it seems quite possible that the entity called TTP may represent a common pathway stemming from more than one causative agent and, therefore, clinical and pathological manifestations may vary accordingly. We nevertheless feel that the case reported here can be considered representative of TTP having the typical clinical presentation of the acute form and disseminated intravascular thrombosis on histological examination. In addition, DIC can be excluded on the basis of the coagulation survey, whereas, pre-mortem and post-mortem information failed to support the possibility of a primary microangiopathic origin or of other disorders that may mimic TTP, such as systemic lupus erythematosus or metastatic malignancy with DIC.

The light- and electron-microscopic observations reported here strongly support the view that a form of TTP is characterized by widespread intravascular platelet aggregation. This finding concurs with the conclusion of a number of earlier workers who studied cases by light microscopy. The electron-microscopic examination in this case, however, clearly defines the nature of the thrombi, and the observations also appear to agree with those of Feldman and co-workers. In our case, however, intravascular platelet aggregation seems to be a prominent feature, while subendothelial fibrin deposition, although occasionally present, is not as prevalent. It is not clear whether these differences are the result of studying different entities or, more likely, the same entity at a different stage.

There is no evidence from coagulation study or histology that disseminated intravascular coagulation is present in this case. This would be in agreement with the majority of the fragmentary coagulation data, as well as with the single serial coagulation study in the literature. However, in some reports, hypofibrinogenemia or evidence of deficiency of some other coagulation factor have been noted. It seems possible that some of these cases were not TTP. On the other hand, these conflicting findings might be explained by activation of the clotting process following platelet aggregation. The deposition of fibrin, as shown by others by immunofluorescence and electron micro-
scopy\textsuperscript{10} and confirmed in this paper (Figs. 5, 6, and 7), could lead to increased fibrinolysis\textsuperscript{28} and possibly hypofibrinogenemia. However, most of the data to date, including recent platelet and fibrinogen consumption studies,\textsuperscript{29} suggest that DIC is an unusual finding in TTP and, therefore, an unlikely factor in the pathogenesis of the disorder.

An alternative view favored by some workers in the pathogenesis of TTP is that it is primarily a vascular disorder.\textsuperscript{47,10} Feldman et al.\textsuperscript{10} showed by electron microscopy subendothelial deposits of fibrin and suggest that TTP is a widespread vascular disease. We believe that subendothelial fibrin can be explained by the increased vascular permeability that follows the release of various substances from aggregating platelets.\textsuperscript{30} The fact that Feldman et al.\textsuperscript{10} occasionally found the lesion unassociated with a thrombus does not negate this conclusion, since the change could result from transient platelet aggregates.\textsuperscript{30,33} In addition, subendothelial fibrin deposits have been noted in vessels occluded by platelet fibrin thrombi subsequent to the experimental production of DIC,\textsuperscript{34} suggesting that this is a nonspecific event secondary to thrombosis. We feel, therefore, that in those cases of TTP in which disseminated intravascular platelet aggregation is a prevalent feature,\textsuperscript{37} the vascular lesion observed is probably secondary to the intraluminal thrombosis.

The cause of the disseminated intravascular platelet aggregation is not known. The frequent acute onset of the disorder, the simultaneous appearance of the disease on two occasions in two individuals\textsuperscript{35,36} (one pair being only related by marriage),\textsuperscript{36} and the known occurrence of platelet aggregation by viruses\textsuperscript{37} suggest that viral infection may be the cause. Viruses could produce platelet aggregation subsequent to phagocytosis by platelets\textsuperscript{40} or following the invasion of endothelial cells.\textsuperscript{41}

The treatment of TTP has been generally unsatisfactory and many forms of therapy have been attempted. These have included heparin,\textsuperscript{20,42} steroids,\textsuperscript{14} steroids and splenectomy,\textsuperscript{17,19,27} fibrinolytic agents,\textsuperscript{43} and, more recently, antiplatelet agents.\textsuperscript{12,13,44} Although the best results have followed steroids and splenectomy,\textsuperscript{27} it is difficult to assess the results of previous therapy, since the cases of TTP described in the literature show such a variable response to treatment and possibly are a group of disorders of different etiology.\textsuperscript{41} It seems that if therapy is to be planned on a rational basis, an attempt should be made to distinguish between disseminated intravascular platelet aggregation and disseminated intravascular coagulation despite the fact that overlap could occur at some stage in the disease process. Recent reports using antiplatelet agents,\textsuperscript{12,13,44} a platelet fibrinogen consumption study,\textsuperscript{29} and the findings in this case suggest that antiplatelet agents may have a place in the treatment of TTP.

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