Thrombotic Thrombocytopenic Purpura
A Case Investigated with $^{111}$In-Oxine-Labelled Platelets

A. DU P. HEYNS, M. G. LÖTTER, P. N. BADENHORST, P. C. MINNAAR, B. J. VORSTER, F. P. RETIEF

SUMMARY

A 34-year-old woman presented with the clinical and laboratory features of thrombotic thrombocytopenic purpura (TTP). Studies with isologous platelets labelled with $^{111}$In-oxine revealed a short half-life of circulating platelets (18.5 hours) and destruction of the transfused platelets in the spleen, liver and bone marrow. There was no scintigraphic evidence of deposition of labelled platelets in the vasculature. The patient was treated with daily fresh frozen plasma transfusions, but no improvement in platelet count or serum urea level was noted. Although there was no clinical evidence of a bleeding tendency at the time, the patient had a fatal cerebrovascular haemorrhage. The findings in this case suggest that an immune type destruction of platelets may occur in TTP.


Thrombotic thrombocytopenic purpura (TTP) is a generally fatal disease characterized by thrombocytopenia, micro-angiopathic haemolytic anaemia and fluctuating neurological abnormalities. Renal failure and fever are usually also present. The aetiology of the disorder is unknown. The primary lesion may be due to vasculitis based on an immune mechanism and there is supporting evidence that the disease occurs in association with collagen and other auto-immune diseases. The thrombocytopenia is thought to result from intravascular coagulation and may be potentiated by intravascular haemolysis.

Therapy is based on administration of a combination of glucocorticoids, splenectomy, heparin, and platelet inhibitors and exchange transfusion. Recently a patient who went into remission after multiple plasma transfusions was reported, and it was suggested that the disease might have been caused by a deficient plasma factor.

We describe a case investigated by the use of $^{111}$In-oxine-labelled platelets to visualize sites of deposition and sequestration of circulating platelets. The patient was treated with repeated plasma infusions to test the hypothesis that the disease may in some cases be caused by a plasma factor deficit.

CASE REPORT

A 34-year-old White woman, the mother of 12-year-old twins who had had no other pregnancies, had been healthy until 2 years before admission, when she had presented to an outlying hospital with an episode of tiredness, headache, paraesthesia of the left hand and transient paralysis of the left leg which lasted about 30 minutes. A diagnosis of thrombocytopenic purpura was made and she was treated with glucocorticoids, with no improvement in the platelet count. The peripheral blood film was not available for inspection and the presence of red cell fragmentation, although not noted, cannot be excluded. Fifteen days before admission to the Universitas Hospital, Bloemfontein, for evaluation, she became acutely ill, with severe occipital headache, menorrhagia and symptoms related to anaemia. She experienced three episodes of transient weakness of the right leg and paraesthesia of the right side of the face. No purpura or haematuria was evident. She smoked about 15 cigarettes per day and occasionally took paracetamol for headaches and dysmenorrhoea. There was no family history of a bleeding tendency. She had not received blood transfusions previously.

On physical examination she was anaemic, but not acutely ill, with a temperature of 38.4°C. Her liver was palpable 3 cm below the costal margin, but there was no splenomegaly or lymphadenopathy. A flame-shaped retinal haemorrhage was noted, but there was no other evidence of a haemorrhagic tendency. No neurological deficit was found.

The laboratory findings included: haematocrit 0.26, haemoglobin 8.5 g/dl, leucocytes 9.0 x 10^9/l, with neutrophils 65%, lymphocytes 28%, eosinophils 4%, basophils 1%, and monocytes 2%; platelets 11.0 x 10^9/l, and ESR (Westergren) 21 mm h. There was marked erythrocyte fragmentation haemolysis with a reticulocyte count of 15%. A bone marrow aspirate revealed abundant megakaryocytes and erythroid hyperplasia. No erythrocyte antibodies were detected and antinuclear and anti-DNA antibodies were absent from the serum. No antinucleotid antibody was detected in the serum with an immuno-injury platelet factor 3 release technique. There was no laboratory evidence of diffuse intravascular coagulation or
fibrinolysis: plasma kaolin partial thromboplastin time, factor VIII:C, factor V, fibrinogen, antithrombin III, plasminogen, euglobulin lysis time, serum fibrinogen degradation products and plasma fibrin monomers were all within normal limits. There was laboratory evidence of chronic intravascular haemolysis with a positive test for serum methaemalbumin and urinary haemosiderin. The serum urea (9.1 mmol/l) and creatinine (120 mmol/l) levels were slightly elevated but electrolyte values were normal. The serum bilirubin level was 25 μmol/l (reference values (RV) 4-21 μmol/l), LDH 349 IU/l (RV 30-90 IU/l), aspartate transaminase 37 IU/l (RV 8-35 IU/l), alanine transaminase 18 IU/l (RV 8-30 IU/l), and protein 72.8 g/l with a normal electrophoretic pattern. There was microscopic haematuria with erythrocytes and granular casts in the urinary sediment.

A diagnosis of TTP was made and treatment with daily fresh frozen plasma transfusions was decided on. No glucocorticoids were given, since she had no clinical evidence of a life-threatening thrombocytopenic purpura. The relevant replacement therapy schedule, platelet counts and haemoglobin concentrations during the period are depicted in Fig. 1.

On the 3rd and 4th days in hospital she experienced two transient episodes of paraesthesiae (each lasting about 30 minutes) in the left side of the face and left arm and difficulty with speech, which was interpreted as being due to right middle cerebral artery ischaemia. During the whole course of the illness she had no purpura, fresh retinal bleeding or other clinical evidence of a bleeding diathesis. Her serum urea increased steadily to a level of 40 mmol/l on the 14th day of hospitalization. After administration of fresh frozen plasma for 6 days (3,000 ml on day 1 and 750 ml daily thereafter) had brought no improvement in the platelet count, arrangements were made for haemodialysis and splenectomy. Before this could be performed, and at a time when there was still no clinical evidence of a bleeding tendency, she suffered a fatal cerebrovascular incident, manifested by a right hemiparesis and rapid evolution of deep coma with death in 30 minutes. Consent for postmortem examination was refused.

\[ ^{111}\text{In}-\text{Oxine}-\text{Labelled Platelets} \]

Blood was collected in an ACD blood pack from an ABO and Rh-compatible donor. Platelet-rich plasma was transferred to sterile 50-ml polypropylene tubes (Falcon, Oxnard, Calif., USA). The pH was adjusted to 6.2-6.5 with ACD, and platelets were sedimented by centrifugation at 00 g for 15 minutes. The supernatant platelet-poor plasma was aspirated and platelets were resuspended in physiological saline. \(^{111}\text{In}-\text{Oxine} \) (Diagnostic Isotopes, Bloomfield, NJ, USA) with an activity of 3 MBq (1 mCi) was added to the platelets in saline and incubated at 22°C for 30 minutes without stirring. Platelet-poor plasma was added to the incubation mixture and platelets were washed by centrifuging at 500 g for 20 minutes. Platelets were re-suspended in platelet-poor plasma, and in vitro platelet aggregation tests and electron microscopy were performed on a small aliquot. Platelets, labelled with 5.6 MBq (150 μCi) \(^{111}\text{In}-\text{oXine} \) were re-injected, and splenic pooling and platelet survival were determined and calculated as recommended by the ICSH. In our experience with this technique in 8 normal subjects, 36 ± 12% (1 SD) of platelets are pooled in the spleen and platelet survival is 7.8 ± 0.7 days.

\section*{Image Acquisition}

The scintillation camera with a high-energy divergent collimator was positioned to visualize the heart, liver and spleen. The camera was interfaced with a computer-assisted imaging system. After injection of labelled platelets images were obtained at 30-second intervals for 15 minutes, at 9-minute intervals for 90 minutes and then daily. Time-activity curves were generated by selecting areas of interest over the heart, liver, spleen and sacrum. The curves for each anatomical region were normalized with reference to the initial values and plotted for interpretation.

Rectilinear scans with a dual detector system were obtained 2 and 24 hours after injection of labelled platelets.

\section*{RESULTS}

No iso-antibodies to donor platelets were detected in the recipient's serum by the immuno-injury platelet factor release technique. Forty-three per cent of the \(^{111}\text{In}\)
oxine added was bound to platelets. Labelled platelets aggregated normally in vitro and on electron microscopy showed only slight dilation of the canalicular system and a few pseudopods. Erythrocytes were not labelled and 4% of the radioactivity injected was bound to plasma. The platelet lifespan was 18.5 hours with 45% of the radioactivity detected in the circulation 5 minutes after injection of the labelled platelets. The 2-hour scan showed accumulation of platelets in the spleen and, to a lesser extent, in the kidneys. Residual activity, interpreted as representing blood flow, was present over the heart and liver (Fig. 2). No evidence of diffuse or localized intravascular accumulation of platelets that could be interpreted as possible thrombosis was observed anywhere in the body. Repeat scintillation camera imaging and rectilinear scanning 24 hours later revealed sequestration of platelets throughout the reticulo-endothelial system, with activity in the spleen, liver and bone marrow (Fig. 3). At this stage the kidneys showed no radioactivity. No deposition of platelets which could be interpreted as being indicative of platelet thrombi was evident in the vasculature.

DISCUSSION

The aetiology of TTP is unknown, but there is evidence to suggest that it may be immunologically mediated. An unusual class of immune complex active against vascular tissue and perhaps platelets may provoke the syndrome by producing a lesion of the subendothelium due to vasculitis based on an immune mechanism. The diffuse disease of the microcirculation causes haemolysis and micro-angiopathic changes in red cell morphology. The thrombocytopenia is generally thought to result from intravascular coagulation and kinetic data suggest that TTP is a syndrome in which intravascular consumption of platelets, rather than of blood coagulation factors, occurs. A recent suggestion has been that plasma exchange may supply a factor, lacking in patients with TTP, which stimulates vascular prostacyclin (PGI) activity. Defective

Fig. 2. Posterior rectilinear scan 2 hours after injection of ¹¹¹In-labelled platelets. The spleen is clearly visualized and both kidneys are imaged.

Fig. 3. Posterior whole-body rectilinear scan 24 hours after injection of ¹¹¹In-labelled platelets. Platelets accumulate in the reticulo-endothelial system: spleen, liver and bone marrow.
PGI activity could favor platelet thrombi in the microcirculation. Descriptions of morphological features of the lesions are not clear. Earlier workers considered the vascular occlusions to be platelet thrombi, but recently they have been regarded as being composed mainly of fibrin. Most of the lesions may actually consist of a mixture of fibrin and platelets. The lesions are morphologically difficult to distinguish from those of disseminated intravascular coagulation. The characteristic laboratory features of the latter are, however, not usually present. Our case also had no laboratory evidence of intravascular coagulation.

"In-oxine" was introduced as a platelet label by McAfee and Thakur. It is a radio nuclide with a short half-life (2.8 days) and its abundance of high-energy gamma photons permits imaging of in vitro distribution of circulating platelets. In our case the distribution of platelet-bound radioactivity could be divided into two important phases, one early and the other after 24 hours. Firstly, within a few hours of injection of the labelled platelets, radioactivity accumulated in the kidneys (Fig. 2). This may have been due to deposition of platelets in the renal microvasculature, or alternatively the platelets may have appeared to have accumulated in the kidneys because of poor renal perfusion. There was no laboratory evidence of intravascular coagulation or active fibrinolysis at this time. Other organs commonly affected by thrombosis in TTP—-the brain, pancreas, adrenals and heart—were not visualized, but this may have been partly due to the difficulty of clearly imaging organs underlying the liver and spleen. The renal outlines were not visible on the day after injection of labelled platelets. Platelets may have been released from vascular thrombi by secondary fibrinolysis or the time elapsed may have allowed clearing of platelets from the vasculature of the poorly perfused kidneys. The second phase revealed prominent sequestration of the platelets in the reticulo-endothelial system (spleen, liver as well as bone marrow) 24 hours after injection of the labelled platelets (Fig. 3). This finding is also open to various interpretations. Circulating platelets may have been damaged by immune complexes with rapid sequestration in the reticulo-endothelial system or have been damaged in the process of harvesting and labelling. Electron microscopic examination of platelets and results of assessments of in vitro platelet function would argue against the latter possibility. We have not seen rapid reticulo-endothelial sequestration of labelled platelets in any of the normal subjects examined by this technique.

Treatment of TTP is largely empirical. Splenectomy, antiplatelet agents, high doses of glucocorticoids and exchange transfusion have all been used, with variable claims of success. Splenectomy has not proved to be of definite value. Glucocorticoid therapy alone has been associated with remissions and splenectomy combined with massive doses of corticosteroids may be the treatment of choice. The observation that remission could be achieved by infusion of large quantities of fresh, frozen, stored or cryoprecipitate-poor plasma, but not albumin, was explained by postulating the replenishment of a deficient plasma factor. It was even suggested that exchange transfusions, which are capable of inducing sustained remissions, work by the same mechanism. A patient with elevated platelet-associated IgG has been described, suggesting that in some cases thrombocytopenia may be due to removal of antibody-coated platelets by the reticulo-endothelial system. Immune complexes may also induce platelet aggregation, thereby aggravating the thrombocytopenia. On testing these hypotheses in our patient, repeated transfusions with fresh frozen plasma in large doses had no effect on circulating platelet count or on the uraemia. Unfortunately the patient died after a cerebrovascular incident before other modes of treatment could be attempted.

Despite the negative results of therapy in our patient, important issues are raised. There was strong evidence of reticulo-endothelial sequestration, but no obvious widespread intravascular deposition of platelets. The findings in this case support the immune complex theory of damage to platelets and the vasculature in TTP. The damaged platelets were sequestered in the reticulo-endothelial system and did not seem to be extensively involved in the intravascular thrombosis. Although it is accepted that there may be a multitude of causes of TTP and that it should be thought of as a syndrome which may appear in the course of various diseases affecting the microcirculation, the demonstration of reticulo-endothelial destruction of platelets influences planning of therapy. With hindsight, this patient might have benefited from splenectomy combined with high-dose glucocorticoid therapy, possibly augmented by exchange transfusion. Aster suggested that the beneficial effect of large quantities of normal plasma may be due to fortuitously contained antibody with high affinity for the antigen component in the plasma. In the case described by Byrnes and Khurana the transfusion of plasma was preceded by exchange transfusion which may have removed immune complexes, and the success of the subsequent plasma transfusion may thus have been facilitated. In our case the titre of antibody or immune complex may have been too high to respond to plasma transfusion alone. It may be prudent not to use plasma infusions as primary therapy for TTP until variants of TTP different from that of our patient are demonstrated.

This study was supported in part by the South African Medical Research Council and the Atomic Energy Board. The technical help of Mr H. Pieters and secretarial assistance of Medames E. Joubert and J. Myburg are gratefully acknowledged.

REFERENCES

Abdominal Apoplexy
A Case Report

D. N. ADAMTHWAITE

SUMMARY

A case of abdominal apoplexy is presented and the literature is briefly reviewed. The diagnosis and management of this rare condition are discussed.


Abdominal apoplexy is a rare condition. Since its first description by Barber in 1909, only 106 cases have been reported. It is defined as a massive, spontaneous, retroperitoneal or intra-abdominal haemorrhage occurring without any predisposing factor or precipitating cause. This definition excludes ruptured ectopic pregnancy, leaking abdominal aneurysm and spontaneous rupture of hepatic, splenic or renal lesions.

Rupture of a splanchnic vessel may cause intraperitoneal haemorrhage, but may also cause an expanding localized haematoma between the leaves of the mesentery. Bleeding commonly occurs from a second- or third-order aortic branch and the middle colic, pancreaticoduodenal and superior mesenteric arteries are the most frequently affected (Table I). Hypertension and arteriosclerosis have been implicated as causes of the condition, the former being noted in 39% of cases. Muscle coat defects in the arteries have been described.

CASE REPORT

A 75-year-old man was admitted with symptoms of chronic prostatism. Although obese, the patient showed no signs of significant vascular disease and was not hypertensive. There was evidence of obstructive airways disease and emphysema. The prostatic enlargement was associated with chronic urinary retention.

Department of Surgery, Addington Hospital, Durban

D. N. ADAMTHWAITE, F.R.C.S.

Date received: 7 March 1979

<table>
<thead>
<tr>
<th>TABLE I. SITE OF ARTERIAL DISRUPTION IN 106 CASES OF ABDOMINAL APOPLEXY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Middle colic artery</td>
</tr>
<tr>
<td>Pancreaticoduodenal arteries</td>
</tr>
<tr>
<td>Superior mesenteric artery</td>
</tr>
<tr>
<td>Left gastric artery</td>
</tr>
<tr>
<td>Gastroduodenal artery</td>
</tr>
<tr>
<td>Left colic artery</td>
</tr>
<tr>
<td>Inferior mesenteric artery</td>
</tr>
<tr>
<td>Right gastric artery</td>
</tr>
<tr>
<td>Ileocolic artery</td>
</tr>
<tr>
<td>Gastro-epiploic artery</td>
</tr>
<tr>
<td>Splenic artery</td>
</tr>
<tr>
<td>Right colic artery</td>
</tr>
<tr>
<td>Coeliac axis</td>
</tr>
<tr>
<td>Hepatic artery</td>
</tr>
<tr>
<td>Renal artery</td>
</tr>
<tr>
<td>Site unknown</td>
</tr>
</tbody>
</table>

Recovery from prostatectomy was uneventful until the 3rd postoperative day. The patient developed severe epigastric pain with progressive tympanitic distension of the abdomen. The pain was constant and radiated to the infra-scapular region of the back. Evidence of free intraperitoneal fluid was found and the haemoglobin level was noted to have dropped to 7.7 g/dl from its postoperative level of 14 g/dl. The apparent loss of blood was much greater than the postoperative urinary blood loss.

Both femoral pulses were present, the circulation to the lower limbs was normal, and no mass could be felt on abdominal examination. Abdominal paracentesis revealed free blood in the peritoneal cavity.

At laparotomy 2 litres of blood was evacuated from the peritoneal cavity, but no bleeding point was immediately apparent. The abdominal aorta and its branches were normal, and the liver and spleen were intact. A mass approximately 12 cm in diameter was found in the right half of the transverse mesocolon. On opening the meso-