Haematology

Platelets

James N George

- **Platelets, derived from megakaryocte cytoplasm, have a critical role in normal haemostasis, and in thrombotic disorders.**
- **The development of megakaryocytes is controlled by thrombopoietin, which binds to c-mpl on the surface of platelets and megakaryocytes.**
- **Platelet membrane glycoproteins mediate binding to subendothelial tissue and aggregation into haemostatic plugs.**
- **Thrombocytopenia and disorders of platelet function cause petechiae and mucocutaneous bleeding.**
- **Drugs causing specific inhibition of platelet function are important in the treatment of cardiovascular and cerebrovascular disease.**

Platelets are the smallest of blood cells, being only fragments of megakaryocyte cytoplasm, yet they have a critical role in normal haemostasis and are important contributors to thrombotic disorders. Understanding of the role of platelets in haemostasis and definition of disorders caused by abnormal platelet function have led to important new therapies for thrombotic disease.

Platelet production

The development of megakaryocytes and production of platelets are unique processes. Megakaryocyte maturation involves nuclear duplication without cell division, resulting in giant cells. Cytoplasmic organelles are organised into domains representing nascent platelets, demarcated by a network of invaginated plasma membranes. Within the marrow, megakaryocytes are localised next to the sinusoidal walls, which facilitates the exit of large segments of cytoplasm into the circulation. The fragmentation of megakaryocyte cytoplasm into individual platelets then results from the shear forces of circulating blood, perhaps largely in the pulmonary circulation.[1]

Thrombopoietin is the dominant hormone controlling megakaryocyte development, but many cytokines and hormones take part, including interleukins 3, 6, and 11.[2] Thrombopoietin was first identified as the ligand for a receptor on the surface membranes of megakaryocytes and platelets, termed c-mpl. This receptor is the normal proto-oncogene of the viral oncogene present in the murine myeloproliferative leukaemia virus (v-mpl). Inhibition of *C-MPL* expression in marrow cells specifically blocks megakaryocyte development. Mice genetically deficient in *c-mpl* have 85% fewer platelets and megakaryocytes than normal but other haemopoietic cells are present in normal amounts; thrombopoietin therefore seems to be the major, but not the only, regulator of platelet production.[3]

Lancet 2000; **355:** 1531–39

Hematology-Oncology Section, Department of Medicine, University of Oklahoma Health Sciences Center, PO Box 26901, Oklahoma City, OK 73190, USA (J N George MD)

(e-mail: Jim-George@OUHSC.edu)

Other clinical observations supporting the importance of thrombopoietin and its receptor are the association of mutations of *C-MPL* with congenital amegakaryocytic thrombocytopenia[4] and the identification of auto-antibodies that neutralise the activity of thrombopoietin in a patient with acquired amegakaryocytic thrombocytopenia.[5]

Platelet structure and function

On activation, platelets change from the normal disc shape to a compact sphere with long dendritic extensions facilitating adhesion (figure 1). The cytoplasm is rich in actin and myosin which bring about the change in shape and retraction of the clot. There are two classes of secretory granules. The first type are dense granules that secrete ADP and calcium, which reinforce platelet aggregation and platelet-surface coagulation reactions. The second type are α granules, which secrete a vast array of proteins: some, such as von Willebrand factor and platelet factor 4, are synthesised by megakaryocytes; others, such as fibrinogen, are acquired from the plasma by receptor-mediated endocytosis; still others, such as the abundant plasma proteins, albumin and IgG, are acquired by fluid-phase pinocytosis.[6] Both secretion and endocytosis are facilitated by a network of deep invaginations of the platelet surface membrane, the surface-connected canalicular system, which is reminiscent of the demarcation membranes that initially defined platelet zones within their parent megakaryocytes.

Platelet-membrane glycoprotein receptors mediate adhesion to subendothelial tissue and subsequent aggregation to form the initial haemostatic plug (panel 1).[7–10] The largest glycoprotein is designated I, the smallest IX. Letters a and b were added when better techniques allowed resolution of single protein bands on electrophoresis into two separate bands (eg, glycoprotein I became glycoprotein Ia and Ib).

Glycoprotein Ib-V-IX is a constitutively active receptor for von Willebrand factor, causing immediate platelet attachment to exposed perivascular von Willebrand factor.[10] Glycoprotein Ia-IIa, a constitutively

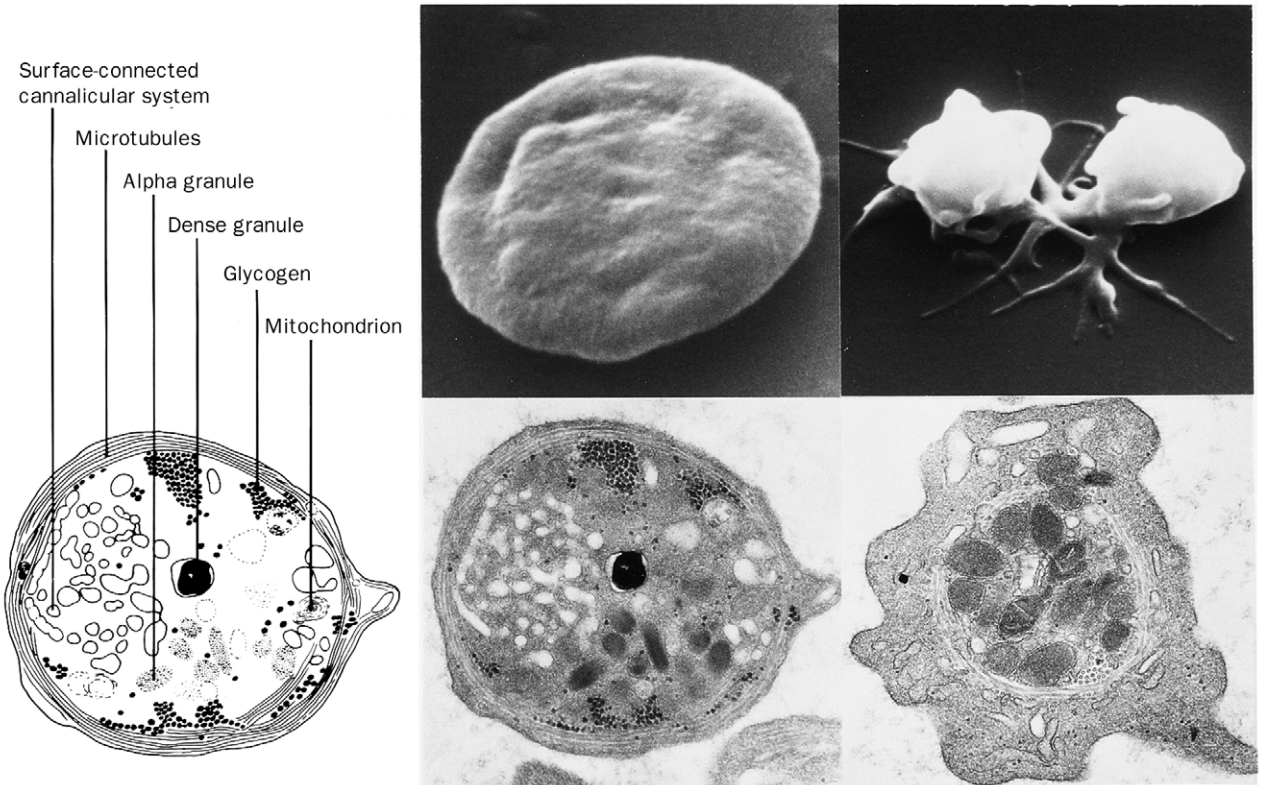

Figure 1: **Electron micrograph of resting and activated platelets**
Top photographs are scanning electron micrographs demonstrating the disc shape of normal circulating platelets (left, ×20 000) and the more spherical form of activated platelets with many long pseudopodia (right, x10 000). Lower left photograph is a transmission electron micrograph of the cross-section of a resting platelet (×21 000) with a matched drawing labelling the normal subcellular structures. Lower right photograph (×30 000) of an activated platelet shows the constriction of the microtubular ring around the centralised granules and the formation of pseudopodia. Electron micrographs courtesy James G White and Marcy Krumwiede, University of Minnesota. Reproduced with permission from: George JN. Haemostasis and fibrinolysis. In: Stein JH, et al, eds. Internal medicine, 5th edn. St Louis: Mosby, 1998: 534–40.

active receptor for collagen, is also involved in initial platelet adhesion to the subendothelial matrix.[8,9] The most abundant surface protein, glycoprotein IIb-IIIa, requires conformational change during platelet activation to express receptor function, mainly for fibrinogen.[7] Fibrinogen binding to glycoprotein IIb-IIIa mediates platelet aggregation.

These reactions define the clinical manifestations of bleeding disorders. Patients with thrombocytopenia have petechiae and mucocutaneous bleeding, resulting from unsealed small endothelial lesions. By contrast, patients with coagulation disorders, such as haemophilia, do not have petechiae or excessive bleeding from small cuts because platelet adhesion and aggregation are sufficient to seal these lesions. Haemostasis for larger wounds requires fibrin formation to reinforce the platelet aggregate. Therefore, in contrast to platelet disorders, coagulation disorders are characterised by the delayed occurrence of large haematomas.

The complementary role of platelets and coagulation factors in haemostasis is well illustrated by the bleeding-time test, the measurement of the duration of bleeding from a small forearm incision. Bleeding times are longer than normal in patients with severe thrombocytopenia or platelet-function disorders, but they are normal, or almost normal, in patients with haemophilia. Aspirin, which causes partial impairment of platelet function, causes a slight increase in bleeding time in some healthy individuals. However, when patients with severe haemophilia were given aspirin, the combination of impaired platelet function and deficient fibrin formation caused extremely prolonged bleeding even from these small wounds; several patients ultimately required factor VIII infusions to stop the bleeding.[11]

Platelet circulation

Platelets survive for about 10 days on average; younger platelets have greater functional ability. The spleen continually but transiently sequesters about a third of circulating platelets. Splenomegaly, particularly when caused by passive congestion due to increased portal venous pressure, greatly increases the fraction of platelets retained in splenic sinusoids, without decreasing overall platelet survival time. This retention causes the mild thrombocytopenia associated with liver cirrhosis and portal hypertension.[12] Most platelets are removed from the circulation after senescence, but a constant small fraction is continually removed by involvement in the maintenance of vascular integrity.

Bleeding disorders

Bleeding can result from defective platelet function or from thrombocytopenia. The abnormalities must be severe for clinically important bleeding to occur, since haemostasis has many redundant functions. For example, the congenital absence of the platelet fibrinogen receptor, glycoprotein IIb-IIIa, has a profound effect on laboratory assessment of platelet function (panel 1), yet patients without this glycoprotein typically have only intermittent and minor bleeding.[13] Also the normal platelet number, $150–350\times10^9$/L (150 000–350 000/μL), far exceeds what is essential for haemostasis. Figure 2 shows the severity of bleeding in relation to the severity of thrombocytopenia

Panel 1: **Major platelet membrane glycoprotein receptors**

Receptor	Structure	Function	Polymorphisms	Disorders
Glycoprotein IIb-IIIa	Integrin family of receptors ($a_{IIb}\beta_3$); 80 000 molecules per platelet	On platelet activation, becomes a receptor for fibrinogen, also for von Willebrand factor, fibronectin, vitronectin, and thrombospondin	Leu_{33}/Pro_{33} creates platelet-specific antigens, HPA-1a/1b, the important antigens for neonatal alloimmune thrombocytopenia	Deficiency results in Glanzmann's thrombasthenia, characterised by absent aggregation in response to all physiological agonists and absent clot retraction; platelet number and morphology are normal
Glycoprotein Ia-IIa	Integrin family of receptors ($\alpha_2\beta_1$); 900–2300 molecules per platelet	Constitutively active receptor for collagen	$C_{807}T$ silent dimorphism affects $\alpha_2\beta_1$ surface density and collagen receptor activity; may influence occurrence of coronary thrombosis and stroke	Deficiency, with absent collagen-induced aggregation, may result in mildly increased bleeding
Glycoprotein Ib-V-IX	Complex of four gene products, each characterised by leucine-rich repeats; covalent heterodimer of Ib_α–Ib_β and non-covalent association with glycoproteins V and IX; 25 000 molecules per platelet	Constitutively active receptor for insoluble von Willebrand factor in the perivascular matrix	None are clinically important	Deficiency results in Bernard-Soulier syndrome, characterised by thrombocytopenia, giant platelets, and lack of binding of von Willebrand factor Mutations with increased function result in platelet-type von Willebrand's disease; characterised by spontaneous binding of von Willebrand factor causing its depletion from plasma

in patients with idiopathic thrombocytopenic purpura: spontaneous major bleeding did not occur until the platelet count was less than 10×10^9/L, and even then it was uncommon. Also, the group of patients represented in figure 2 may be more severely affected than current unselected patients with idiopathic thrombocytopenic purpura, since they were treated at the University of Michigan Medical Center, a tertiary referral centre, so may have been more severely affected; automated platelet counts were not widely used at the time of the study and therefore mildly affected patients were not recognised; and aspirin was not fully appreciated as a risk factor for exacerbation of bleeding.

Disorders of platelet function

Although hereditary disorders of platelet function are rare, they define the bleeding symptoms caused by platelet abnormalities.[13] Mucocutaneous bleeding, such as purpura, epistaxis, gingival bleeding, and menorrhagia, are prominent features; gastrointestinal bleeding is common; visceral haematomas, haemarthroses, and intracerebral haemorrhage rarely, if ever, occur in the absence of trauma. Even when the genetic defect is severe, as in patients with Glanzmann's thrombasthenia who have undetectable platelet glycoprotein IIb-IIIa, bleeding symptoms are sporadic; this observation emphasises that other platelet receptors can compensate for the absent fibrinogen receptor.

Acquired platelet function defects are mild and ubiquitous, considering, for example, the number of people who take aspirin regularly and who therefore have impaired platelet function caused by irreversible inhibition of cyclo-oxygenase-dependent thromboxane formation. More than 100 other drugs, foods, spices, and vitamins have been reported to impair platelet function.[14] For almost all agents, the data are limited to descriptions of abnormal in-vitro platelet aggregation tests or a long bleeding time, which may have no clinical importance. Only for aspirin has an increased risk of bleeding been clearly documented, and that proof came from a study of 22 071 physicians followed up for 5 years, to assess the efficacy of aspirin (325 mg on alternate days) for the primary prevention of myocardial infarction. 27% of the physicians taking aspirin reported bleeding symptoms consistent with impaired platelet function; however, perhaps more important was the observation that 20% of those taking placebo also reported "excessive" bleeding.[15] These data emphasise the simple, familiar facts that bleeding symptoms occur in normal people and that excessive bleeding is difficult to define. Negligible or no excessive bleeding may be expected in acquired platelet-function disorders, since the impairment of platelet function is much less severe than in Glanzmann's thrombasthenia, and since patients with thrombasthenia may have no bleeding for many years.[13] However, aspirin, and presumably other causes of abnormal platelet function such as chronic renal failure and cardiac surgery,[14] can profoundly exacerbate bleeding in patients who already have compromised haemostasis from any cause, such as a coagulation disorder[11] or anticoagulant therapy.

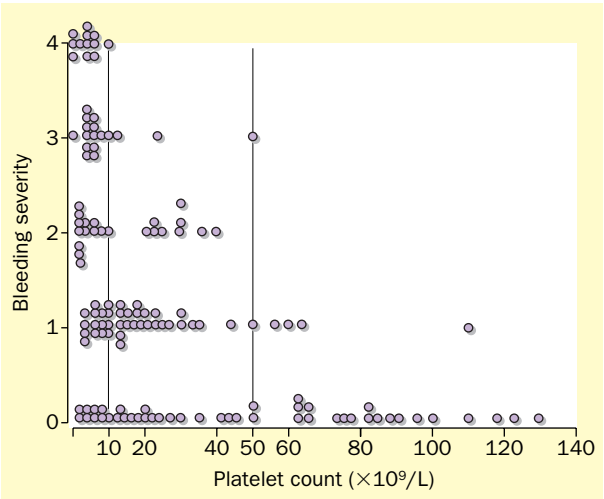

Figure 2: **Bleeding severity in relation to the platelet count in patients with idiopathic thrombocytopenic purpura**
Bleeding symptoms: 0=no bleeding or purpura; 1=minor bruising with trauma; 2=spontaneous but self-limited bleeding (eg, purpura, epistaxis); 3=spontaneous bleeding requiring medical attention (eg, epistaxis requiring nasal packing); 4=major bleeding requiring emergency treatment. Reproduced and modifed with permission from Lacey and Penner, *Seminars in Thrombosis and Hemostasis* 1977; **3:** 3.

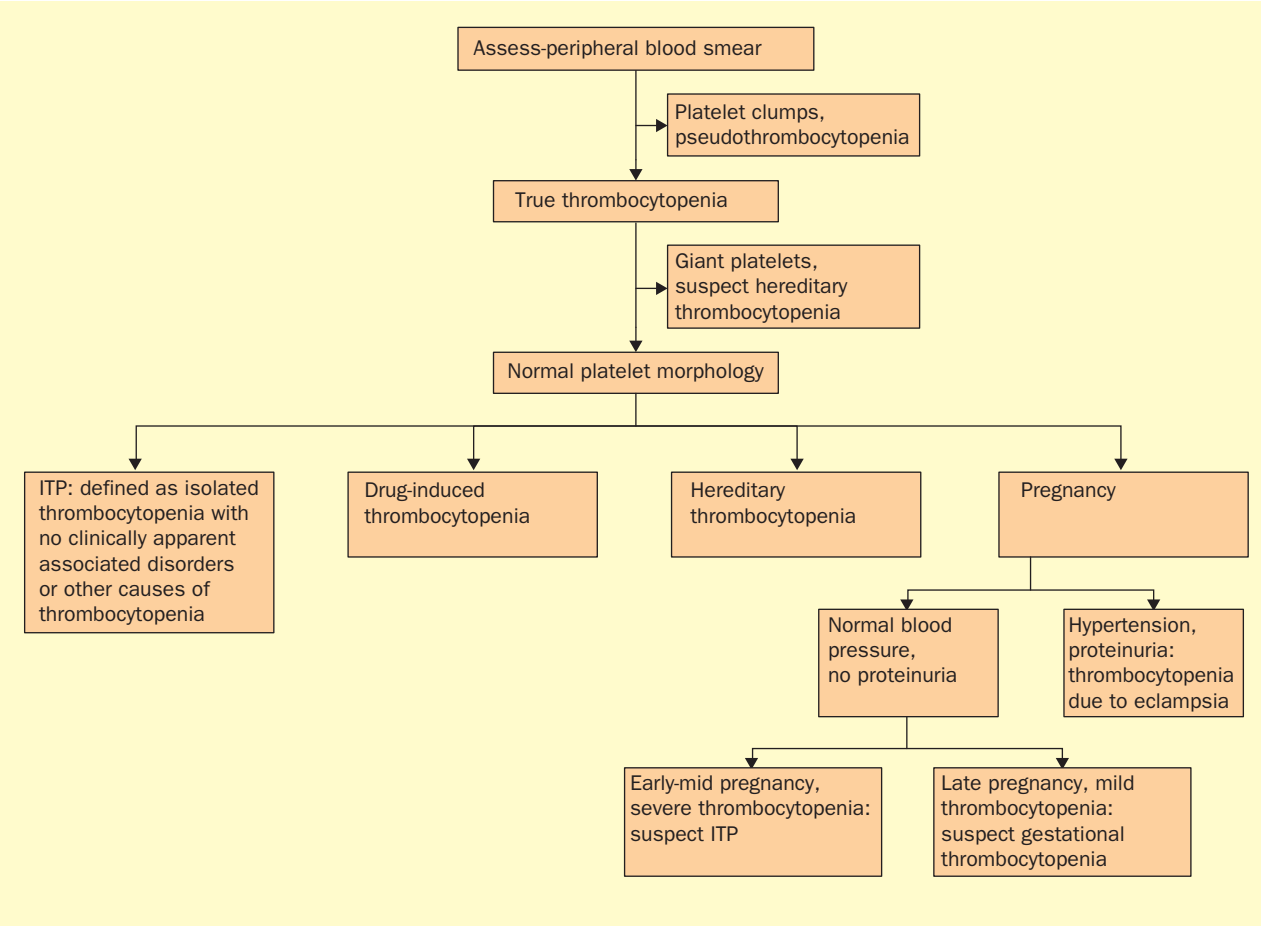

Figure 3: **Algorithm for investigation of isolated thrombocytopenia in an otherwise healthy person**

The low risk of major bleeding in patients with hereditary and acquired disorders of platelet function has been exploited in the use of aspirin and platelet glycoprotein IIb-IIIa blockers as antithrombotic agents.

Thrombocytopenia occurring as an isolated abnormality

Thrombocytopenia in an otherwise healthy person

Thrombocytopenia may be suspected from bleeding symptoms, or may be discovered by a routine blood count in a person without symptoms. The incidental discovery of thrombocytopenia, which has occurred since platelet counting became routine, has shown the existence of pseudothrombocytopenia and expanded the clinical range of thrombocytopenic disorders to include many symptom-free patients. The investigation and management of patients with isolated thrombocytopenia is illustrated by the algorithm in figure 3 (the algorithm does not include other disorders associated with thrombocytopenia, such as chronic liver disease with hypersplenism, and autoimmune, lymphoproliferative, and infectious diseases, because they are generally accompanied by signs and symptoms suggesting systemic disease). Isolated thrombocytopenia initially diagnosed as idiopathic thrombocytopenic purpura may subsequently be diagnosed as congenital thrombocytopenia or myelodysplasia. Congenital thrombocytopenias are rare but their recognition is critical for avoiding unnecessary treatment; commonly they manifest giant platelets, but platelet size may be normal or small.

Pseudothrombocytopenia

Many large studies have shown that falsely low platelet counts, due in most cases to platelet agglutination caused by the anticoagulant edetic acid (EDTA), used for routine blood counts (figure 4), occurs in about one person in 1000, irrespective of the presence or absence of any disease. Therefore the diagnosis of thrombocytopenia must always be confirmed by examination of the peripheral-blood smear. In patients with edetic-acid-induced pseudothrombocytopenia, a naturally occurring antibody causes platelet agglutination by binding to normally concealed epitopes on glycoprotein IIb, which are revealed when edetic acid chelates calcium and thereby alters the glycoprotein IIb-IIIa molecule.[16] Other anticoagulants, such as sodium citrate used for coagulation assays, generally do not perturb glycoprotein IIb-IIIa and therefore do not lead to platelet agglutination in these individuals. Edetic-acid agglutinins are not clinically important,[17] except for the risk of a mistaken diagnosis of thrombocytopenia and resulting inappropriate treatments.

Hereditary thrombocytopenias

Although rare, many types of hereditary thrombocytopenia have been described. Some are autosomal dominant traits, such as the May-Hegglin anomaly (characterised by giant platelets and neutrophil inclusions) and type 2B von Willebrand's disease. Others are X-linked, such as the Wiskott-Aldrich syndrome (characterised by small platelets and

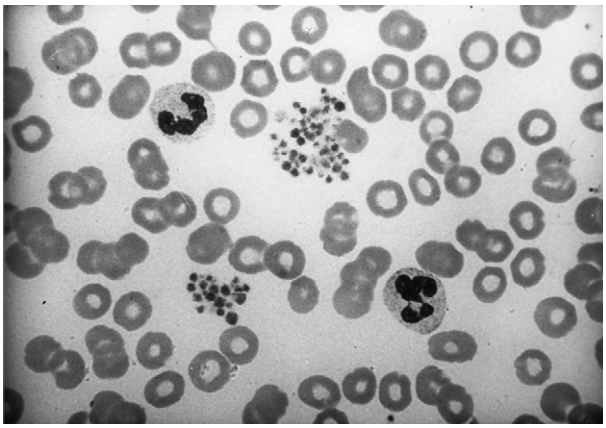

Figure 4: **Blood film showing platelet clumps, made from blood anticoagulated with edetic acid**
Reproduced with permission from: George JN. Thrombocytopenia. In: Beutler E, Lichtman MA, Coller BS, Kipps TJ, eds. Hematology, 5th edn. New York: McGraw-Hill, 1995: 1356.

immunodeficiency). And yet others are autosomal recessive traits, such as the Bernard-Soulier syndrome (also characterised by giant platelets; figure 5). Most hereditary thrombocytopenias are apparent in infancy, though some are symptomless and not detected until adulthood. The important issue is to distinguish these disorders from idiopathic thrombocytopenic purpura and to avoid inappropriate treatment. A hereditary disorder should be considered in patients who have a diagnosis of idiopathic thrombocytopenic purpura with persistent thrombocytopenia, particularly children and adolescents in whom chronic idiopathic thrombocytopenic purpura is uncommon.[18] Hereditary thrombocytopenias should also be considered when giant platelets are present. Although some patients with idiopathic thrombocytopenic purpura have platelets that are somewhat larger than normal, truly giant platelets approaching the diameter of red cells (figures 3 and 5) occur only in congenital disorders.

Drug-induced thrombocytopenia

In patients with unexpected, isolated thrombocytopenia, a drug-induced mechanism must be considered. The best-described mechanism is platelet destruction by drug-dependent antibodies against platelets formed when binding of the drug to a platelet-membrane molecule exposes a normally concealed aminoacid or carbohydrate sequence that becomes antigenic (neoepitope). In affected patients the offending drug may bind only weakly and reversibly, if at all, to either the antibody or platelets, but it can promote high-affinity binding of the antibody to the platelet antigen. Drug-dependent antibody-binding sites (the neoepitopes) are highly specific and are restricted to small domains on glycoprotein IX, glycoprotein Ib, and less commonly on glycoprotein IIb-IIIa.[19,20]

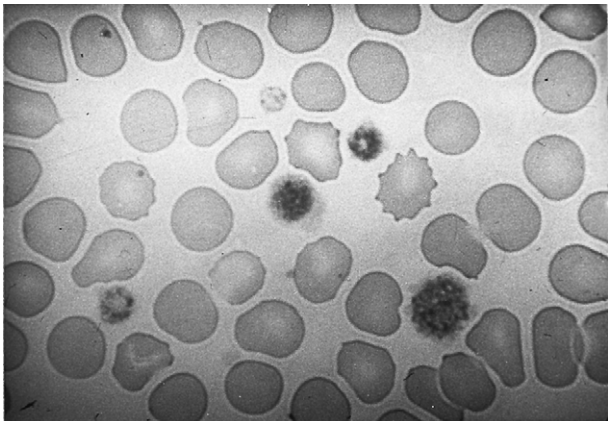

Figure 5: **Blood film showing giant platelets, made from a patient with Bernard-Soulier disease**
Reproduced with permission from: Hoffbrand AV, Pettit JE. Sandoz slide atlas of clinical haematology. London: Gower Medical Publishers, 1990.

The suspicion of drug-induced thrombocytopenia is confirmed by recovery from thrombocytopenia after the suspected drug is withdrawn, which is predictable within 5–7 days.[21] Laboratory tests for drug-dependent antibodies are not routinely and promptly available. To address the issue of which drugs are most likely to cause thrombocytopenia, a systematic review analysed all published case reports with defined levels of evidence to document the causal relation between the drug and thrombocytopenia.[21] In that review, case reports describing 98 drugs provided definite or probable evidence that the drug caused the thrombocytopenia. The most commonly reported drugs with definite evidence were quinidine, quinine, rifampicin, and trimethoprim-sulphamethoxazole.[21] The database established by this systematic review, including the full list of drugs and articles and data on clinical course and severity of bleeding, is updated annually and is available at the website: http://moon.ouhsc.edu/jgeorge.

Heparin-induced thrombocytopenia needs specific emphasis because of its frequency and the variability of its clinical manifestations, which include thrombotic complications. Antibodies are specific for heparin bound to platelet factor 4, a positively charged platelet-secreted protein that binds tightly to the negatively charged heparin. The complex of these three molecules can cause platelet activation; activated platelets then increase the risk of further thrombosis.[22] Obviously, since these patients are being treated with heparin, they already have, or are at risk of, thrombosis. In most patients the thrombocytopenia is mild and transient, occurring several days after the occurrence of thrombosis when heparin can safely be discontinued. Severe thrombocytopenia with thrombosis is much less common, and its frequency is further decreasing with shorter durations of heparin treatment and increased use of low-molecular-weight heparins, which are less commonly associated with thrombocytopenia.[22]

Idiopathic (or immune) thrombocytopenic purpura

Idiopathic thrombocytopenic purpura is defined as isolated thrombocytopenia with no clinically apparent associated disorders (eg, HIV infection, systemic lupus erythematosus). No specific criteria establish the diagnosis of idiopathic thrombocytopenic purpura; the diagnosis relies on the exclusion of other causes of thrombocytopenia.[23] Idiopathic thrombocytopenic purpura is caused by autoantibody destruction of platelets, but tests for antiplatelet antibodies have not yet been shown to be important for diagnosis and management.[23] If the history, physical examination, and initial blood counts with examination of the blood smear are compatible with the diagnosis and do not include atypical findings that are uncommon in idiopathic thrombocytopenic purpura or suggest other causes for the thrombocytopenia, further diagnostic tests are

unnecessary in most patients. Bone-marrow examination may be appropriate in older patients, to exclude myelodysplasia (which can present as isolated thrombocytopenia), and in patients with severe thrombocytopenia in whom initial treatment is unsuccesful and in whom splenectomy is considered.[23] A normal bone marrow is consistent with the diagnosis of idiopathic thrombocytopenic purpura.

In adults, idiopathic thrombocytopenic purpura typically has an insidious onset and a chronic course. The incidence of the disorder is increasing, especially among older adults, with the routine reporting of platelet counts;[24] currently 30–40% of adult patients are symptom-free and the diagnosis is made incidentally. Major bleeding is not an important risk unless the platelet count is less than 10×10^9/L (figure 2). Intracerebral haemorrhage and death occur, but too rarely for an incidence to be calculated.[23] Adult patients with severe and symptomatic thrombocytopenia are treated initially with prednisone 1 mg/kg daily; however, when symptomless thrombocytopenia is incidentally discovered and the platelet count is over 30×10^9/L, patients can safely be observed with no specific treatment.[23] The aim of treatment is not cure of the idiopathic thrombocytopenic purpura, but prevention of bleeding. Splenectomy is the conventional treatment for adults who continue to have severe and symptomatic thrombocytopenia despite treatment with prednisone. Although long-term remissions, with normal platelet counts on no further treatment, occur in only about 50% of patients, the results are better than with other available treatments. For patients who continue to have severe and symptomatic thrombocytopenia after splenectomy, various immunosuppressive regimens have been used, all with some reports of success, but most patients have incomplete responses.

Emergency treatment for life-threatening bleeding consists of platelet transfusions, high doses of glucocorticoids given intravenously (eg, 1000 mg methylprednisolone), and intravenous immunoglobulin.

In children younger than 10 years, idiopathic thrombocytopenic purpura typically has an acute onset and resolves spontaneously within 6–12 months. Therefore initial management is typically even more conservative than that in adults. Some paediatric haematologists recommend no specific treatment even for children with severe thrombocytopenia, in the absence of clinically important bleeding.[23,25,26] However, although the clinical benefit is uncertain, others treat children who have severe thrombocytopenia with intravenous immunoglobulin or glucocorticoids, even in the absence of bleeding symptoms.[25,27] This variation in practice must be resolved by a randomised clinical trial, documenting the efficacy (or lack of efficacy) of treatment on prevention of major bleeding as well as the complications of the treatment itself. Persistent thrombocytopenia is uncommon in children, and splenectomy is rarely done, because most children eventually have a spontaneous remission.[25]

Thrombocytopenia in a patient with acute systemic illness

Thrombocytopenia caused by infection

The most common causes of thrombocytopenia are infections. Thrombocytopenia can occur in infections caused by viruses (eg, HIV, cytomegalovirus, Epstein-Barr virus, hantavirus), mycoplasmas, bacteria, mycobacteria, rickettsiae, or protozoal parasites (eg, malaria). In most cases the mechanism is decreased platelet production, though hypersplenism can contribute. In HIV infection, thrombocytopenia is caused by infection of marrow stromal cells that facilitate haemopoiesis.[28] Thrombocytopenia is common in critically ill patients with sepsis, in whom the dominant cause is platelet phagocytosis mediated by increased concentrations of macrophage colony-stimulating factor.[29]

Thrombotic thrombocytopenic purpura-haemolytic uraemic syndrome (TTP-HUS)

Before effective treatment with plasma exchange became available in the 1970s, the mortality from TTP-HUS was 90%.[30] At that time, when the clinical course was long and fatal in most cases, the diagnosis was made by the presence of five cardinal clinical features: thrombocytopenia, microangiopathic haemolytic anaemia, renal and neurological abnormalities, and fever, as well as the characteristic arteriolar hyaline thrombi on histopathology.[30] Now that effective plasma exchange treatment is available, lowering mortality to about 20%, establishment of the diagnosis is urgent; therefore the stringency of diagnostic criteria has inevitably decreased.[31] In a case series reported in 1991, only the presence of thrombocytopenia and micro-angiopathic haemolytic anaemia, without a clinically apparent cause, were required for initiation of treatment.[32] Earlier diagnosis and use of fewer diagnostic criteria have, in turn, resulted in a large increase in the number of patients treated for TTP-HUS[33] and a wider clinical range of disease. TTP and HUS were initially described as different syndromes (with TTP having more severe neurological abnormalities and HUS having more severe renal abnormalities) but a clear distinction is typically not apparent. Even the distinction between TTP-HUS and other diseases with acute thrombocytopenia and anaemia (eg, viral, bacterial, or rickettsial sepsis) is initially unclear in many cases.[31] This overlap of clinical syndromes is understandable, since the characteristic abnormality of thrombotic microangiopathy is not specific for TTP-HUS, but is also seen in other disorders with distinct causes and outcomes, such as malignant hypertension, acute scleroderma, anti-phospholipid antibody syndrome, and renal allograft rejection.[34] Currently TTP-HUS can best be described as a syndrome, not a specific disease, which can result from many causes and can be associated with a variety of clinical disorders (panel 2). As more specific causes for TTP-HUS are documented, such as hypersensitivity to quinine and ticlopidine,[35,36] the proportion of patients in the idiopathic category will decrease.

Endothelial-cell damage seems to be a central feature in the pathogenesis of the TTP-HUS syndromes. Plasma from patients diagnosed as having TTP or sporadic HUS, but not HUS associated with childhood epidemic diarrhoea (panel 2), can cause apoptosis of microvascular endothelial cells isolated from organs typically affected by TTP-HUS.[37] Endothelial cells synthesise and secrete large von Willebrand factor molecules that are later decreased in size by a plasma protease. A deficiency of the protease that cleaves von Willebrand factor may allow unusually large multimers

Panel 2: **TTP-HUS: a classification of clinical presentations and associated conditions**

Childhood epidemic HUS
Association with haemorrhagic colitis (typically *E coli* O157:H7 infection)

Adult TTP-HUS syndromes
Idiopathic
Associations with other disorders:
- Haemorrhagic colitis
- Drug-induced TTP-HUS
  - Hypersensitivity reactions (quinine, ticlopidine)
  - Cumulative dose-related toxicity (mitomycin A)
  - Pregnancy
- Autoimmune diseases (systemic lupus erythematosus, antiphospholipid antibody syndrome, scleroderma)
- Allogeneic bone-marrow transplantation

of von Willebrand factor to be present in plasma, which could agglutinate circulating platelets, causing arteriolar thrombi.[38,39]

Epidemic HUS in children caused by shiga toxins from enterohaemorrhagic infections, mostly caused by *Escherichia coli* O157:H7,[40] is clinically distinct among the disorders in panel 2. Acute renal failure is the dominant abnormality in these children, and most survive with only supportive care, without plasma exchange. The other disorders in panel 2 occur mainly in adults; all have similar clinical presentations; all are potentially fatal without plasma-exchange treatment; therefore all are treated with plasma exchange, even haemorrhagic colitis due to *E coli* O157:H7 in adults. Although the basis for the efficacy of plasma exchange remains unknown, this treatment has had a remarkable effect on the clinical course of patients with TTP-HUS. The long-term risk of recurrent episodes has now become apparent,[41] but recurrence was almost unknown in the era before plasma exchange.[30] These recurrences seem to be restricted to adult patients with idiopathic TTP-HUS; they are unlikely to occur among patients who have haemorrhagic colitis or drug-induced TTP-HUS (unless the drug is taken again, of course).

Thrombocytopenia associated with pregnancy

The occurrence of thrombocytopenia during pregnancy raises important diagnostic and management issues.[23] Mild, symptomless thrombocytopenia (gestational thrombocytopenia), is common at term, occurring in 5% of women.[42] If thrombocytopenia is more severe (platelet counts less than 70×10^9/L) or occurs early in pregnancy, idiopathic thrombocytopenic purpura is diagnosed. This distinction is not precise but nor is it important for management of the woman; severe, symptomatic thrombocytopenia during pregnancy is treated in the same way as that occurring at other times, and observation without treatment is appropriate for mild, symptomless thrombocytopenia. The distinction is important for the infant, however, because fetal thrombocytopenia does not occur with gestational thrombocytopenia but infants born to mothers with idiopathic thrombocytopenic purpura have a 4–10% risk of having severe thrombocytopenia at birth or during the first week of life.[23,42] Gestational thrombocytopenia may be simply a mild, transient manifestation of idiopathic thrombocytopenic purpura. This idea is supported by several observations. First, platelet counts in some women with an established diagnosis of idiopathic thrombocytopenic purpura decrease during pregnancy and recover after delivery. Second, concentrations of antibodies against platelets are increased in both idiopathic thrombocytopenic purpura and gestational thrombocytopenia.[43] Finally, severe neonatal thrombocytopenia is more common in infants born to mothers with more severe idiopathic thrombocytopenic purpura.[44] Follow-up studies are needed to find out the long-term clinical outcomes of women who have been diagnosed as having gestational thrombocytopenia.

Pre-eclampsia, defined by hypertension and proteinuria, occurs in 5–10% of all pregnancies, and thrombocytopenia occurs in about 15% of women with pre-eclampsia.[42] In addition, some women with severe pre-eclampsia have microangiopathic haemolysis and liver dysfunction (a syndrome described by the acronym, HELLP), and neurological abnormalities, such as hyperreflexia and visual disturbances. Characteristically, all manifestations of pre-eclampsia resolve promptly after delivery, but some women continue to be affected for some time post partum.[45] Severe pre-eclampsia/HELLP syndrome can be indistinguishable from TTP-HUS, and intervention with plasma exchange may be appropriate. This similarity explains why case series of TTP-HUS describe frequent occurrences during pregnancy and post partum.[30,31]

Thrombocythaemia

Essential thrombocythaemia is a clonal disorder that originates from a multipotent stem cell and is characterised by an isolated, persistently high platelet count, typically greater than 600×10^9/L, without the presence of features diagnostic for other myeloproliferative disorders or clinical evidence for reactive thrombocytosis. Many patients have no symptoms, and the diagnosis is made incidentally. Although patients with essential thrombocythaemia do not have the Philadelphia chromosome t(9;22), which defines chronic myelocytic leukaemia, the chimeric *BCR-ABL* transcript mRNA from this translocation has been identified in patients with clinically typical essential thrombocythaemia.[46] The important management issue is whether to start treatment, either to lower the platelet count or to inhibit platelet aggregation, or to observe the patient without specific treatment.

Current options for lowering the platelet count are hydroxyurea, an alkylating agent, and anagrelide, which selectively inhibits platelet production. The potential benefit of treatment is prevention of thrombotic complications such as stroke and myocardial infarction. However, these agents do not permanently control thrombocytosis, so they must be given indefinitely. An important concern is that long-term or lifetime exposure of young patients to an akylating agent such as hydroxyurea may increase the risk of acute leukaemia.[47] Anagrelide is associated with side-effects of vasodilatation (eg, headache, cardiac failure). Aspirin can prevent thrombotic complications, but it also increases the risk of bleeding, especially in patients with very high platelet counts. There are no data documenting a clinical benefit from treatment with aspirin or anagrelide. Standard practice is to use hydroxyurea to lower the platelet count to less than 600×10^9/L if any of the following criteria apply: age over 60 years, platelet count over 1500×10^9/L, or a history of thrombosis.[47,48]

Pharmacological inhibition of platelet function to prevent thrombosis

Since the demonstration that aspirin is effective in the primary prevention of myocardial infarction,[15] the prophylactic use of aspirin for thrombotic disorders has increased enormously. Aspirin has also become a standard treatment for patients with both cardiovascular and cerebrovascular diseases. However, the use of angioplasty and stent placement to open obstructed coronary arteries has necessitated even more effective antithrombotic agents to prevent restenosis. Ticlopidine, which blocks the platelet ADP receptor, has become a standard agent in addition to aspirin for patients with coronary-artery stents, and it shows greater efficacy than aspirin for prevention of recurrent stroke.[49] However ticlopidine has greater risks than aspirin, with the potential for causing neutropenia and TTP-HUS.[36,49] A newer analogue of ticlopidine, clopidogrel, is now used for these indications; its safety compared with that of ticlopidine is not yet certain.[49]

The most effective antithrombotic agents for coronary-artery disease are those that block the platelet fibrinogen receptor, glycoprotein IIb-IIIa.[50] These drugs have been approved for use only within the past 3 years but already are used in most coronary-angioplasty and stent procedures. All currently approved glycoprotein IIb-IIIa blockers, including monoclonal antibodies, peptides, and other small molecules, are given intravenously, but oral agents for long-term use are in clinical trials.[50] An adverse effect of these agents is the occurrence of profound thrombocytopenia in about 1% of patients.[51] Thrombocytopenia can occur immediately after the initial exposure, distinct from typical drug-induced thrombocytopenia, which requires sensitisation by repeated administration. A possible mechanism for the acute thrombocytopenia after initial exposure to a glycoprotein IIb-IIIa blocker is that "naturally occurring" antibodies to glycoprotein IIb-IIIa (described above as causing platelet agglutination in edetic-acid-anticoagulated blood samples and resulting in pseudothrombocytopenia) react with platelets in which the glycoprotein IIb-IIIa is perturbed by interaction with the blocking agent.

References

1 Behnke O, Forer A. From megakaryocytes to platelets: platelet morphogenesis takes place in the bloodstream. *Eur J Haematol* 1998; **60:** 3–23.

2 Kaushansky K. Thrombopoietin: the primary regulator of platelet production. *Blood* 1995; **86:** 419–31.

3 Gurney AL, Carver-Moore K, De Sauvage FJ, Moore MW. Thrombocytopenia in c-mpl-deficient mice. *Science* 1994; **265:** 1445–47.

4 Ihara K, Ishii E, Eguchi M, et al. Identification of mutations in the c-mpl gene in congenital amegakaryocytic thrombocytopenia. *Proc Natl Acad Sci USA* 1999; **96:** 3132–36.

5 Shiozaki H, Miyawaki S, Kuwaki T, Hagiwara T, Kato T, Miyazaki H. Autoantibodies neutralizing thrombopoietin in a patient with megakaryocytic thrombocytopenic purpura. *Blood* 2000; **95:** 2187–88.

6 George JN. Platelet immunoglobulin G: its significance for the evaluation of thrombocytopenia and for understanding the origin of alpha-granule proteins. *Blood* 1990; **76:** 859.

7 Shattil SJ, Kashiwagi H, Pampori N. Integrin signaling: the platelet paradigm. *Blood* 1998; **91:** 2645–57.

8 Santoro SA, Zutter MM. The alpha2beta1 integrin: a collagen receptor on platelets and other cells. *Thromb Haemost* 1995; **74:** 813–21.

9 Santoso S, Kunicki TJ, Kroll H, Haberbosch W, Gardemann A. Association of the platelet glycoprotein Ia C807 T gene polymorphism with nonfatal myocardial infarction in younger patients. *Blood* 1999; **93:** 2449–53.

10 Lopez JA, Andrews RK, Afshar-Kharghan V, Berndt MC. Bernard-Soulier syndrome. *Blood* 1998; **91:** 4397–418.

11 Kaneshiro MM, Mielke CHJr, Kasper CK, Rapaport SI. Bleeding time after aspirin in disorders of intrinsic clotting. *N Engl J Med* 1969; **281:** 1039–42.

12 Aster RH. Pooling of platelets in the spleen: role in the pathogenesis of "hypersplenic" thrombocytopenia. *J Clin Invest* 1966; **45:** 645-57.

13 George JN, Caen JP, Nurden AT. Glanzmann's thrombasthenia: the spectrum of clinical disease. *Blood* 1990; **75:** 1383–95.

14 George JN, Shattil SJ. The clinical importance of acquired abnormalities of platelet function. *N Engl J Med* 1991; **324:** 27–39.

15 Steering Committee of the Physicians' Health Study Research Group. Final report of the aspirin component of the ongoing Physicians' Health Study. *N Engl J Med* 1989; **321:** 129–35.

16 Fiorin F, Steffan A, Pradella P, Bizzaro N, Potenza R, De Angelis V. IgG platelet antibodies in EDTA-dependent pseudothrombocytopenia bind to platelet membrane glycoprotein IIb. *Am J Clin Pathol* 1998; **110:** 178–83.

17 Bizzaro N. EDTA-dependent pseudothrombocytopenia: a clinical and epidemiological study of 112 cases, with 10-year follow-up. *Am J Hematol* 1995; **50:** 103–09.

18 Najean Y, Lecompte T. Hereditary thrombocytopenias in childhood. *Semin Thromb Hemost* 1995; **21:** 294–304.

19 Gentilini G, Curtis BR, Aster RH. An antibody from a patient with ranitidine-induced thrombocytopenia recognizes a site on glycoprotein IX that is a favored target for drug-induced antibodies. *Blood* 1998; **92:** 2359–65.

20 Burgess JK, Lopez JA, Berndt MC, Dawes I, Chesterman CN, Chong BH. Quinine-dependent antibodies bind a restricted set of epitopes on the glycoprotein Ib-IX complex: characterization of the epitopes. *Blood* 1998; **92:** 2366–73.

21 George JN, Raskob GE, Shah SR, et al. Drug-induced thrombocytopenia: a systematic review of published case reports. *Ann Intern Med* 1998; **129:** 886–90.

22 Warkentin TE, Chong BH, Greinacher A. Heparin-induced thrombocytopenia: toward consensus. *Thromb Haemost* 1998; **79:** 1–7.

23 George JN, Woolf SH, Raskob GE, et al. Idiopathic thromboctyopenic purpura: a practice guideline developed by explicit methods for the American Society of Hematology. *Blood* 1996; **88:** 3–40.

24 Frederiksen H, Schmidt K. The incidence of ITP in adults increases with age. *Blood* 1999; **94:** 909–13.

25 Bolton-Maggs PHB, Moon I. Assessment of UK practice for management of acute childhood idiopathic thrombocytopenic purpura against published guidelines. *Lancet* 1997; **350:** 620–23.

26 Lilleyman JS. Management of childhood idiopathic thrombocytopenic purpura. *Br J Haematol* 1999; **105:** 871–75.

27 Vesely S, Buchanan GR, George JN, Raskob GE, Cohen A. Self-reported diagnostic and management strategies in childhood idiopathic thrombocytopenic purpura: results of a survey of practicing pediatric hematology/oncology specialists. *Am J Pediatr Hematol Oncol* 2000; **22:** 55–61.

28 Bahner I, Kearns K, Coutinho S, Leonard EH, Kohn DB. Infection of human marrow stroma by human immunodeficiency virus-1 (HIV-1) is both required and sufficient for HIV-1-induced hematopoietic suppression in vitro: demonstration by gene modification of primary human stroma. *Blood* 1997; **90:** 1787–98.

29 Francois B, Trimoreau F, Vignon P, Fixe P, Praloran V, Gastinne H. Thrombocytopenia in the sepsis syndrome: role of hemophagocytosis and macrophage colony-stimulating factor. *Am J Med* 1997; **103:** 114-20.

30 Amorosi EL, Ultmann JE. Thrombotic thrombocytopenic purpura: report of 16 cases and review of the literature. *Medicine* 1966; **45:** 139–59.

31 George JN, Gilcher RO, Smith JW, Chandler L, Duvall D, Ellis C. Thrombotic thrombocytopenia purpura-hemolytic uremic syndrome: diagnosis and management. *J Clin Apheresis* 1998; **13:** 120–25.

32 Rock GA, Shumak KH, Buskard NA, et al. Comparison of plasma exchange with plasma infusion in the treatment of thrombotic thrombocytopenic purpura. *N Engl J Med* 1991; **325:** 393–97.

33 Clark WF, Rock GA, Buskard N, et al. Therapeutic plasma exchange: an update from the Canadian Apheresis Group. *Ann Intern Med* 1999; **131:** 453–62.

34 Laszik Z, Silva F. Hemolytic-uremic syndrome, thrombotic thrombocytopenia purpura, and systemic sclerosis (systemic scleroderma). In: Jennett JC, Olson JL, Schwartz MM, Silva FG, eds. Heptinstall's pathology of the kidney, 5th edn. Philadelphia: Lippincott-Raven, 1998: 1003–57.

35 Gottschall JL, Neahring B, McFarland JG, Wu G-G, Weitekamp LA, Aster RH. Quinine-induced immune thrombocytopenia with

hemolytic uremic syndrome: clinical and serological findings in nine patients and review of literature. *Am J Hematol* 1994; **47:** 283–89.

36 Bennett CL, Weinberg PD, Rozenberg-Ben-Dror K, Yarnold PR, Kwaan HC, Green D. Thrombotic thrombocytopenic purpura associated with ticlopidine: a review of 60 cases. *Ann Intern Med* 1998; **128:** 541–44.

37 Dang CT, Magid MS, Weksler B, Chadburn A, Laurence J. Enhanced endothelial cell apoptosis in splenic tissues of patients with thrombotic thrombocytopenic purpura. *Blood* 1999; **93:** 1264–70.

38 Furlan M, Robles R, Galbusera M, et al. Von Willebrand factor-cleaving protease in thrombotic thrombocytopenic purpura and the hemolytic-uremic syndrome. *N Engl J Med* 1998; **339:** 1578–84.

39 Tsai H-M, Lian ECY. Antibodies to von-Willebrand factor-cleaving protease in acute thrombotic thrombocytopenic purpura. *N Engl J Med* 1998; **339:** 1585–94.

40 Mead PS, Griffin PM. *Escherichia coli* O157:H7. *Lancet* 1998; **352:** 1207–12.

41 Shumak KH, Rock GA, Nair RC, Canadian Apheresis Group. Late relapses in patients successfully treated for thrombotic thrombocytopenic purpura. *Ann Intern Med* 1995; **122:** 569–72.

42 Burrows RF, Kelton JG. Fetal thrombocytopenia and its relation to maternal thrombocytopenia. *N Engl J Med* 1993; **329:** 1463–66.

43 Lescale KB, Eddleman KA, Cines DB, et al. Antiplatelet antibody testing in thrombocytopenic pregnant women. *Am J Obstet Gynecol* 1996; **174:** 1014–18.

44 Valat AS, Caulier MT, Devos P, et al. Relationships between severe neonatal thrombocytopenia and maternal characteristics in pregnancies associated with autoimmune thrombocytopenia. *Br J Haematol* 1998; **103:** 397–401.

45 Martin JN Jr, Files JC, Blake PG, Perry KG Jr, Morrison JC, Norman PH. Postpartum plasma exchange for atypical preeclampsia-eclampsia as HELLP (hemolysis, elevated liver enzymes, and low platelets) syndrome. *Am J Obstet Gynecol* 1995; **172:** 1107–27.

46 Blickstein D, Aviram A, Luboshitz J, et al. BCR-ABL transcripts in bone marrow aspirates of Philadelphia-negative essential thrombocythemia patients: clinical presentations. *Blood* 1997; **90:** 2768–71.

47 Cortelazzo S, Finazzi G, Ruggeri M, et al. Hydroxyurea for patients with essential thrombocytopenia and a high risk of thrombosis. *N Engl J Med* 1995; **332:** 1132–36.

48 Ruggeri M, Finazzi G, Tosetto A, Riva S, Rodeghiero F, Barbui T. No treatment for low-risk thrombocythaemia: results from a prospective study. *Br J Haematol* 1998; **103:** 772–77.

49 Sharis PJ, Cannon CP, Loscalzo J. The antiplatelet effects of ticlopidine and clopidogrel. *Ann Intern Med* 1998; **129:** 394–405.

50 Topol EJ, Byzova TV, Plow EF. Platelet GPIIb-IIIa blockers. *Lancet* 1999; **353:** 227–31.

51 Berkowitz SD, Sane DC, Sigmon KN, et al. Occurrence and clinical significance of thrombocytopenia in a population undergoing high-risk percutaneous coronary revascularization. *J Am Coll Cardiol* 1998; **32:** 311–19.