UT Southwestern Medical Center

Immune thrombocytopenic purpura: an update

Internal Medicine Grand Rounds

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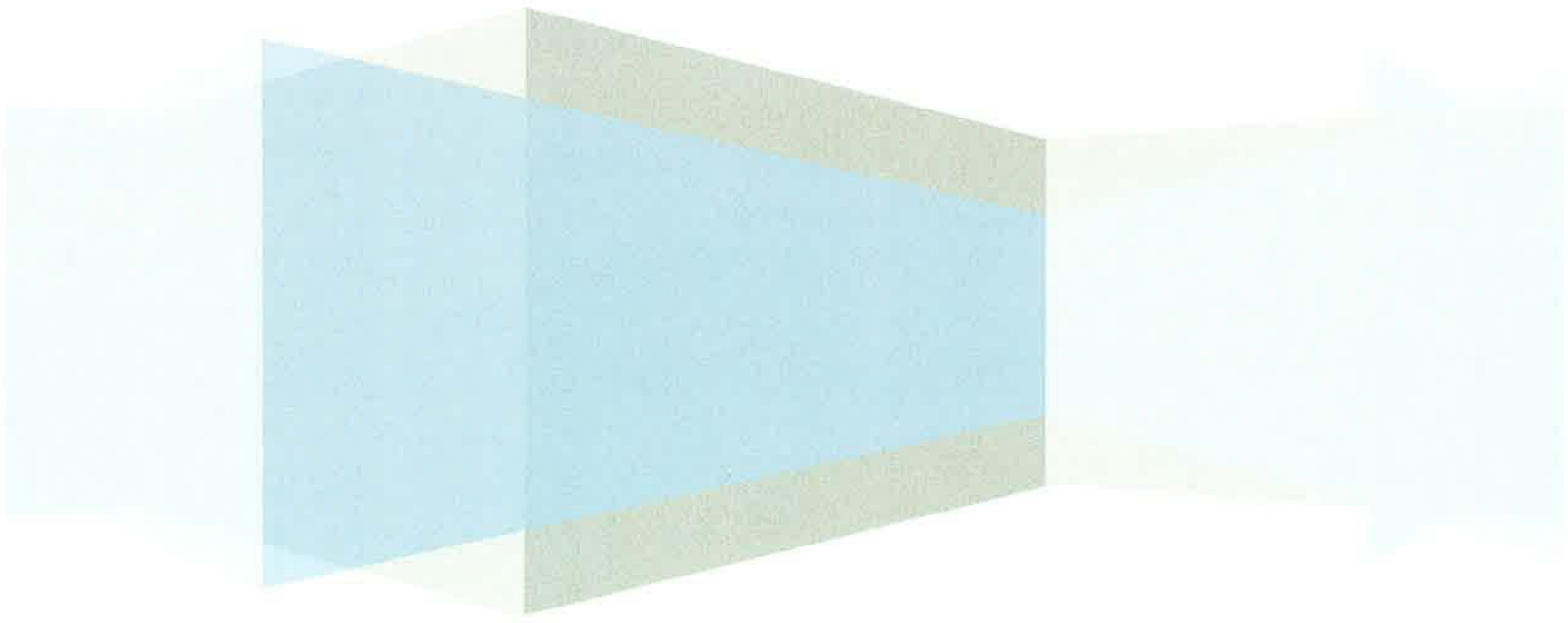

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Dr. Rutherford's clinical and research interests are in non-malignant hematologic disorders, particularly immune thrombocytopenia, bleeding disorders and sickle cell disease.

This is to acknowledge that Cynthia Rutherford, M.D. has not disclosed any financial interests or other relationships with commercial concerns related directly or indirectly to this program. Dr. Rutherford will not be discussing off-label uses in her presentation.

A. A CASE OF REFRACTORY ITP

A 43 year old African American woman presented to the Parkland ER in June 2009, having noted an enormous bruise on her leg following a fall, after she had fainted three days previously. She gave a history of menorrhagia, unchanged since her teenage years, but had noted increased bruising for approximately the last year. Six months earlier she had started taking aspirin three times weekly. In the ER she was noted to be very pale and to have a 10 inch bruise on her thigh, but had no other bruising and no petechiae on skin or mucous membranes.

Laboratory studies showed to have a profound iron deficiency anemia, with hemoglobin of 7.3g/dl, ferritin of 7 ng/ml; her platelet count was only 8,000/mm^3. Other than the iron deficiency changes, there were no blood smear abnormalities. She was admitted, a presumptive diagnosis of immune thrombocytopenic purpura was made, and she was started on Prednisone 1 mg/kg and oral iron. She was discharged three days later, by which time her platelet count had already risen to 34,000, eventually reaching a high of 207,000 2 weeks later.

Because her response to steroids was rapid, and because of concerns with steroid side effects in this morbidly obese woman, steroid therapy was tapered quite rapidly, so that 30 days later she was off all steroids. Her platelet count promptly dropped back to 9,000/mm^3. Steroids were restarted, again with an immediate response, and tapered more gradually. However, again the platelet count dropped, even before steroid withdrawal was complete.

By now she was iron replete; her blood smear was normal except for thrombocytopenia with rather large platelets. Steroids were restarted in fairly low dose to get the platelet count to a "safe" level and she underwent vaccination with Pneumococcal, meningococcal and hemophilus influenza B vaccine. After waiting the requisite two weeks following vaccination, and confirming her bone marrow had normal numbers of megakaryocytes without any morphological abnormalities, she underwent laparoscopic splenectomy in mid-December 2009, six months after her initial presentation.

Unfortunately, she had no response to splenectomy, now meeting the definition of "refractory ITP". In February 2010 she received 4 doses of the monoclonal antibody, rituximab. She was tapered off corticosteroids by April, at which time her platelet count remained normal, and she has been followed regularly in the clinic off all treatment.

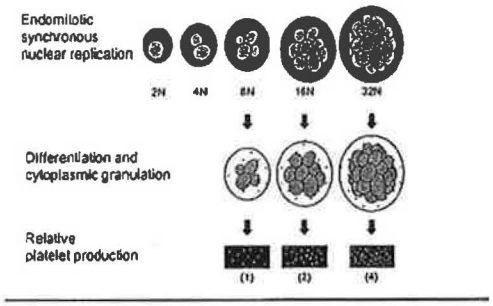
Since early July her platelet count has slowly fallen, most recently measuring 38,000/mm^3 three weeks ago. At this level she was asymptomatic and surveillance was continued off treatment with close follow-up.

This case will be discussed further at the end of the presentation.

B. NORMAL PLATELET PRODUCTION AND LIFE SPAN

Platelets are formed from megakaryocytes in the bone marrow, where the megakaryocyte precursor is one of the progeny of the hemopoietic stem cell. Initially this diploid precursor is a small nondescript cell. Under the sequential influence of multiple cytokines, together with its specific growth factor Thrombopoietin, the megakaryocyte precursor undergoes a series of unique endomitotic divisions, resulting in the gigantic bone marrow cell, the megakaryocyte, a cell with 16N or even 32N chromosomes, all contained within one huge, complex nucleus. This development takes 7-10 days. While the nucleus is progressively enlarging there is a commensurate increase in the amount of cytoplasm. In this cytoplasm the organelles and granules develop which contain the compounds, or their precursors, vital for the platelet's role in primary and secondary hemostasis.

Figure 1: Megakaryopoiesis



Stimulation of Megakaryocyte development

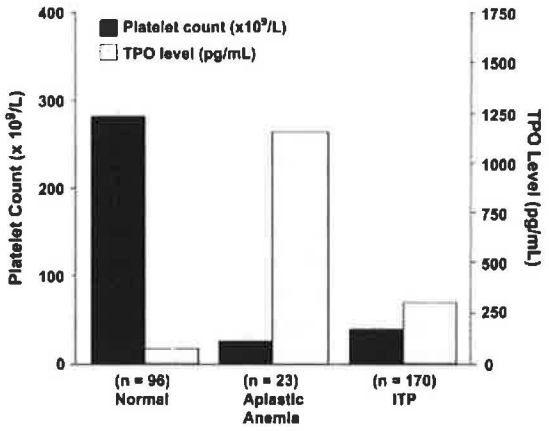
The cytokines which induce the megakaryocyte to develop and mature include interleukin (IL)-6. IL-11, stem cell factor (c-kit ligand), GM-CSF and the cell has a receptor, *(c-mpl)* on its surface membrane for its specific growth factor, Thrombopoietin (TPO). When TPO binds to this *c-mpl* receptor, it induces endomitotic division and maturation via tyrosine phosphorylation, and the JAK/STAT signal transduction pathway is triggered. Thrombopoietin is the major growth factor for megakaryocytes. Mice with the TPO gene knocked out have only 10% of the platelet production of normal controls.

Thrombopoietin [1]

Thrombopoietin (TPO) is produced constitutively, predominantly by the liver and there is little inducible Thrombopoietin. Its levels are controlled by the number of megakaryocytes and by binding to their *c-mpl* receptor. Unlike the red cell growth factor, erythropoietin, there is no feedback loop. When megakaryocyte numbers are low, such as after chemotherapy or in aplasia, the TPO level will be high. In states of platelet consumption, when there are normal numbers of megakaryocytes such as in ITP the TPO levels are not increased. See Figure 2.

When the megakaryocyte cytoplasm reaches maturity, the megakaryocyte membrane proliferates and invaginates into the cytoplasm. This forms a network, called the demarcation membrane system, which divides the cytoplasm into areas called pro-platelets Individual platelets are released from long pseudopodia which extend from the megakaryocytes into the lumen of the marrow sinusoid. Each megakaryocyte is able to produce 2-3000 platelets. These platelets then enter the peripheral blood, where they circulate for approximately seven to ten days.

Figure 2: Thrombopoietin levels in ITP [2]



Under normal circumstances approximately one-third of the released platelets are sequestered within the spleen. There is also a random loss of platelets as they repair breaches in the endothelium, consuming the equivalent of 25-40,000 platelets per day. Individual platelets are enucleate structures, approximately 2 microns in diameter, 10 femtoliters in volume. Their complex membranes, formed from the demarcation membrane system contain a number of functionally important glycoproteins, including adhesive molecules such as GPIb-IX (Von Willebrand factor), GPIa/IIa (collagen), GPIc/IIa (fibronectin), and others such as GPIIb-IIIa, involved in platelet-platelet aggregation via fibrinogen "bridging". The membrane also contains receptors for the Fc (constant) portion of immunoglobulin molecules and complement receptors. Platelet membranes also contain the TPO *c-mpl* receptor and will bind circulating TPO.

C. HISTORY OF ITP [3, 4]

In 1881, Bizzorzero first correctly identified platelets as the essential component for primary hemostasis. It was a decade later before megakaryocytes were described, and it was not until the turn of the century that their role in platelet production was recognized. In 1915, Frank observed patients with immune thrombocytopenic purpura (ITP), or as it was known then, *purpura haemorrhagica (*non-febrile purpura*)* had normal or even increased numbers of megakaryocytes in the bone marrow, but a markedly diminished number of platelets in the peripheral blood. Frank believed this was evidence of defective platelet production. This belief was reinforced because the megakaryocytes looked different from normal, in that there was a shift to immaturity. Others believed the low platelet count was due to excessive platelet destruction in the periphery, a situation analogous to hemolytic anemia. They believed this idiopathic thrombocytopenic purpura (ITP) was the platelet equivalent of hemolysis, with platelets rather than red cells being destroyed prematurely in the spleen. The cause of this premature destruction was not known.

This line of reasoning led to a trial of splenectomy for ITP, in which the surgeon's maxim "in wet, out dry" recognized that hemostasis was usually dramatically improved as soon as the splenic artery was cross clamped. The earliest reported studies of splenectomy date back to the 1930s and 1940s, prior to the availability of corticosteroids. [5, 6] The platelet count was observed to rise rapidly, often reaching supranormal levels in the first 24-48 hours. The reason for the platelets' premature removal by the spleen

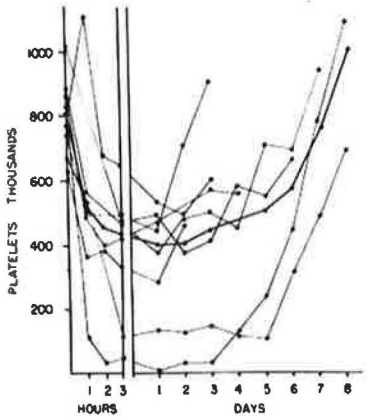
was still not understood, although many believed some circulating factor was responsible, especially when it was observed that a pregnant woman with ITP could give birth to a baby with thrombocytopenia.

In 1950, William Harrington, a 27 year old Boston-trained physician [7] began a hematology fellowship with the eminent Dr. Carl V. Moore at Washington University Medical School in St. Louis. There was such intense competition for this position that the two finalists, Harrington and James Hollingsworth agreed to share the salary. Harrington had become very interested in ITP while a medical student at Tufts in Boston, and was convinced that some substance circulating in the blood was responsible.

He was determined to test this theory. One weekend, while Dr. Moore was away, a patient with severe ITP was admitted. This patient had previously had her spleen removed, but continued to hemorrhage. Harrington realized this was the case he needed to prove his theory, and persuaded Hollingsworth to join him in the experiment, which is graphically described by Lawrence Altman in *Who Goes First.* [8] Harrington determined that they would take a pint of this patient's blood and administer it to the one who matched her blood type. This individual would donate a pint of blood to be infused into the patient. Scientific rigor demanded a bone marrow examination before and after the infusion, to confirm the numbers of megakaryocytes had not been affected, and serial platelet counts on both patient and subject would be measured. Harrington, who as luck would have it, had the same blood type as the patient, donated his pint of blood to be given to the patient; he was so excited he barely felt the sternal bone marrow Hollingsworth performed on him. He was then infused with the patient's blood and both young physicians set out to perform the serial platelet counts. Harrington began to feel increasingly unwell, and was alarmed to find his platelet count had fallen to almost unrecordable levels. Hollingsworth was even more aghast when he observed Harrington have a *grand mal* seizure. It finally dawned on them that the experiment they had undertaken was potentially very dangerous. Fortunately Harrington recovered and went back to counting platelets, without further untoward events. When Dr. Carl Moore returned the next day they confessed, and the alarmed Moore insisted Harrington needed to be hospitalized, as his platelet count remained essentially zero. He sat bolt upright in bed until his platelet count returned to normal over the next four to five days, without further incident.

Once the worry about Harrington was over the three became very excited about the success of this "experiment". Over the next few months nearly everyone working in the department, from Dr. Carl Moore himself to the department secretaries, became subjects for further experiments, although they used much smaller volumes of patient plasma. Harrington gave himself 35 more infusions of ITP plasma, proving without doubt that it contained an anti-platelet factor. By the time his paper describing this was published [9], the thrombocytopenic factor was known to be part of the globulin fraction of the plasma

Figure 3: Infusion of normal subjects with blood or plasma from a patient with ITP [9]



D. PATHOPHYSIOLOGY OF ITP

Antiplatelet antibodies

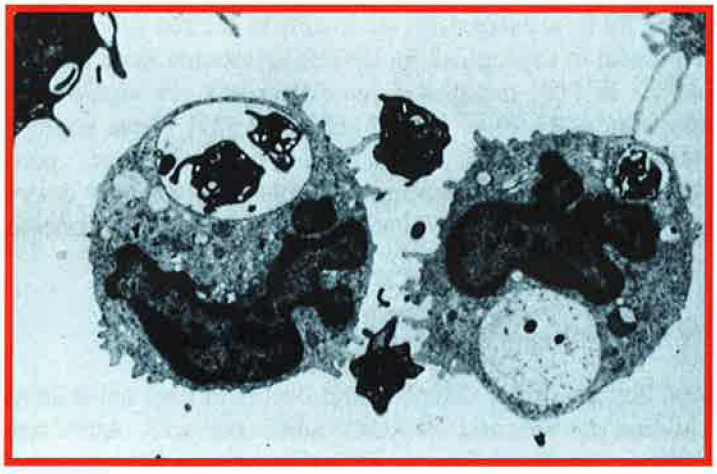
Patients with ITP have been shown to have increased numbers of platelet-associated IgG and IgM molecules, which are both adherent to the platelet membrane and found within the platelet cytoplasm. [10] [11] However, even in normal subjects, immunoglobulins, particularly IgG, adhere non-specifically to the platelet membrane, through its many immunoglobulin Fc receptors. [12] Thrombocytopenia from platelet consumption of any cause is usually associated with an increased number of molecules of IgG per platelet, some of which may reflect their larger size. Patients with an increased serum IgG for any cause (myeloma, liver disease, AIDS) similarly show increased amounts of IgG bound to their platelets through membrane Fc receptors. Thus measurement of platelet-associated IgG, a test akin to the direct antiglobulin (Coombs') test for red cells has been too non-specific to be clinically useful. [13] Serum measurements of circulating anti-platelet antibodies have been even less helpful and are not routinely carried out.

More recently auto-antibodies in ITP have been found to be directed at important functional glycoproteins, particularly antibodies to GPIIb/IIIa and GPIa/IX. [14-16] Antigen capture techniques, using monoclonal antibodies to specific glycoproteins, enable measurement of antibodies bound through their Fab component. These techniques involve exposing purified glycoproteins, immobilized on beads or in microtiter wells, to ITP serum or solubilized platelets, and demonstrating their presence by adding a labeled anti-human antibody. It is possible, especially in the acute ITP seen in children that the viral infection or other alterations of these glycoproteins, renders them antigenic.

Mechanisms of Platelet destruction

Phagocytosis of antibody-coated platelets is well recognized, with the platelet-membrane-associated antibody binding to the Fc receptor of the macrophage. [17] These events have been dramatically captured by electron microscopy. [18, 19] The early recognition of splenectomy as the most effective treatment modality in ITP suggested that much of this phagocytosis occurred in the spleen. However, other parts of the reticulo-endothelial system (RES) are clearly involved, suggested by the 20 – 30% of patients who do not respond to splenectomy and by studies using labeled platelets which demonstrate uptake in liver also. There may also be some intravascular destruction of platelets via the complement pathway. [20]

Figure 4: Macrophages with adherent and engulfed platelets[18]



Abnormalities in cellular immunity may also contribute to platelet destruction. [21] For example: T-helper cells may show increased proliferation and IL-2 production when incubated with autologous platelets and antigen-presenting cells. In other in vitro systems, T lymphocytes can cause lysis of platelets or react with specific platelet autoantigens.

Removing the spleen has two potential benefits: it removes an important source of lymphocytes, especially those capable of antibody production, and it removes a stringent micro-environment, wherein the antibody-coated platelets are in close contact with avid phagocytes.

Experiments using infusion of ITP plasma in splenectomized and non-splenectomized subjects, show that a significantly higher concentration of antibody is needed to produce thrombocytopenia in splenectomized subjects; the presences of the spleen increases the efficiency of destroying antibody-coated platelets. Chromium-labeling of platelets has shown they have a very short survival in ITP, with a half-life of only a few minutes in severely affected individuals. As expected, the half-life is proportional to the platelet count. [22]

Platelet production in ITP

It was always assumed that production of platelets was increased in ITP, because of the frequently observed increase in number of megakaryocytes in the marrow and the larger-than-normal platelets observed in the peripheral blood. In 1977, Harker demonstrated that in ITP platelet production from megakaryocytes could increase up to five fold. However, evidence suggests there is impaired platelet production or release in up to half the cases of ITP. Antibodies from ITP spleens have been found to bind to megakaryocytes. This may interfere with platelet production, possibly by binding to the membrane demarcation system, or by labeling the released platelets so that they are removed by marrow macrophages, before they get a chance to enter the peripheral circulation. [23-25]

Electron microscopy of megakaryocytes in ITP also shows abnormalities in their ultrastructure, with evidence of apoptosis. [26]. The stimulus to this megakaryocyte proliferation in ITP is poorly understood. Although Thrombopoietin (TPO) levels are a little higher than normal, they are significantly less than in hypoproliferative thrombocytopenic states. [2]

Function of platelets in ITP

Because of their short half life, most of the platelets circulating in ITP are newly produced. This can be confirmed by determining the reticulated platelet count, whereby the percentage of young platelets, still containing RNA is measured by flow cytometry, analogous to the red cell reticulocyte. This technique is a research tool that can be useful in distinguishing thrombocytopenia from defective production from that of consumptive disorders. In ITP, reticulated platelet counts are significantly higher than those in patients thrombocytopenic from AML or in normal subjects. [27] These young platelets are larger than normal size when seen with electron microscopy or in the peripheral blood smear. The young platelets in ITP function very well and patients with immune destruction of platelets have significantly less bleeding at a given platelet count that patients with thrombocytopenia from hypoplastic causes.

E. EPIDEMIOLOGY OF ITP [26] [28]

ITP is a relatively common hematological disorder, and occurs in both children and adults. Its estimated incidence is 5/100,000 children per year and 2/100,000 adults per year. Adult cases are appreciated more

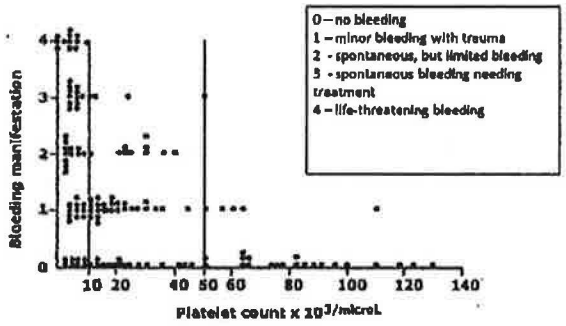
often now since mild asymptomatic cases are picked up by automated blood counting. ITP in children is significantly different from that seen in adults, and is usually an acute and short-lived illness following a viral infection. This review will focus on the disorder as it occurs in adults, so-called chronic ITP.

Aggregated data from multiple studies show a moderate female predominance, but in the middle years only, and an increased incidence with age. There is no racial variation. In untreated patients, presumably the milder cases, about 5% will spontaneously remit. For patients who do receive therapy and achieve a platelet count over 30,000/mm^3 long term outcomes are similar to those of a non-ITP population. In contrast, patients whose platelet counts remain below 30,000 have four times the mortality rate of the general population, due to both infectious complications, usually from treatment, or thrombocytopenic bleeding. There is close correlation of morbidity and mortality from bleeding and the degree of thrombocytopenia. The literature suggests 1.6 to 3.0 fatal cases per 100-patient-years in patients with a platelet count under 30,000/mm^3 one year after diagnosis, with older patients having significantly higher rates.

F. THE CLINICAL PRESENTATION OF ITP

The clinical presentation of chronic ITP is essentially that of a defect in primary hemostasis. Bleeding manifestations correlate with the degree of thrombocytopenia and include, in order of severity: skin bruising, muco-cutaneous bleeding such as epistaxis, petechiae in skin and mucous membranes. The most feared bleeding complications, fortunately rare, are internal, particularly intracerebral bleeding and bleeding obstructing the airway, seen in 1-2% of cases. With the highly functional platelets in this condition, few serious clinical manifestations are seen unless the platelet count is very low; even then many patients have modest symptoms [29]

Figure 5: Bleeding manifestations in relation to platelet count in ITP [29]



ITP is essentially a diagnosis of exclusion, both in its clinical presentation and in the laboratory. Clinical manifestations are limited to signs of bleeding. Clinical features such as splenomegaly should raise other diagnostic possibilities. Table 1 gives a differential diagnosis. Most conditions can be excluded by evaluation of the history, basic clinical examination and CBC with smear review. History taking should include questions about symptoms of viral infection, about medications, alcohol intake, HIV risk factors and symptoms suggestive of systemic lupus erythematosus. Physical examination should search carefully for splenomegaly, which is not expected in primary ITP, and for manifestations of the other disorders associated with thrombocytopenia.

Table 1: Differential diagnosis of chronic ITP

Spurious thrombocytopenia – EDTA or other agglutinins

Common causes of thrombocytopenia that mimic ITP
- Pregnancy – gestational thrombocytopenia
- Drugs – quinine, quinidine, heparin. . .
- Viral infections, especially AIDS
- Hypersplenism due to chronic liver disease

Disorders with 2^0 immune thrombocytopenia
- Systemic lupus erythematosus
- Lymphoproliferative disorders: CLL, Non-Hodgkin's lymphoma
- Hepatitis C infection
- HIV infection

Less common causes – most of which have other obvious clinical or laboratory features
- TTP / HUS
- Myelodysplasia
- Wiskott Aldrich syndrome
- Type 2B von Willebrand disease
- Chronic disseminated intravascular coagulation (DIC)

Laboratory assessment with CBC should show an isolated thrombocytopenia, without abnormalities in other cell counts, unless there is anemia from significant bleeding. The blood smear should always be examined and should be normal apart from thrombocytopenia, usually with large platelets. It is particularly important to examine the smear for the platelet clumping of "spurious" thrombocytopenia, and for schistocytes, to exclude thrombotic thrombocytopenic purpura (TTP).

G. GUIDELINES FOR DIAGNOSIS AND MANAGEMENT IN ITP

In 1996, the first treatment guideline ever published by the American Society of Hematology (ASH) provided specific recommendations for diagnosis and management of ITP in adult patients. [30]

In Hematology practice, ITP is a common disorder, with a copious literature. However, as the editorial accompanying the publication of the ASH guideline stated what was true then, and unfortunately remains true:

. . . our eyes were opened to the fact that most of what we do with ITP patients is based on case series of selected patients, whose course cannot be evaluated in the absence of a control group, and whose outcomes were only evaluated by platelet counts. There is a lack of knowledge about the natural history of untreated ITP and of the effect of treatment on clinical outcomes of major bleeding and death. [31]

These guidelines were developed by a panel of 15 hematologists, experienced in the management of ITP, who evaluated the available literature, using specific criteria to assess validity. They then voted on nearly 2000 questions regarding diagnosis and treatment, and presented the majority vote as their recommendations, namely:

If the history, physical examination and the initial CBC and peripheral smear are compatible with a diagnosis of ITP, minimal further testing is indicated. They proposed the only further tests needed to diagnose ITP were:

1. HIV antibody, if there are risk factors of HIV
2. Bone marrow examination in patients over 60 years, and in patients being considered for splenectomy.
3. Thyroid function tests before elective splenectomy – to exclude Grave's disease

The guidelines also recommended against measuring bleeding time, performing abdominal imaging studies to detect splenomegaly or routine platelet antibody tests, because of the latter's poor sensitivity and specificity.

They recommended corticosteroids as the first line of treatment, initiated at a platelet count of 30,000/mm3, or higher if there were bleeding manifestations. If no response to steroids was noted after four weeks, or if the platelet count fells when steroids were withdrawn, they recommended a prompt splenectomy.

In 2003 the British Committee for Standards in Haematology (BCSH) weighed in with a further guideline [32] with more wide-ranging recommendations, including for pregnant patients. Their recommendations, like those of the ASH group were derived from a combination of then available studies and from expert opinion. They concurred with 30,000/mm^3 platelets as the level at which treatment should be initiated, even in asymptomatic patients, although used 20,000/mm^3 for asymptomatic pregnant patients. They recommended splenectomy as the major second line therapy, but did not make specific recommendations when it should be undertaken, whereas the ASH guideline felt this should be undertaken earlier, particularly if there was no response to corticosteroids..

A lot has happened since 2003 in terms of new therapeutic options for ITP, and a new set of guidelines, with the same scientific rigor and lack of bias as these earlier guidelines is sorely needed. The "consensus report" published in Blood this year by a panel of twenty-two international hematologists is definitely not that document. [33]. Perhaps the least helpful aspect of this "consenus" was their listing the second line ITP treatments in alphabetical order, putting splenectomy low on the list. In a stinging rebuke in last week's editorial published in the New England Journal of Medicine, Dr. James George, who led the panel that published the original ASH guidelines, stated

> "Interpretation of these recommendations should take into account that this report was supported by the companies that produce Romiplostim and Eltromobopag. Conflict-of-interest issues have arisen in relation to clinical-practice guidelines. Therefore, it will be important to compare these recommendations for immune thrombocytopenia with the revised guideline of the American Society of Hematology (currently in preparation), which was drafted with neither commercial support nor participation by anyone with a commercial conflict of interest". [34]

The good news is that an update of the 1996 ASH guideline is in the works.

H. GENERAL PRINCIPLES OF TREATEMENT IN ITP

Many patients diagnosed with ITP do not require treatment. The decision to begin treatment needs to be carefully considered, as the disease is chronic, and the first-line treatment, corticosteroids, have major long and short term side effects, including acne, weight gain, fluid retention, hyperglycemia and osteoporosis. The decision to treat should be based on both the degree of thrombocytopenia and associated bleeding symptoms. Treatment is not recommended in patients with platelet counts of $30,000/mm^3$ or more, unless there are significant bleeding symptoms. Once the platelet count gets below $30,000/mm^3$, at which level most patients are symptomatic, therapy is recommended by both ASH and BCTH guidelines. [30, 32]

Treatment options for ITP can be divided into first line therapy, emergency therapy and second line therapy.

1. FIRST LINE TREATMENT IN ITP

This is essentially corticosteroids, which are initially effective in 60 – 70% of patients. Corticosteroids have been the sheet anchor of initial treatment of ITP since they were first introduced in the 1950s. They appear to work in three ways: in the early phase of treatment they reduce the number of Fc receptors on macrophages, particularly in the spleen; long term they appear to suppress platelet antibody production. Older studies suggest they may reduce bleeding by a direct effect on blood vessels. [35]

However, even after using these agents for 60 years, there is no major randomized study of this treatment modality. There is consistent evidence, from uncontrolled studies, that glucocorticoids improve platelet counts in most patients; unfortunately only about 30% of responses to daily prednisone are durable.

Both the ASH and BCSH panels concluded that glucocorticoid therapy with prednisone 1-2 milligrams per kilogram, was the appropriate initial treatment for patients with platelet counts less than $30,000/,mm^3$, or at higher platelet levels if significant bleeding attributable to thrombocytopenia was present. They recommended that steroids should be continued until there is a response and then tapered over a month.

Because of concerns with the troublesome side effects of the protracted corticosteroid therapy which is sometimes needed, more recent studies have looked at the use of short pulses of high dose dexamethasone. Initial reports appeared very promising: Cheng et al [36] administered a single four-day course of oral dexamethasone (40mg a day) to 125 consecutive adults newly diagnosed with ITP. All had a platelet count below $20,000/mm^3$, or below 50,000 with bleeding: 80% of the patients had a good initial response, namely a platelet count that rose at least 30,000 after initiation of treatment, however at longer term follow-up (mean 30 months) only 42% of patients showed a sustained response. Subsequent studies have used multiple dexamethasone pulses with longer relapse free intervals of 8-15 months, in 60-80% of patients. [37] Although high dose steroid pulses appear to be a promising option for initial treatment, there has not been a randomized trial comparing this treatment with daily Prednisone, and long term follow-up data is lacking.

2. EMERGENCY TREATMENT IN ITP

a. Intravenous immunoglobulin: Patients with chronic ITP may present with acute life-threatening bleeding. In this situation intravenous immunoglobulin (IVIG) can be life-saving. Probably the most important mechanism of action of IVIG is through RES blockade. Fc receptors, occupied by the infused immunoglobulins, cannot bind to anti-platelet antibodies on the platelet surface so platelets escape phagocytosis. Other possible mechanisms, extrapolating from murine ITP models, include cytokine regulation, and neutralization of pathogenic autoantibodies mediated by anti-idiotypic antibodies in the IVIG preparation. [38]

Our usual strategy is to administer 2 grams/kilogram of IVIG over 2-4 days. One small study demonstrated that 60% of patients will respond after a single dose of 1 gram/kilogram. [39] Thus one could start with an initial dose of 1 g/Kg and only if response is inadequate by 48 hours would a second be given. In patients with life-threatening bleeding, the standard 2 gram/kilogram dose over 2 days should still be undertaken. IVIG is also helpful in the situations where emergency surgery or delivery is needed. Unfortunately, IVIG has a short duration of action, usually 2-4 weeks at the most, but this is often long enough to achieve a response to corticosteroids or to have a surgical procedure.

Studies have shown that a more rapid rise of platelet counts occurs with IVIG than with Prednisone alone. Apart from the expense of IVIG there are some untoward side-effects, including fevers, chills, headaches, and vomiting. Some IVIG preparations contain immune complexes or sucrose compounds, which can produce renal and other problems.

b. Rh immune globulin: This is an alternative method for inducing temporary RES blockade.[40-42] This preparation contains antibody directed at the Rh(D) antigen, and is only effective in patients who are Rh (D)-positive: approximately 85% of the population. In such patients the administration of intravenous Rh(D) immune globulin, in a single dose of 75 micrograms/kilogram, binds to the red cells, causing mild hemolysis, usually with a hemoglobin drop of 1 gram/dl; these antibody-coated red cells block macrophage Fc receptors. Occasionally a much more severe hemolytic anemia occurs, so vigilance is needed, and there is a Black Box warning for this product regarding this complication. As with IVIG, this effect is relatively short-lived (1-3 weeks) and it is only appropriate for patients with acute bleeding problems or patients being prepared for surgery although a randomized study showed that repeated administration might obviate, or at least delay, splenectomy. [43] There has not been a controlled trial of Rh(D) immune globulin in comparison to IVIG; moreover it is only licensed for use in patients who still have a spleen.

c. High dose intravenous methylprednisolone in doses of 1 gram daily for three days was recommended as an emergency strategy by the ASH guidelines. More recently a randomized study comparing 3 days of methylprednisolone at 15 mg/kilogram and 3 days of IVIG at 0.7 g/kilogram showed that IVIG gave a more rapid and more sustained increase in platelet counts. [44]

d. Platelet transfusion usually gives very little increment in platelet counts, as expected from the tagged platelet survival studies, but will often be included in emergency management, particularly if there is intracerebral bleeding. [45]

3. SECOND LINE TREATMENT IN ITP

Table: 2 Second line therapy in ITP

Therapy	Mechanism of action	References
Splenectomy	Removes stringent microenvironment for antibody-coated platelets Reduces auto-antibody production	[30, 32, 34, 37, 46, 47]
Rituximab	Immunosuppression by B-lymphocyte depletion	[48-51]
Romiplostim	Thrombopoietin analogue	[2, 52-58]
Eltrombopag	Thrombopoietin analogue	[2, 55, 58, 59]
*Azathioprine	Immunosuppression	[60]
*Cyclosporine	Immunosuppression	[61]
*Cyclophosphamide	Immunosuppression	[62, 63]
*Vinca alkaloids	Immunosuppression	[30]
*Mycophenolate mofetil (CellCept)	Immunosuppression	[64, 65]
*Danazol	Unknown	[66]
*Dapsone	Immunomodulatory	[67]

* = "third line" therapy

a. Splenectomy:

Splenectomy remains the mainstay of treatment of ITP in adult patients, and a high proportion of patients with symptomatic ITP eventually require a splenectomy. Kojouri et al reviewed the role of splenectomy in ITP in detail, collating data on efficacy, durability of responses and surgical complications from 135 case series from 1966 to 2004, an enormous endeavor that looked at these three outcomes in thousands of patients. [68] They concluded that two-thirds (66%) of patients responded to splenectomy, in studies that reported follow-up for 1-153 months. The response rate did not vary with the duration of follow-up. No pre-operative characteristics consistently predicted who would respond. Characteristics evaluated included age, response to corticosteroids, response to IVIG, splenic sequestration confirmed by radioisotopic techniques and the magnitude of the platelet rise immediately after splenectomy.

They reported surgical complication rates within 30 days of surgery or during the original hospitalization. These occurred in of 12.9% of patients undergoing open splenectomy and 9.6% of those undergoing laparoscopic splenectomy. Deaths from splenectomy occurred much more frequently in patients having open splenectomy, though many of these were from a much earlier era, and perioperative care would not be comparable with that in the last 2 decades: laparoscopic splenectomy was first undertaken in 1991.

Splenectomy for ITP may have long term complications, particularly infection and thrombosis, though these are fortunately relative rare. Recommended practice is to vaccinate patients who are going to have splenectomy for the three encapsulated organisms, pneumococcus, hemophilus influenza B and meningococcus. This should be given 2 weeks prior to splenectomy, and our practice is to administer it in the early weeks of treatment, as soon as the platelet count is at a safe level for injection. We also supply patients with an emergency penicillin prescription to use in the setting of fever prior to seeking medical attention after splenectomy.

Thomsen et al were able to identify all 3812 patients who underwent splenectomy in Denmark for the decade 1996-2005 and compare them in terms of infection requiring hospital contact with non-splenectomized patients with the same condition, with patients who had appendectomy and with the general population [69]. ITP was the indication for splenectomy in 7.1% of these patients; other more common diagnoses included traumatic splenic rupture, cancer and splenomegaly. The risk of 90-day infection post splenectomy was increased 2-3 fold, compared with their matched patient group. Long term hazard ratios for infection for patients with ITP remained increased with a hazard ratio of 1.4

Table 3: Deaths from splenectomy for ITP [68]

Cause of death	**Open splenectomy (81 papers)**	**Laparoscopic splenectomy (29 papers)**
Postoperative bleeding	11	1
Cardiovascular	10	1
Infection	6	1
Venous thromboembolism	5	0
Other – including pancreatitis	16	0
Mortality	48/4955 1%	3/1301 0.2%

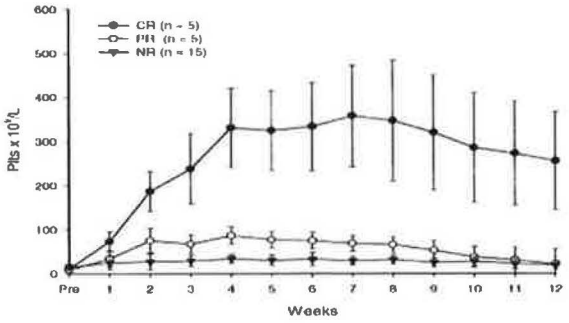
Thomsen et al subsequently used their same cohort to look at long term risks of venous thromboembolism after splenectomy [70] As with infection they found the highest risk in the 90 days after splenectomy, occurring in 1.9% of the splenectomized patients, giving a relative risk (RR) of 32.6. Even a year post-operatively the relative risk remained increased, albeit modestly; for ITP patients the RR was 2.7, in line with that of traumatic splenectomy patients, whose risk was 3.1 fold.

Splenectomy has been a surgical staple in ITP for 80 years, but has never been compared in randomized studies with newer therapies. However, its high rate of permanent correction of the thrombocytopenia of ITP still makes it the leading treatment option in ITP in patients who fail corticosteroids and who are surgical candidates. The 30% of patients with symptomatic ITP do not respond to the combination of steroids and splenectomy, and are considered "refractory ITP". They constitute a major treatment challenge.

b. Rituximab

Studies of immune suppression with Rituximab administered to patients with ITP were first published in 2001. [48] Rituximab is a chimeric, monoclonal antibody directed at the CD20 antigen on B-lymphocytes. It was initially developed for treatment of B-cell lymphoproliferative disorders, for which it remains an important treatment, usually administered in combination with chemotherapy. The initial Italian study of rituximab included patients with ITP who had failed 2-5 treatments, including 5 who had failed splenectomy. They were treated with rituximab in the same dosing schedule used in the B-cell malignancies, namely 375 mg/M^2 weekly for 4 weeks. This demonstrated benefits in approximately 40% of these refractory ITP patients, including 20% achieving normal platelet counts and 20% having a platelet count 50-100,000/mm^3.

Figure 6: Response to rituximab in patients with ITP [48]



This initial study led to widespread use of rituximab for patients with refractory thrombocytopenia or those who were unsuitable or unwilling to undergo splenectomy. Multiple single arm trials have since been reported; unfortunately no randomized studies have been undertaken, comparing it with splenectomy. [49-51]

Arnold et al collated 19 reports involving five or more patients who had received rituximab for ITP, and looked at safety and efficacy in more than 300 patients, of whom about half had already undergone splenectomy. [51] They documented that 62.5% of patients responded with a platelet count over 50,000/mm3; 43% actually achieved a normal platelet count. Responses lasted 2-48 months. They assessed a number of factors they hoped might predict a response – pre-treatment platelet count, number of prior treatments, sex and age – but found none. Toxicity was evaluable in 306 patients, of whom 66 (22%) had grade 1-2 toxicity, 10 (3%) had grade 304 toxicity and 9 (3%) died, three with hemorrhage. These authors concluded that "controlled trials of rituximab for ITP are urgently needed", but they have still not occurred.

Rituximab causes unpleasant infusion reactions, with chills, fever, rash and anaphylaxis-type reactions in 60% of patients with the initial infusion, although subsequent infusions are better tolerated; we try to pre-empt these reactions by premedicating patients with acetaminophen, diphenhydramine and decadron. We use intravenous ranitidine with the first infusion and for those who have prior reactions. Moreover we always have meperidine and an anaphylaxis kit handy. Rituximab carries has Black Box warnings for fatal infusion reactions and severe mucocutaneous reactions.

Rituximab results in marked B-lymphocyte depletion, yet does not alter immunoglobulin levels, and does not appear to be associated with an increased risk of infection in ITP patients. [50] However, it was recently was assigned a Black Box warning for the devastating neurological complication Progressive multifocal encephalopathy (PML) caused by the JC virus; one of the 57 cases of post-rituximab PML

described recently occurred in a patient with ITP, although the majority (52) were in patients with lymphoproliferative disorders. [71]

Because of the lack of a randomized trial comparing Rituximab to splenectomy, it is unclear whether this is an alternative to splenectomy, which when effective usually results in a protracted remission, unlike rituximab, where relapses occur sooner or later. My own practice is to use splenectomy as second line treatment, and to reserve Rituximab for those patients who fail splenectomy, unless there are compelling medical reasons to avoid surgery.

c. Immunosuppresive and other agents for ITP

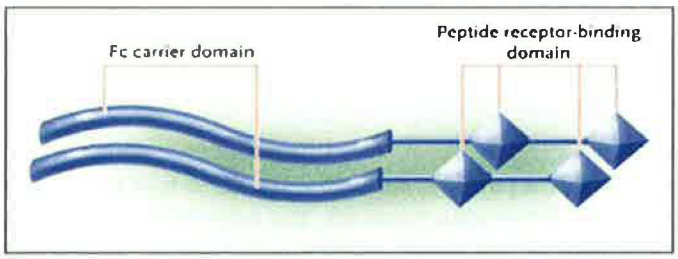
These are invoked relatively infrequently. See table 2 for these and the references supporting their use. Most are currently best described as "third line" treatments.

d. Thrombopoietin analogues (Thrombomimetics)

With the recognition that platelet production from megakaryocytes is often reduced in ITP, recent efforts have focused on increasing platelet production. When Thrombopoietin was recognized as the specific growth factor for platelet production, and subsequently produced in purified form in the mid 90s, investigators were hopeful it would be as useful for treating patients with thrombocytopenia as erythropoietin was for many types of anemia. Unfortunately these hopes were dashed by two studies, one with normal volunteers and one with thrombocytopenic patients, which showed that a number of individuals developed neutralizing antibodies to TPO, rendering them profoundly thrombocytopenic, often for months. [72]

The search then began for a new type of thrombopoietic agent, with a different chemical structure to thrombopoietin, but which would bind and activate the *c-mpl* receptor. Recombinant peptide libraries were screened to find those that had different sequences from native TPO, but which would bind to the *c-mpl* receptor. A potent 14-amino acid sequence was identified, then dimerized to increase efficacy. To increase its circulating half-life it was bound to a portion of Fc portion of immunoglobulin, making a so-called "peptibody".

Figure 7: Peptibody structure as in Romiplostim [54]



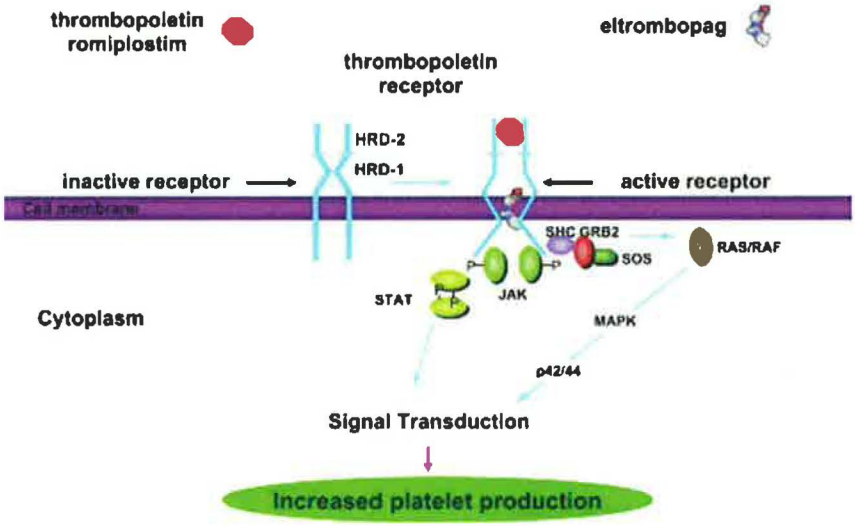
The first of these to be used successfully was an agent we now know as Romiplostim, more usually called by its simpler trade name, Nplate. Romiplostim needs to be administered subcutaneously. Initial dose-finding trials demonstrated that there was a dose-dependent increase in platelet counts using this agent, which could even drive the platelet count to supra-normal levels. [54] However, the effect of romiplostim is short-lived, and platelet counts plummet when the treatment stops. In a few patients platelet counts even dropped below the starting level (rebound thrombocytopenia). Once a safe dose was demonstrated, which stimulated megakaryopoiesis, but did not increase the platelet count to unsafe levels this agent was approved by the FDA approval as the first thrombomimetic agent – approved for use in ITP only. This agent is administered subcutaneously and has a circulatory half-life of 120-160 hours; it is usually

administered on a weekly basis, with the dose determined according to the platelet count. All prescribers and patients must be registered in an ongoing post-release monitoring study.

An international phase III trial of this agent has recently been reported. [56] This study randomized 234 adult patients with ITP and a platelet count of <50,000/mm3, who had not undergone splenectomy to receive weekly subcutaneous injections of romiplostim (157 patients) and standard of care (77 patients) for 52 weeks. The primary end points were the incidence of treatment failure and the incidence of splenectomy. Romiplostim was associated with higher rates of platelet response, lower rates of treatment failure and splenectomy and less need for other medical treatment. There were few serious treatment-related serious adverse events.

One long term concern is that the increased megakaryopoiesis may cause increased reticulin formation in the bone marrow. This has been seen in a number of patients who were followed retrospectively, but has not been noted in prospectively followed patients. [57] The reticulin fibrosis seen in both animal models and patients appears to be reversible once treatment is stopped. Bone marrow to assess this is recommended annually currently.

Figure 8: Mechanisms of Thrombopoietin and its analogues [2]



A second thrombomimetic was soon released, Eltrombopag, which has the advantage of oral administration. Eltombopag is not a peptide, and activates the *c-mpl* receptor by directly binding to the adjacent transmembrane region. From this point on, the activation of megakaryopoiesis appears to be identical to that of Romiplostim. Although somewhat easier to administer than the subcutaneous Romiplostim, Eltrombopag must be taken on an empty stomach and patients cannot eat for 2 hours afterwards. Moreover it too requires regular visits for platelet count monitoring. It carries a Black Box warning for hepatotoxicity and two-weekly measurement of bilirubin and transaminases is recommended.

The oral nature of this agent lent itself to a placebo-controlled double blind phase III trial of this agent, which was recently reported by Bussel et al. [59] The trial involved 197 ITP patients, with initial platelet counts under 30,000/mm^3, who received daily Eltrombopag or placebo for six months. These patients were well matched on terms of age, sex, prior splenectomy and the number of prior treatments. Results

are summarized in Table 3 below, which shows improvement in ITP-related issues, at the expense of a modest increase in thrombotic risk and liver function abnormalities in the treated patients.

The two available thrombomimetics have not been compared head to head, nor with the other second line therapies, splenectomy or rituximab. They are extraordinarily expensive medications, not just the high price of the medication itself, but the frequent monitoring. Moreover, their effect is only noted during their administration, as platelet counts will rapidly drop to baseline or below if the medication is stopped.

Table 3: A randomized double-blind trial of Eltromobopag vs. placebo[59]

Result	**Eltombopag** 135 patients	**Placebo** 62 patients
Reduction in concomitant therapy	37 (59%)	10 (32%)
Rescue therapy	24 (18%)	25 (40%)
Bleeding	1 (<1%)	4 (7%)
Thromboembolism	3 (2%)	0
Increase in alanine aminotransferase (ALT)	9 (7%)	2 (3%)
Increase in total bilirubin	5 (4%)	0

However, the thrombopoietin mimetics are an enormous step forward in many ways and a whole new approach to treatment of ITP. As Dr. George said in his recent editorial [34]

> "The availability of the Thrombopoietin-mimetics has been a great advance for patients with immune thrombocytopenia. Thrombopoietin mimetic agents can be effective in inducing safe platelet counts when all other therapy, including splenectomy and rituximab, has failed, providing hope for patients with the most severe thrombocytopenia"

In addition to the agents discussed above, there are many thrombopoietin analogues in development and clinical trial. We hope their use will not be limited to patients with ITP, but will benefit our thrombocytopenic patients with aplastic anemia, chronic hepatitis C requiring treatment, myelodysplasia and patients thrombocytopenic after chemotherapy or other myelosuppressive agents.

SUMMARY

The last decade has been a time of great progress in both understanding and treating immune thrombocytopenic purpura. An important new immunosuppressive agent, rituximab, is available and in wide use, although its place in the ITP armamentarium lacks rigorous study. Important insights into megakaryopoiesis have led to the first of two new thrombopoietin analogues to treat thrombocytopenia by stimulating platelet production.

Within a few months I am hopeful of a new ASH-sponsored ITP Guideline, which will incorporate these new agents into the long-established treatment options of corticosteroids and splenectomy.

Appendix

Costs of Emergency and Second Line treatment in ITP

Emergency Treatment (70 kilogram patient)

*Intravenous immunoglobulin (IVIG) 2 grams/kg	PHS	$10300
	AWP	$19300
*Rh-Immune globulin 75 mcg/kg		$1550
*Single apheresis platelet transfusion		$560

Second Line Treatment

Laparoscopic splenectomy		$10,000
*Rituximab 375 mg/M^2 x 4	PHS	$7740
	AWP	$19600
*Romiplostim 3 mcg/kilo weekly for one year	PHS	$41900
	AWP	= 70400)
*Eltrombopag 50 mcg dose daily for one year	PHS	$30000
	AWP	$57000)

* does not does include dispensing, administration or monitoring costs

PHS = Public Health Service AWP = Average Wholesale Price

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