The Clinical Spectrum of Thrombocytosis and Thrombocythemia

Eugene P. Frenkel, M.D.

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It is now clear that megakaryocytopoiesis (and resultant platelet production) is an ordered and controlled sequence which begins at the level of a pluripotent hematopoietic stem cell and ends with a megakaryocyte actively producing platelets. That this sequence is highly regulated is supported by the constancy of the normal circulating platelet count, the evident capacity of the bone marrow to respond to clinical circumstances of accelerated platelet turnover, and by the qualitative alterations in megakaryocytes and platelets that accompany various forms of thrombocytopenias and thrombocytosis (1). Although far from completely characterized, our current knowledge of megakaryocytopoiesis allows us to correlate clinical events with alterations or abnormalities at specific steps in the maturation cascade.

Regulation of Megakaryocyte and Platelet Production

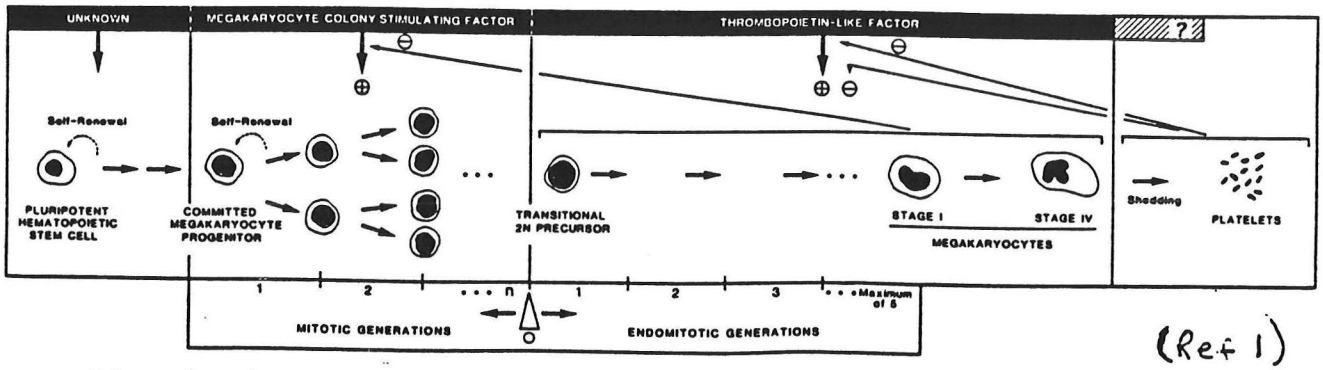
In vitro systems now exist to examine growth of megakaryocyte progenitor cells (2, 3, 4), immature megakaryocytes (4) and mature megakaryocytes (5). In a clinical analysis of thrombokinetics in patients in 1968 Larry Harker and Clem Finch proposed a "two-tiered" mechanism regulating megakaryopoiesis; that is, separate "activities" appear to control expansion and size of the [maturing] megakaryocyte (6). All of the current observations on regulation of megakaryopoiesis confirm control by (at least) two factors, one acting on a megakaryocyte colony-forming unit affecting the proliferation of progenitor cells, and a second modulating megakaryocyte maturation and development (7). Parenthetically, it merits note that a similar requirement for dual hemopoietic regulatory molecules characterize erythroid and macrophage production.

Megakaryocyte Progenitor Cells:

It is now clear that the ultimate origin of the megakaryocyte is the pluripotent hematopoietic stem cell (8). In vitro assay systems have been developed for a hematopoietic progenitor cell, the so-called colony forming unit (CFU) blast cells (9, 10). As expected of "blast cells" they have an extensive capacity for self renewal as well as an ability to differentiate into a number of different hematopoietic cell types. Whether it is the "pluripotent hematopoietic cell" is not certain.

A developmental hierarchy of megakaryocytic progenitor cells appears to exist (8, 11), and presently three classes of megakaryocyte progenitor cells have been recognized, although not fully characterized. It should be stressed that none of these precursors is recognizable as a megakaryocyte in the examination of a bone marrow preparation.

A working model of megakaryocytopoiesis has been depected by Mazier (1):



(Ref 1)

Schema of megakaryocytopoiesis.

Classes of Megakaryocyte Progenitor Cells

	Burst Forming Unit - Megakaryocyte BFU-MK	Colony Forming Unit - Megakaryocyte CFU-MK	Light Density Megakaryocyte (progenitor) LD-CFU-MK
Characteristics:	Most Primative	Intermediate Form	Most Differentiated Low Proliferative Capacity
In-Vitro Incubation Time	21d	12d	?
Colony Character	Multiple and Large Clusters	Unifocal Colonies	Small Colonies
In-Vivo Pre-Rx 5FU	Cloning Efficiency Not Affected	Cloning Efficiency Markedly Reduced	?
Ploidy of Colonies	Low Ploidy	Intermediate Ploidy	High Ploidy
Phenotype Characterization:			
	No HLA-DR	Expresses: HLA-DR	?
	Expresses: CD_{34} Antigen	CD_{34} Antigen	?
		? Platelet Membrane Glycoprotein	? Platelet Membrane Membrane Antigens

An attractive maturational "sequence" can be made from these classes with the concept that the LD-CFU-MK cell is at a stage of development where mitosis ends and the classical feature of the megakaryocyte, that of endomitosis, begins.

It should be emphasized that during megakaryocyte development a small mononuclear cell emerges that expresses platelet-specific markers, but still is not morphologically recognizable as a megakaryocyte (5, 11). These cells, in the presence of growth factors transition, into morphologically identifiable megakaryocytes.

Humoral Regulation of Megakaryocytopoiesis:

It is now clear from a variety of studies in different species that platelet production is altered, and probably largely regulated, by (a variety of) cytokines (1, 7, 8, 11).

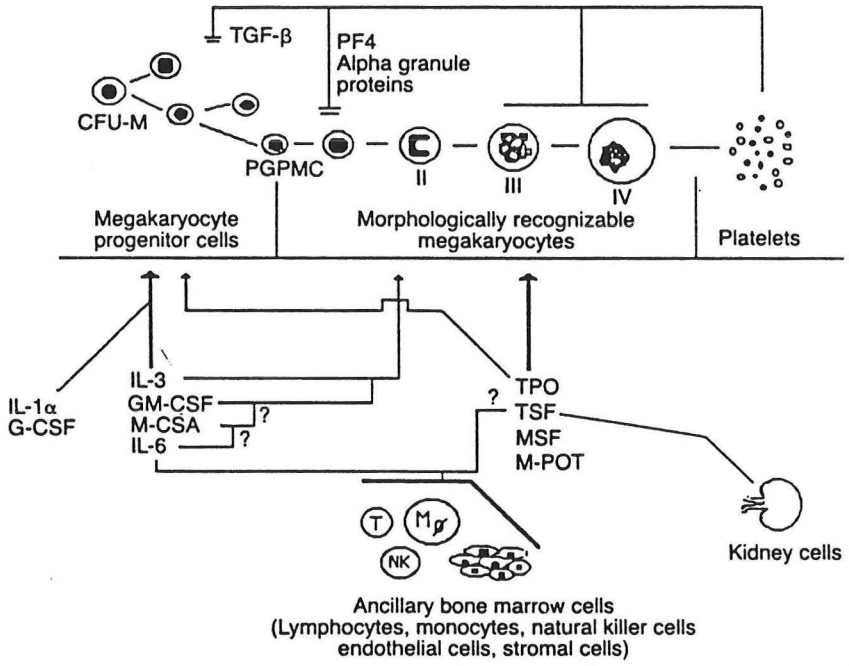


Figure 1. Diagrammatic representation of human megakaryocyte ontogeny and the regulatory influences that impact on this process. Megakaryocyte development from CFU-M to platelets is illustrated at the top of the figure; putative regulators are shown above and below. IL-3, GM-CSF, IL-6, and M-CSA exert their predominant effect (*dark line*) on progenitor cells. They may also affect cell maturation (*light line*). IL-α and G-CSF do not have progenitor cell stimulatory activity of their own but may synergize with the other cytokines. TPO, TSF, MSF, and M-POT exert their primary effect on maturing megakaryocytes, although some effect on progenitor cell proliferation has been reported. Such activity may also be primarily of a synergistic nature. Inhibitors include TGF-β and autocrine alpha granule products such as PF4. Increases in megakaryocyte and platelet mass likely inhibit production of the trophic regulators and may lead to increased inhibitor levels. Decreases in megakaryocyte and platelet mass likely stimulate production of trophic regulators and perhaps inhibitors. Mechanisms for sensing changes in cell mass and for stimulating regulatory activities are unknown. Putative sources of the various factors are as indicated. [PGPMC = platelet glycoprotein–bearing

(Ref. 11)

A candidate molecule for primary regulation of platelet production, thrombocytopoiesis stimulating factor or thrombopoieten, has been pursued for over 3 decades (12). In spite of intense efforts to delineate a single pivotal molecule, convincing identification and characterization of such a moiety has not been achieved (8). In addition, it is now evident that

a variety of cytokines affect platelet production and exactly how these are orchestrated or interactive is not clear (8, 11, 13). Nevertheless, the collective data to date support the concept of the existence of a megakaryocyte-specific growth factor: M-CSF

Such a "factor" has been isolated from urine and serum of patients with hypomegakaryocytic thrombocytopenic states (1, 7, 8, 11) regardless of course. Its production has been correlated with megakaryocyte mass rather than circulating platelet numbers. Yamaski, et al (14) have shown that "M-CSF" in plasma peaks 7 to 21 days after delivery of chemo-radiotherapy. When marrow engraftment occurs with transplantation, the activity falls to normal. Final confirmation of existence M-CSF still awaits purification and amino acid sequence determination to prove that this factor is indeed unique in light of the existence of a growing number of cytokines prepared by recombinant technology that had been considered single hematopoietic linage specific but are now know to affect multiple linages (8, 13).

There is no question that a variety of other cytokines affect *in vitro* and *in vivo* megakaryocytopoiesis. Recombinant erythropoietin, for instance, has been shown to stimulate platelet production in animals (15). This has not been the experience in man, thus making the translation of animal studies to man difficult.

Three cytokines appear to have important stimulatory biological activity:

1.) GM-CSF

2.) IL-3:
- actually more active than GM-CSF in colony production
- its effect is additive to GM-CSF. In primates it will result in a nearly 2-fold increase in platelet numbers.

3.) IL-6:
- a multifunctional cytokine originally characterized as a T cell derived factor capable of promoting terminal maturation of activated B cells to Ig-producing cells (16)

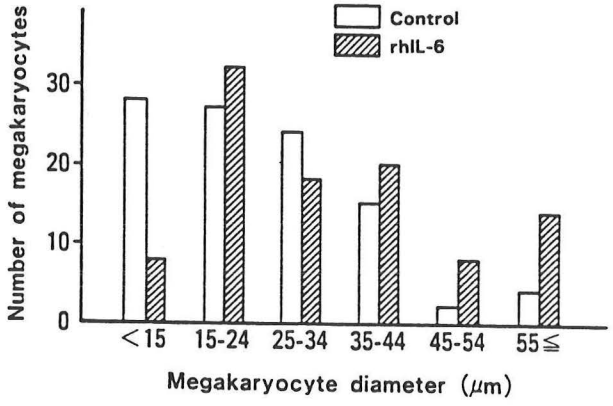


Fig 5. The effect of rhIL-6 administration (80 μg/kg/d SC, twice daily for 7 days) on the size of bone marrow megakaryocytes. Bone marrow samples were obtained 1 day after the last day of administration.

(Ref 17)

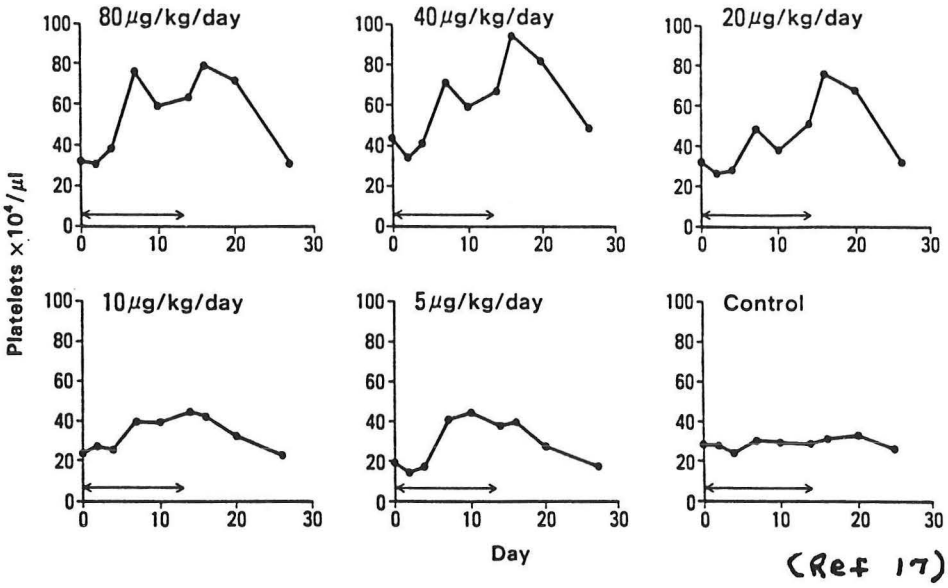


Fig 1. Changes in peripheral blood platelet counts in cynomolgus monkeys treated SC with rhIL-6 for 14 days (as indicated by arrows). Monkeys were treated twice daily with 5 to 80 μg/kg/d of rhIL-6. Control monkey received HSA.

- augments maximum megakaryocytic colony formation stimulated by IL3.

- produces an increase in megakaryocyte size but no change in numbers (17a) and its use results in a dose-dependent increment in platelet numbers.

Thus, IL-6 appears to function as a maturational stimulating agent (i.e. thrombopoietin) rather than as a stimulus to megakaryopoiesis proper.

Inhibitors of megakaryopoiesis further complicates our delineation of the regulation of platelet production. These were initially proposed by the recognition that plasma is superior to serum as a growth factor for megakaryocytes *in vitro* (18, 19). One attractive concept in the characterization of this observation is that the "products" of platelets down regulate platelet production by megakoryocytes, thereby providing an autocrine regulatory loop.

Several cytokines have been implicated in such an inhibitory - regulatory role:

1.) TGF-β:

- the cytokine transforming growth factor β exists in high concentrations in platelets; however, its inhibitery effects are not limited to megakoryocytes (20).

2.) Platelet-released glycoprotein:

- a 12,000 to 17,000 dalton platelet released glycoprotein which appears to specifically inhibit CFU-MK (21).

3.) Platelet factor 4:

- also a platelet alpha granule product. Its regulatory role is not clear (22)

4.) Interferon - α and γ:

- non-platelet derived cytokines may also have a role in regulating megakaryocyte colony formation (23, 24).

The specific role and biologic importance of these moieties is uncertain.

Is Megakaryocyte Maturation Regulated?

The maturation of megakaryocytes extends from a hematopoietic precursor cell to a cell with a progressive degree of nuclear endoreplication; this latter form is the stage in maturation where the cell is no longer in active proliferation (25). Cytoplasmic organelles and the development of membrane antigens and glycoproteins occur during this sequence. The resultant megakaryocytes have ploidy values of 4N, 8N, 16, and 32N, and the ploidy distribution has been shown to vary with circulating platelet numbers.

Gewertz and Hoffman (8, 11) have extensively reviewed the studies relating to regulation of megakaryocyte maturation. All of the evidence supports a reciprocal relationship between mitotic events (where replication results in an amplification of the immature cell pool) and endomitotic events (which result in platelet production).

IL-6 (and "thrombopoieten", if it turns out to be different from IL-6), as mentioned, clearly has been shown to have a "maturational effect" (17). Its interactions with IL3 and probably GM-CSF may serve to be the "effectors" of megakaryocyte maturation. Although these steps are not yet well characterized, if the observations in animals extend to man the maturation of megakaryocytes is highly regulated.

Clinical Characterization of Circumstances of Excess Platelet Numbers

The nomenclature used to describe patients with increased platelet numbers has been widely accepted in clinical practice, although it is a quite arbitrary terminology. Three groups can be delineated:

1.) Pseudothrombocytosis
2.) Thrombocytosis
3.) Thrombocythemia

Pseudothrombocytosis

Routine platelet counts in clinical medicine became common approximately 15 years ago with the availability of electronic cell counters.

In clinical circumstances where cryoglobulinemia is present (idiopathic or secondary) protein precipitates may occur in the tube used for the blood count, and these precipitates often are enumerated as "platelet - sized particles" by the electronic counter (26). As is true in pseudothrombocytopenia, examination of the peripheral blood smear by the careful physician will provide the correct interpretation of the reported platelet numbers. In addition, this sequence helps suggest the patients' correct underlying disorder.

Thrombocytosis

Elevated platelet counts have been recognized in a variety of clinical circumstances. In some the elevation is acute and transient; in others the finding is chronic and persistent. The term "reactive"thrombocytosis is frequently applied to define the concept that these patients have increase circulating platelet numbers in response to some underlying disease, in contrast to those circumstances where an autonomous drive to platelet product exists, terms thrombocythemia.

Clinical Circumstances Associated with Thrombocytosis ("Reactive Thrombocytosis"):

I. Acute and Transient:

A. Persisting minutes to hours:

1.) Epinephrine
2.) Exercise

B. Persisting hours to a few days:

1.) Acute Blood Loss
2.) Recovery from Acute Infection
3.) Post ("Rebound") Thrombocytopenia:

a.) Post-immune
b.) Post-cytoreductive chemotherapy (esp. Methotrexate and Vinca Agents
c.) Post Megaloblastic Anemia
d.) Post alcohol associated thrombocytopenia

II. Chronic:

A. Persisting during a significant duration of:

1.) Chronic Blood Loss with Iron Deficiency
2.) Chronic Inflammatory Disease
3.) Chronic Infectious Disease
4.) Cancer
5.) Hemolytic Anemia

B. Potential Life-long:

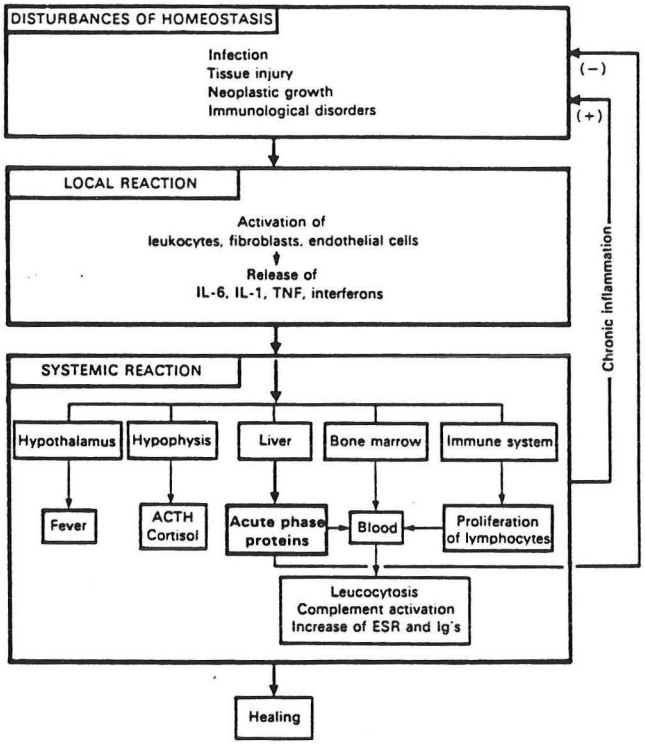
1.) Post Splenectomy (auto or surgical)

This long differential diagnosis can be simplified, since in many clinical circumstances the thrombocytosis appears to be a "rebound" occurring after a period of bone marrow suppression that was the result of nutritional, immune or toxic circumstances. This "rebound" usually peaks at 10 to 20 days following removal or repair of the suppressive event. The mechanism of rebound excess is unknown, although the current vogue is to implicate cytokines as the basis.

Although the term "thrombocytosis" can be appropriately applied to any patient with platelet numbers in excess of 400,000/ μl, clinical attention is commonly not stimulated unless the counts are in excess of 650,000/ μl. Platelet "millionaires" are a well recognized event and Dr. Robert Schilling, who coined this term, was able to identify 102 such cases in a random 18 month survey of laboratory results at the University of Wisconsin (27). Significant mystique has been applied to the recognition of thrombocytosis and its presence has been used in differential diagnosis. For instance, the presence of increased platelet numbers has been suggested as a

clue to the diagnosis of malignant plural mesothelioma (ie in the setting of pleural disease, recurrent pleural effusion, malaise, and chest symptoms) (28, 29). Recent studies have failed to confirm such an interesting a correlation (30).

The mechanism(s) for "reactive" thrombocytosis is unknown. Current understanding of IL-6 productionin "acute" phase responses suggests that it is the major cytokine responsible for the amplification of the megakaryocyte progenitor population (31).



Acute phase response of the organism (Ref 31)

Acute phase plasma proteins in human and rat

Species	Increase			No change	Decrease
	10–100-fold	2–10-fold	⩽ 2-fold		
Human	C-reactive protein Serum amyloid A	α_1-Proteinase inhibitor α_1-Acid glycoprotein α_1-Antichymotrypsin Fibrinogen Haptoglobin	Caeruloplasmin C3 of complement α_2-Antiplasmin C_1-inactivator	α_2-Macroglobin Haemopexin Serum amyloid P Prothrombin	Inter-α-antitrypsin Transferrin α_1-Lipoprotein Prealbumin Albumin
Rat	α_2-Macroglobulin α_1-Acid glycoprotein	Fibrinogen Haptoglobin Cysteine proteinase inhibitor	α_1-Proteinase inhibitor Caeruloplasmin Prekallikrein Haemopexin C-reactive protein	α_1-Macroglobulin Antithrombin III Serum amyloid P Prothrombin	α_1-Inhibitor 3 Transferrin Prealbumin Albumin

(Ref 31)

An important issue when thrombocytosis is recognized in a patient is its clinical relevance. Four simple questions can be used as clinical parameters for physician action:

1.) ? DO INCREASED PLATELET NUMBERS POSE A CLINICAL RISK?

An interesting and consistent observation is that virtually regardless of the platelet number reactive thrombocytosis has not been associated with an increased risk for hemostatic complications (27, 32-35) specific exceptions have been seen and these are increased platelet numbers occurring in setting of:

- underlying hematopoietic disease
- pre-existent arterial disease
- prolonged immobility

Thus, clinical concern when an elevated platelet count is identified can generally be approached quickly with the data available at the bedside. The term "underlying" hematopoietic disease is focused to mean the presence of a clonal disorder of the myeloproliferative or myelodysplastic type. It is wise however to consider the broader concept of the hematopoietic abnormality that can include circumstances of altered platelet function and/or hemolytic anemia (especially of the congenital type).

An example, for instance, is that following splenectomy 40% of patients have prolonged (greater than 2 months) elevated platelet counts; and, almost 15% have platelet counts over 1,000,000/ μl (36). Nevertheless, in the absence of an underlying hematopoietic lesion or an underlying hematologic disease there is no increase in thromboembolic or hemorrhagic events (37).

Thus, a given platelet number alone does not pose a clinical risk.

2.) ? WHAT PLATELET NUMBER IS SIGNIFICANT?

Since platelet excess is not necessarily associated with clinical risk, the decision concerning the extent of clinical evaluation of a patient with an elevated platelet count has some arbitrary aspects.

Since patients with underlying hematopoietic lesions and post-splenectomy patients with existent platelet abnormalities or (congenital or acquired hemolytic states) have thrombohemorrhagic events at platelet numbers in excess of 650,000/ μl, this value has become the benchmark denoting clinical concern.

3.) ? HOW SHOULD SUCH PATIENTS BE EVALUATED?

In most patients with an elevated platelet count the clinical history and routine laboratory studies will provide the data to identify those patients with thrombocythemia, since the complete blood count will almost always identify an underlying myeloproliferative or myelodysplastic state, the primary "mechanisms" associated with an autonomous proliferative drive (ie thrombocythemia). Banal as it may sound, the characterization of the underlying disease is the best focused approach in the evaluation. Indeed, it is the absence of definable hematologic

and/or hemostatic laboratory abnormalities that characterizes thrombocytosis and separate this lesion from thrombocythemia.

Laboratory Studies in Thrombocytosis and Thrombocythemia

	Thrombocytosis	Thrombocythemia
Platelet Hyperaggregability (38)	Normal	Increased
Defective Aggregation Response to Epinephrine (39-42)	Normal	Defective
Defective Signal Transduction Pathway for Calcium in Platelets (43)	Normal	Defective
Platelet Derived Growth Factor Activity in Platelets (44)	Normal	Decreased
Serum Lactic Dehydrogenase (45)	Normal	Elevated
Bleeding time (46)	Normal	Normal to Prolonged
Plasma fibrinogen (39)	Elevated	Normal
Factor VIII Activity (39)	Elevated	Normal

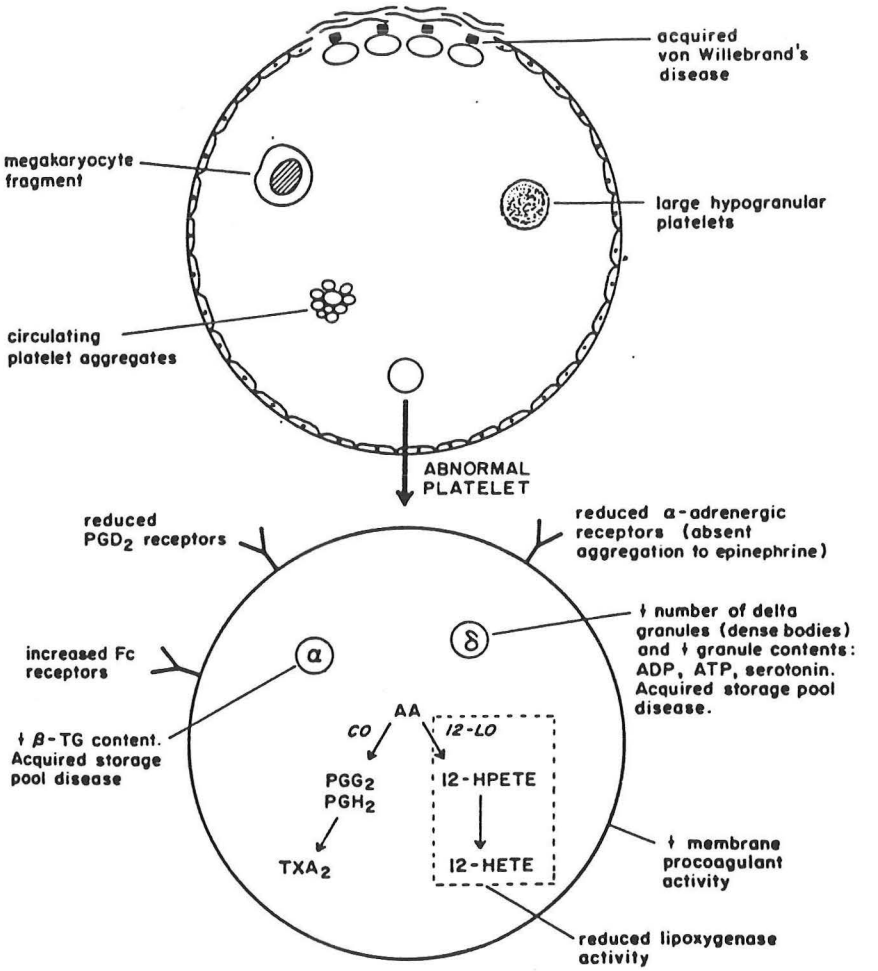


Figure 3. The qualitative platelet defects in ET. Depicted on top are abnormalities in platelet-vessel wall interactions (acquired von Willebrand's disease) and platelet morphology (platelet clumping, large agranular platelets, and megakaryocyte fragments). Biochemical changes in an enlarged platelet are shown below: defects in membranes and receptors (increased Fc receptors, decreased alpha [α] adrenergic and prostaglandin D_2 [PGD_2] receptors, and alteration in platelet procoagulant activity); changes in granule content and number (decreased number of dense granules and concentration of adenine nucleotides and serotonin as well as platelet specific β-thromboglobulin [β-TG]), all resulting in a form of acquired storage pool disease; and abnormalities in arachidonic acid (AA) metabolism (decreased lipoxygenase [LO] activity). (Ref 32)

4.) ? HOW SHOULD SUCH PATIENTS BE MANAGED?

Since by definition "reactive" thrombocytosis is secondary to some underlying lesion, control of that primary disease is the initial step. Except for those with pre-existent or significant arterial disease no other therapy is needed or desirable. Since the common thombohemorrhagic sequelae do occur in patients with reactive thrombocytosis and vascular disease it is appropriate to treat with aspirin (or a similar such arachidonate pathway inhibitor) plus a second agent, usually one which interferes with platelet phosphodiesterase activity (47-49). Only exceedingly rarely would direct efforts to reduce platelet numbers be indicated.

Common Drugs Effecting Platelet Dysfunction (47)

Inhibition of Arachidonate Pathway [inhibition of cyclo-oxygenase activity]	Aspirin Ibuprofen Indomethacin Naproxen	Quinidine Steroids
Interference with Membrane Receptor Sites	Alcohol Amitrpyline Cocaine Imipramine Phenothiazine	Carbenicillin Penicillin Ticarcillin Propranolol Lidocaine
Interference with Phosphodiesterase Activity [↑ cAMP and interfere with phosphodiesterase action]	Caffeine Theophylline	Dipyridamole Prostaglandin Prostacycline

Thrombocythemia

The term thrombocythemia has been used to define two groups of clonal hematopoietic disorders: Myeloproliferative lesions and selected Myelodysplastic lesions:

Clonal Hematopoietic Disorders Associated with Thrombocythemia:

I. Myeloproliferative Disorders:

- Primary (Essential) Thrombocythemia
- Polycythemia Vera
- Chronic Myelogenous Leukemia
- Primary (Idiopathic) Myelofibrosis

II. Myelodysplastic Disorders:

Especially:

- $5q^-$ lesions
- Primary (Idiopathic) Sideroblastic Anemia

Fortunately, significant thrombocythemia (platelet counts in excess of 650,000/ μl) are quite uncommon in the myelodysplastic states and their characteristic clinical, morphologic, cytochemical and cytogenetic patterns easily identify this subset.

The separation of the forms of myeloproliferative disorders is generally not difficult. Thus, the significant leukocytosis, shift to the left in the granulocytic series, low leukocyte alkaline phosphatase and moderate splenomegaly usually helps suggest that the patient has chronic myelogenous leukemia with associated thrombocythemia. In addition, the presence of the Philadelphia chromosome (i.e. the characteristic reciprocal chromosome translocation t(9;22) or 22q⁻ chromosome) has classically been used to confirm that clinical diagnosis. Recent studies of patients with the classical clinical and laboratory features of primary (essential) thrombocythemia have identified the Philadelphia chromosome in a small subset of cases (50-55). Initially, it appeared that these could be early cases of chronic myelogenous leukemia, since acute leukemic transformation was common after a 4-7 years follow-up, (54-57) an otherwise rare circumstance in primary thrombocythemia. The alternative view of some was that the true cytogenetic abnormality was at a point different from that found in Classical chromic myelogenous leukemia (57).

However, a recent molecular analysis has been done of material from seven patients with Philadelphia chromosome positive primary thrombocythemia in John Goldman's laboratory (58). They confirmed another DNA study (59) that a rearrangement existed within the major (5.8 kb) breakpoint cluster region (M-bcr) typical of the changes in classical chronic myelogenous leukemia.

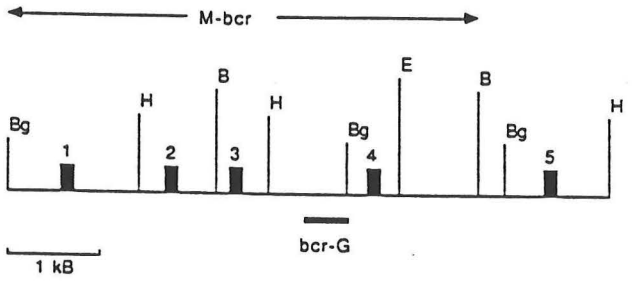


Figure 1. Structural map of the M-*bcr* part of the *bcr* gene. The solid blocks numbered 1–5 mark the positions of the M-*bcr* exons b1–b5. Restriction enzyme sites are shown together with the position of the *bcr*-G probe used for Southern analysis. Bg = *Bgl*II; H = *Hind*III; B = *Bam*HI; E = *Eco*RI.

(Ref 58)

Furthermore, their study of mRNA, using the polymerase chain reaction, demonstrated evidence of the B2A2 junction in one case and the B3A2 junction in five cases (58): these findings are identical to the changes at the RNA level in chronic myelogenous leukemia.

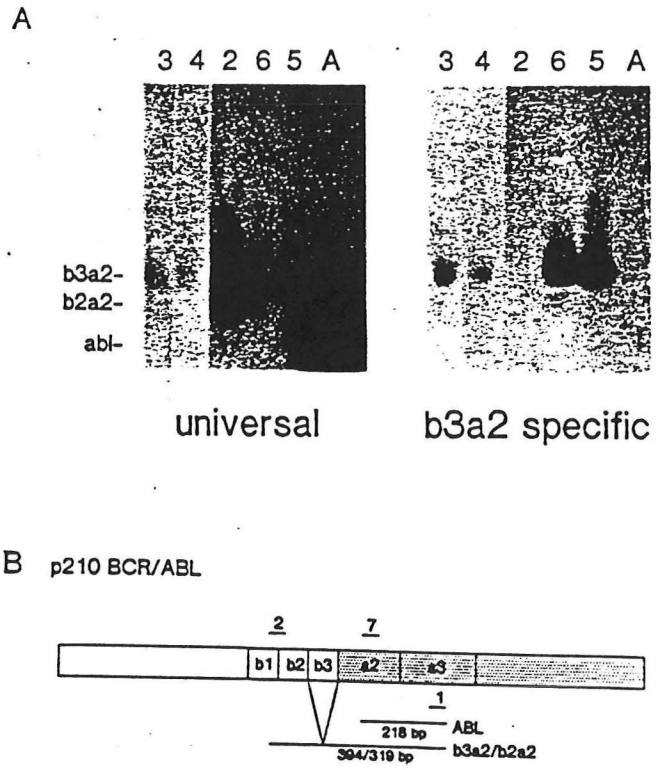


Figure 2. PCR analysis for presence of *bcr/abl* mRNA in patients with Ph-positive essential thrombocythemia. A, products from *bcr* analyses were first size fractionated by agarose gel electrophoresis, transferred to nylon membrane, and hybridized to oligomer 7 (left) or a b3a2 junction-specific oligomer (right). Lane numbers correspond to the individual patients in the study. Patient 2 has a band corresponding to a b2a2 junction; the other four patients show b3a2 bands. Lane A contains amplified *abl* sequences. B, Schematic drawing of the *bcr/abl* junction mRNA with position of oligomers numbered 1, 2, and 7 used in the PCR reaction; *bcr* specific sequences are indicated by the open boxes; *abl* specific sequences by the stippled boxes. The products bracketed by the primers used for PCR are indicated. Exon nomenclature is as previously described (26).

(Ref 58)

Table 3. M-*bcr* Rearrangement and PCR Studies

Patient No.	M-*bcr* Rearrangement			PCR Studies (Type of Junction)
	*Bam*HI	*Bgl*II	*Hind*III	
1	−	+	+	ND
2	+	+	−	b2a2
3	+	+	+	b3a2
4	+	+	+	b3a2
5	+	+	−	b3a2
6	+	+	−	b3a2
7	+	+	+	b3a2

ND = not done.

(Ref 58)

Thus, in some cases the precise separation of chronic myelogenous leukemia with associated thrombocythemia and primary thrombocythemia may be difficult. Such a circumstance is not too surprising to the hematologist, since the generic term "myeloproliferative disorder" is, at times, the most descriptive nomenclature until further evolution of the clinical and laboratory features occurs to a more clearly delineated form.

Similarly, accurate distinction between primary thrombocythemia and the proliferative phase of polycythemia vera (i.e. polycythemia vera with marked thrombocytoses) may be difficult. Usually a determination of the red cell mass and the adequacy of tissue iron stores can identify patients with polycythemia vera. Since these tests may not be available in some clinical centers or clinical bleeding may have affected the interpretation of the results, the Polycythemia Study Group undertook comparative analysis of their collected cases to evaluate whether a clinical impact exists to merit such a distinction. In addition, they sought to define a single parameter capable of reliably separating these two groups (57). Although in many respects the clinical patterns were similar, a remarkable difference in survival was noted between patients with primary thrombocythemia and polycythemia vera with thrombocytosis.

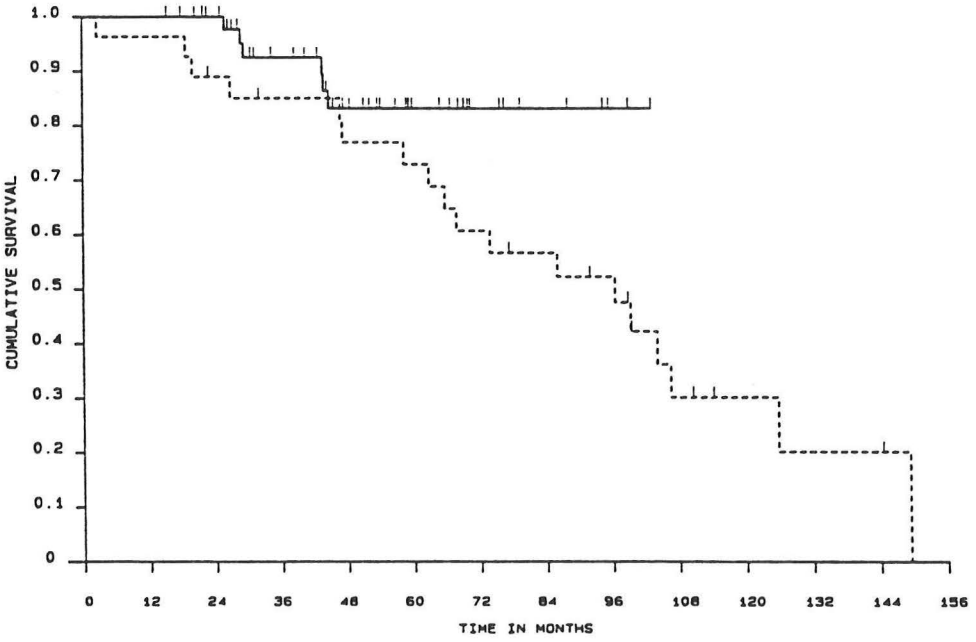


Fig. 2. Cumulative survival of the PVSG essential thrombocythemia (ET, —) and polycythemia vera with thrombocytosis (PVT, ---) cohorts. Tics indicate censored patients. (Ref 57)

In addition, clinical responses to platelet anti-aggregating agents appeared quite different (57). Thus in polycythemia vera with thrombocythemia aspirin and dipyridamole did not prevent thrombotic complications, and, in fact, had significant side effects in the form of major gastrointestinal hemorrhage (60). In primary thrombocythemia, aspirin has been shown to reverse vascular occlusions and even incipient gangrene (61), and to improve neurologic function even in the continued presence of elevated platelet numbers (62). These features suggest a true biological difference between these entities. This led to the development of a logistic regression algorithm (which incorporates hematocrit, white cell count, and spleen size) to differentiate between primary thrombocythemia and polycythemia vera with marked thrombocythemia (57).

The algorithm can be applied with a pocket calculator that has exponential capabilities or with a micro computer:

TABLE V. Simple BASIC Computer Program for Calculation of $P_{et/pvt}$*

```
10 REM Program for calculating algorithm for ET versus PVT
20 CLS : PRINT
30 PRINT "LOGISTIC REGRESSION ALGORITHM FOR P(ET/PVT)" : PRINT
40 INPUT "HEMATOCRIT (%) ="; HCT
50 INPUT "WHITE CELL COUNT (× 10 ↑ 9/1) ="; WBC
60 INPUT "SPLEEN SIZE (cm) ="; SPL
70 A = 23.0188 — (0.374255*HCT) — (0.227586*WBC) — (0.833028*SPL)
80 PROB = (EXP(A))/(1 + EXP(A))
90 PRINT : PRINT "P(ET/PVT) ="; PROB,
100 IF PROB >= 0.65 THEN PRINT "ET PREDICTED" : GOTO 130
110 IF PROB <= 0.35 THEN PRINT "PVT PREDICTED" : GOTO 130
120 PRINT "INTERMEDIATE PROBABILITY"
130 END
```

(Ref 57)

Finally, as one might expect, difficulties have been identified in the differentiation of primary thrombocythemia from idiopathic myelofibrosis and thrombocytosis. Parenthetically, since myelofibrosis is now known to be a reactive event to other clonal hematologic lesions, one can say that this is an artificial separation. Nevertheless, patients have been reported to initially meet all of the clinical and laboratory features of primary thrombocythemia, but then after 4 to 6 months progressively developed as peripheral blood leukoerythroblastosis (i.e. nucleated red cells, a shift to the left in the granulocyte series, etc.), falling platelet numbers, progressive splenomegaly and increasing marrow fibrosis (63). Although separation of these two clinical patterns is difficult at initial presentation, the different biologic patterns may in part explain the shorter survival of a small subset of patients.

Thus, the "myeloproliferative" concept recognized the overlapping clinical and laboratory features of these disorders, the common finding of generalized expansion of all the bone marrow elements (panmyelosis), and the transitions from one form to another that may occur during the course of disease. As is evident, the overlap of findings may make it difficult to pinpoint the individual entity, and the generic term myeloproliferative syndrome is occasionally used to reflect this clinical difficulty. Although all of the myeloproliferative disorders appear to be the result of a basic abnormality in the stem cell, the mechanisms that determine amplification of the "selected" cellular population (e.g. platelet, red cell, etc.) are unknown (64).

The still unclear relationships in this clinical circumstance are emphasized by a recent report of primary thrombocythemia occurring in two sisters (64a). Clonal hematopoiesis was confirmed in both cases (by x-chromosomal inactivation analyses) using DNA polymorphism of the phosphoglycerate-kinase (PGK) gene. In one sister a common ancestor appeared true for granulocytes, monocytes and T lymphocytes; in the other, monoclonality could be shown only for the granulocyte fraction. Their conclusion was that the lesion arose from heterogenous stem cell levels (64a)

Primary (Essential) Thrombocythemia

Primary ("essential")thrombocythemia is an autonomous clonal proliferation of a multipotential stem cell that results in an absolute increase in the numbers of circulating platelets. The term primary implies that the megakaryocyte expansion occurs in the absence of an identifiable mechanism, thereby separating it from non-autonomous drives to hematopoiesis.

Neither the pathogenetic mechanisms nor the molecular biologic events are known. A defect in the multipotent stem cell appears to cause generalized hyperplasia of the bone marrow, with a marked prominence of megakaryocytes and megakaryocyte precursors. Since such clonal panmyelosis is seen in other myeloproliferative disorders as well, a critical question is how perturbation of the multipotential stem cell results in a selective amplification of platelets in primary thrombocythemia, as opposed to red cells in polycythemia vera or granulocytes in chronic myelogenous leukemia.

One concept for lineage specific amplification in the myeloproliferative disorders is the multistep model of transformation. An early event, presumably common to all the myeloproliferative disorders, would confer a proliferative advantage to a multipotential stem cell, resulting in clonal hematopoiesis. A second, different event would then provide lineage specificity. Although no consistent chromosome abnormality has been described in primary thrombocythemia, the model of another myeloproliferative disorder, chronic myelogenous leukemia, supports this hypothesis. Thus, there is evidence that the Philadelphia chromosome, although characteristic of chronic myelogenous leukemia, is not the initial pathogenetic event in this leukemia. Presumably in primary thrombocythemia an analogous second mutation or other molecular event, although not cytogenetically recognizable, could account for the selective involvement of the megakaryocyte mass. An alternative model is that a single etiologic event could produce both altered regulation of stem cell proliferation as well as amplification of a specific cell population at a later level of differentiation (65).

A. Diagnostic Criteria:

Diagnostic criteria were established by the Polycythemia Study group (66) and have largely remained intact. The major change is the "diagnostic" level for platelets. They had used 1,000,000/μl. Most investigators currently consider 650,000/μl more appropriate (66a, 67).

Diagnostic Criteria for Primary ("Essential")Thrombocythemia

1. Platelet count 650,000/ μl or greater

2. Megakaryocytic hyperplasia in a marrow with panmyelosis (generalized hyperplasia)

3. Presence of Stainable iron (or no more than 1g/dl increase in hemoglobin following one month of oral iron therapy.

4. Absence of:
   - an identifiable cause
   - Philadelphia chromosome
   - Elevated RBC mass (> 36 ml/kg ♂
     > 32 ml/kg ♀)
   - Significant Fibrosis (> 1/3 cross-section of marrow)

5. No more than two of the following:
   - mild marrow fibrosis (< 1/3 area of marrow)
   - splenomegaly
   - leukoerythroblastic reaction

As already mentioned, the restriction associated with identification of the Philadelphia chromosome is unclear. Although a different cytogenetic abnormality was described earlier (a 21q$^-$ lesion) (57), the recent data of Philadelphia chromosome positive cases discussed earlier appears to identify a clear subset of patients with primary thrombocythemia with a poor prognosis.

Simplified working clinical criteria are:

Primary Thrombocythemia Diagnostic Criteria

Platelet count > 650,000/ μl	No defined cause
Hemoglobin ≤ 13g/dl or normal RBC mass	No Leukoerythroblasos
Marrow Panmyelosis and megakaryocytic hyperplasia	Limited marrow fibrosis (< 1/3 area)
Stainable Iron in Marrow	Less than 1g/dl increase in Hb. on 1 month of iron supplement

?Subset:

Splenomegaly
Presence of the Philadelphia Chromosome

B. Clinical Characteristics:

Historically Epstein and Goedel (68) are credited with the description in 1934, of a hemorrhagic disorder associated with increased numbers of circulating platelets. This may well have been a case of "reactive thrombocytosis" since post-mortem examination identified an atrophic spleen weighing 7g. in this 56 year old man. In 1960, Fred Gunz (69) from New Zealand carefully reviewed 5 of his own cases and 50 previously reported patients and delineated the clinical features of primary ("hemorrhagic" or "essential" thrombocythemia. This syndrome with the unusual presentation of either a thrombotic or hemorrhagic (or thrombohemorrhagic) diathesis in the presence of increased numbers of platelets was quickly recognized and reported by a variety of investigators (70-72). Nevertheless, the absence of a specific diagnostic marker has continued to place primary thrombocythemia as a diagnosis of exclusion.

Clinical Features of Primary Thrombocythemia

1.	Age: - most common over age of 50	Seen in children as young as 2 yrs. (73) and families (73, 73a). Had been considered relatively benign in those < 40 years (74). Recent evidence now consistent with symptomatic (primary thrombotic) and often severe disease in the young (75, 76).
2.	Bleeding diathesis: - epistaxes - gi/gu bleeding - ecchymosis	Bleeding was the most evident presenting symptom in the earlier literature (77); it appears to be more common with thrombocythemia associated with other myeloproliferative lesions, especially polycythemia vera (57).
3.	Thrombotic lesions: - erythromelalgia	Erythromelalgia (red-burning hands or feet) is common; this platelet-mediated arteriolar inflammation has a specific response to aspirin (78).
	- intermittent claudication; digital infarction (gangrene)	
	- occlusive lesions of the micro-circulation	Transient ischemic attacks are common; retinal vascular occlusions; significant cerebral vessel involvement.
	- increased incidence of both arterial and venous occlusions	Least common in large size vessels. Central nervous systems occlusions more common than myocardial infarctions.
4.	Splenomegaly - in 30-50%	Very modest increase in size; rarely more than 4 cm beneath the costal margin. (79)

5. Hypertension — Seen in up to 1/3 of patients (79, 80). May significantly contribute to the vascular lesions.

6. Special Problems:

a.) Surgery: — Significant operative morbidity and mortality when surgery is done before platelet numbers are controlled ($< 600{,}000/\mu l$) (81)

b.) Pregnancy: — Very high incidence of obstetrical complications associated with placental thromboses and infarction (82-84). Spontaneous declines in platelet counts during pregnancy have been observed and these pregnancies have been successfully carried to term (75), although the mechanism is not known. Eaves et al (85) sequentially examined such a case with restriction fragment length polymorphism analysis and showed that the red cell precursors were unchanged, but granulocyte precursors became polyclonal at mid pregnancy at a time when her platelet numbers had returned to normal; post-partum the pattern again returned to its original monoclonal pattern. They suggested "a differential" sensitivity of normal and neoplastic hematopoietic precursor cells to physiological changes associated with pregnancy (85). Control of platelet numbers and aggregation results in successful pregnancies. Aspirin appears to have significant value in pregnancy (82).

c. Development of an acquired coagulation defect:

- Protein S deficiency (acquired) (86)
- Acquired van Willebrand's disease (76, 87)

Although these clinical manifestation define the symptomatic patient, in virtually every clinical series 30-40% of all cases are identified by a (routine) laboratory examination in a patient with no symptoms reasonably referable to the thrombocythemia. Several groups have now analyzed

their patient populations to try to determine the risk of such an asymptomatic patient to develop clinical manifestations of the disease, and to characterize the natural history of primary thrombocythemia.

Clinical Course of Primary Thrombocythemia

Some heterogeneity has been noted in the clinical course of patients in the collected series. In most series, thrombotic events punctuated the clinical picture (75, 76 77, 88, 89, 90) although in the French series hemorrhage was a more common occurrence during the course of disease (79). In most series, the thromboembolic episodes and complications were considered dangerous to survival (75, 76, 77, 79, 88, 89, 90).

In spite of these episodes during the course of primary thrombocythemia, many investigators have not been able to strictly define a direct correlation between the number of circulating platelets and the specific clinical event (76, 79, 88, 90). Nevertheless most investigators have provided data to support the view that reduction in platelet numbers results in fewer symptomatic episodes (75, 77, 88, 89, 90). This confusion has led to correlative observations:

1.) the total circulating platelet mass may be a better parameter, than actual platelet count (75).

2.) The longer the patient remains in clinical control, the lower the rate of thrombotic events (88).

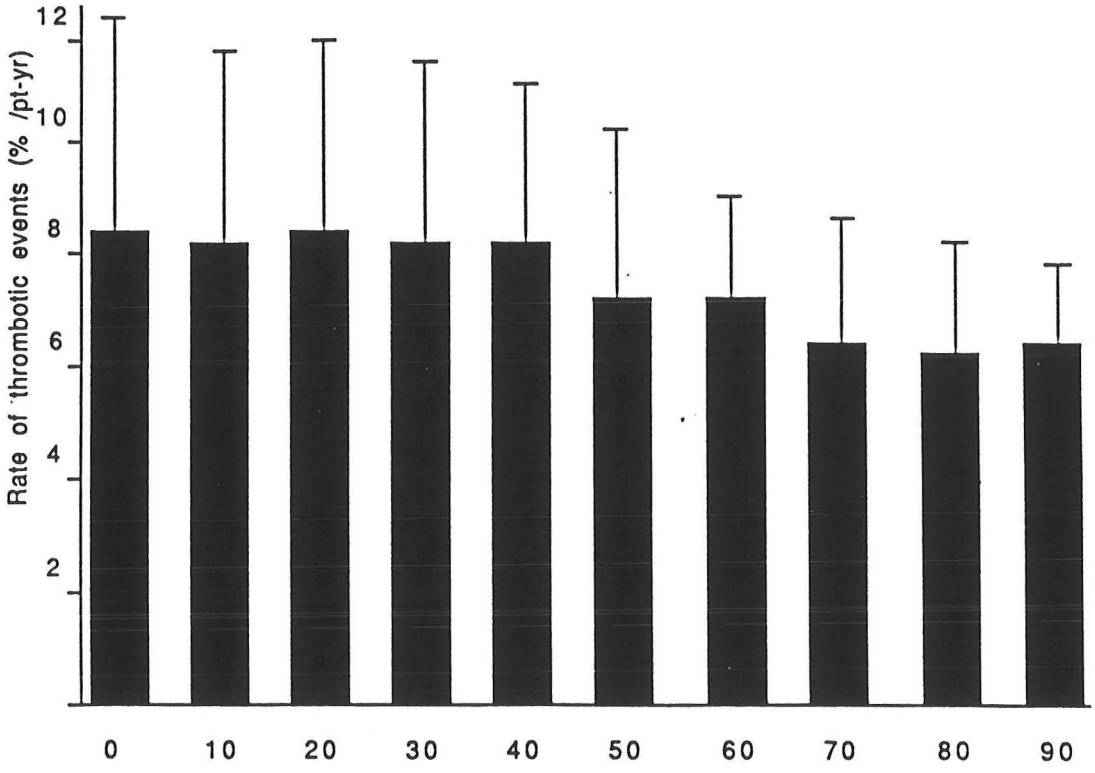


Fig 1. Rate of thrombotic events according to the percentage of the follow-up period spent in remission (platelet count ≤ 600 x 10⁹/L). The bars represent SE.

(Ref. 88)

In spite of these problems of correlation, virtually all experienced investigators feel patients circulating platelet numbers should be kept below 650,000/ μl, particularly in those who present with clinical symptoms.

An important aspect of the degree of the severity of the disease is the overall survival following diagnosis. A reasonably typical survival curve is that of the polycythemia study group (57) shown earlier. Thus, long term (10-25 year) survival is commonly seen. In fact, a factor is the survival pattern from some institutions appears to be the subset of patients treated with alkylating agents with the subsequent development of secondary leukemia or those who present with an identified Philadelphia chromosome.

Therapy of Primary Thrombocythemia:

The management of patients with primary thrombocythemia has focused upon reduction of circulating platelet numbers and the use of antiaggregating agents.

1.) Antiaggregating agents:

Aspirin: has been shown to reduce clinical signs and symptoms in patients with vascular occlusive lesions (35, 48, 49, 75, 79). In addition, it has become an important therapeutic approach during pregnancy (75, 82-84). In that circumstance, it has resulted in patients being able to carry pregnancy to successful delivery. Too little experience exists with the other agents that affect platelet function listed above (91).

2.) Approaches to Reduction of Platelet Numbers:

Virtually every method capable of reducing platelet numbers has been employed:

A. Platelet-pheresis:

Provides a rapid lowering of platelet numbers (in hours), but is of short duration (hours to days). Uncommonly needed, but appears to have its major value in rapidly evolving microvascular thrombotic lesions (35, 92).

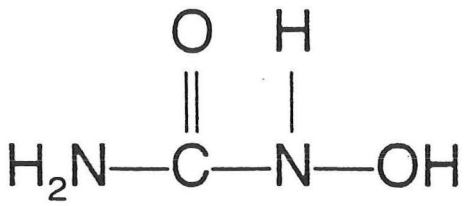
B. Myelosuppressive Cytoreductive Drugs:

Virtually every cytoreductive chemotherapeutic agent (^{32}Phosphorous; alkylating agents; antimetabolites; etc.) has been used to reduce platelet numbers. These agents have been successful and still are in use in some parts of the world.

As the evidence that these drugs have leukemogenic potential grew and as the experience in polycythemia vera confirmed a higher incidence of leukemic transformations when patients were treated with most of these agents; they have been abandoned, except for Hydroxyurea.

Hydroxyurea:

Hydroxyurea has emerged as the cytoreductive agent of choice. This simple molecule:



Structure of hydroxyurea.

was synthesized over 100 years ago, but its entry into clinical medicine did not come until the 1960's, when it was shown to be a ribonucleotide reductase inhibitor (94). It is relatively non-toxic with a broad dose response range. It does induce megaloblastic changes as one might expect from its biochemical site of action (95, 96). The enthusiasm for it use is the belief that it is non-leukemogenic. It however should best be considered to be an agent with uncertain carcinogenic potential (94). It is of some note that at least one case of primary thrombocythemia conversion to acute leukemia has been linked to hydroxyurea therapy (97).

In daily oral doses of 500-1000 mg most thrombocythemia patients will have a decrease in platelet values, and then drug dosage can be titrated to the desired circulating platelet number. It is the preferred cytoreductive agent of choice. An interesting recent report suggests that long term Hydroxyurea may reverse marrow fibrosis; if confirmed this may be an important advantage in, at least, some forms of myeloproliferative lesions with thrombocythemia.

C. Biologic Response Modifiers:

Interferon-alpha

Interferon has been shown to lower platelet counts in patients with primary thrombocythemia and thrombocythemia associated with other types of myeloproliferative lesions (99, 100). Its mechanism of action appears to be mediated by an inhibitory effect of the interferon on megakaryopoiesis.

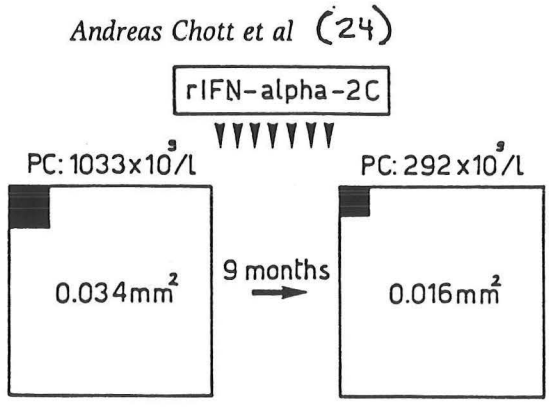


Fig 4. Graphic demonstration of a marked rIFN-alpha-2c therapy-induced reduction of total megakaryocyte area per square millimetre bone marrow in an individual case of AMM. PC = platelet count.

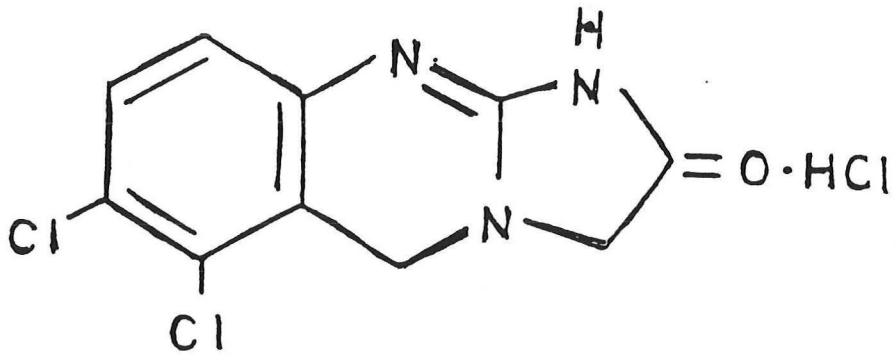
Unfortunately only approximately 50% of patients achieve a stable state of remission with interferon therapy. In addition, on cessation of the therapy, recurrence of the clinical and laboratory findings is usual (100).

Usual dose: 25 million units S.C. per week given in divided (4 to 6) doses.

D. Anagrelide:

A new agent with an anti-aggregating effects on platelets, Anagrelide, has recently been in clinical trials in the United States (102). In humans the drug has proven to be exceedingly effective at reducing platelet numbers, but the mechanism of action is not certain.

Anagrelide:



Anagrelide is a member of the imidazo (2, 10-b) quinazolin-2-one series of compounds which inhibit platelet aggregation. It is active against both ADP and collagen stimulated platelet aggregation *in vitro*, whether the platelets come from an anagrelide treated patient or are collected and then treated with the drug *in vitro*.

The important features of Anagrelide are:

1.) effective anti-aggregating agent

2.) produces a dose dependent decrease in platelets

3.) non-mutagenic and non-leukemogenic

4.) Produces no other hematologic (peripheral blood or bone marrow) changes.

5.) Has a mild positive inotropic effect on the heart

Average daily dose: 0.5 to 4 mg/d
Mean: 2 mg/day

Side effects: During institution of therapy many patients note a "fullness in their chest" particularly when the anagrelide is taken with coffee. Mild increases in pancreatic enzymes have been noted in a few cases.

To date, our experience is very positive with this agent. There is great ease in achieving excellent platelet control in patients with thrombocythemia regardless of the form of associated myeloproliferative disorder. In addition, many of our patients who had previously failed alpha-interferon or hydroxyurea have achieved excellent control with Anagrelide.

Leukemic Transformation in Primary Thrombocythemia

Acute leukemic transformation has been a feature of all of the subtypes of myeloproliferative lesions. Since 60-90% of patients with primary thrombocythemia have an essentially normal life expectancy, examination of the subset who do not is merited. Aside from the mortality from the thrombohemorrhagic lesions, acute leukemic transformation makes up the important second group (97, 103, 104). Examination of the patients who transition suggest that almost all have either been exposed to cytoreductive agents with their expected leukemogenic potential, or they had the Philadelphia chromosome evident early in their course. These latter cases, as mentioned previously, may indeed represent a different (form of) disease. In those patients whose disease had been treated with cytoreductive agents (radiophosphorous, alkylating agents, etc.) their leukemic transformations occured 4 to 7 years after beginning therapy; this temporal pattern is quite consistent with the known sequalae of these drugs.

These observations emphasize the potential value of cytogenetic analysis at diagnosis to help define prognosis. Present evidence suggests that those patients with Philadelphia chromosome positive primary thrombocythemia merit aggressive therapeutic considerations to attempt to erodicate that marker clone. In the majority of patients it appears wise to use non-mutagenic agents. Presently, Anagrelide appears to be the most exciting agent available.

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