THROMBOCYTOSIS: TOO MUCH OF A GOOD THING?

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ABSTRACT

Thrombocytosis is due to (a) a variety of disorders that cause reactive stimulation of platelet production, (b) familial mutations, or (c) essential thrombocythemia (ET) and other myeloproliferative disorders (MPDs). The MPDs are clonal abnormalities of the pluripotent hematopoietic stem cell. Dysregulation of megakaryocytopoiesis in ET involves defective binding of thrombopoietin by platelets and megakaryocytes resulting in increased levels of plasma free thrombopoietin, and increased sensitivity of megakaryocytes to thrombopoietin leading to their increased proliferation. Bleeding and thrombosis are the major causes of morbidity and mortality in ET and the other MPDs. The elevated platelet count and qualitative platelet defects have been implicated in the pathophysiology of these hemostatic problems. However, these platelet abnormalities do not correlate well with clinical complications. It is proposed that bleeding and thrombosis could be due to vascular abnormalities that result from dysfunctional hematopoietic stem cell-derived endothelial cells.

INTRODUCTION

Dismissed by early microscopists of the 19th century as merely particles of "blood dust" (1), platelets today have earned proper respect. They are metabolically versatile cell fragments that circulate in blood passively, but are constantly poised to respond instantaneously to vascular injury with a powerful armamentarium of prothrombotic and vasoactive mediators that can staunch blood loss. Despite their indispensable role in hemostasis, however, certain clinical disorders are characterized by an excessive number of circulating platelets that may be directly or indirectly harmful. The prototype of such disorders is essential thrombocythemia (ET), one of the so-called myeloproliferative disorders (MPDs). This article will focus on the pathophysiology of ET and the hemostatic complications that are associated with it.

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CAUSES OF THROMBOCYTOSIS

With the advent of automated platelet counting, thrombocytosis is not infrequently detected on routine blood testing as a coincidental finding. A classification of major causes of thrombocytosis is provided in Table 1.

In most cases, thrombocytosis is a secondary or reactive phenomenon. Even extreme thrombocytosis (platelet count >1,000,000/μL) is most frequently a reactive process, as was found in more than 80% of 280 consecutive hospitalized patients in one reported series (2). In these situations, the elevated platelet count *per se* is generally harmless, and does not itself directly pose a risk of thrombosis. However, secondary thrombocytosis does reflect a potentially serious underlying disease which should be correctly diagnosed and treated.

Familial thrombocytosis (or hereditary thrombocythemia) is a genetically heterogeneous disease. Most cases described to date involve autosomal dominant transmission of mutations in the thrombopoietin (TPO) gene that lead to markedly elevated plasma TPO levels due to derepression of gene translation and overproduction of TPO. These cases can be regarded as *nonclonal* myeloproliferative disorders restricted to the megakaryocyte-platelet lineage. For unclear reasons, patients with familial thrombocytosis may be clinically affected with thrombohemorrhagic complications, as are patients with essential thrombocythemia (3).

Clonal thrombocytosis includes essential thrombocythemia (ET) and related MPDs (polycythemia vera, chronic myelogenous leukemia, and myeloid metaplasia with or without myelofibrosis) or myelodysplastic syndromes which may be also accompanied by elevated platelet counts. The diagnosis of ET is presently one of largely exclusion as no specific diagnostic tests are available. After reactive and familial causes have

TABLE 1
Classification of Major Causes of Thrombocytosis

1. Reactive (secondary) thrombocytosis
   - Iron deficiency
   - Recovery from thrombocytopenia ("rebound")
   - Acute blood loss
   - Postsplenectomy, asplenic states
   - Malignancies
   - Inflammatory and infectious diseases
2. Familial thrombocytosis
3. Clonal thrombocytosis
   - Essential thrombocythemia
   - Other myeloproliferative disorders
   - Myelodysplastic syndromes

been ruled out, patients with thrombocytosis can be considered to have ET if they do not have a myelodysplastic syndrome (usually diagnosed by bone marrow and cytogenetic analysis) or one of the other MPDs. For example, chronic myelogenous leukemia (CML) can occasionally masquerade as ET, presenting with only thrombocytosis, but the finding of a Philadelphia chromosome on cytogenetic studies or the BCR-ABL rearrangement by reverse transcriptase-polymerase chain reaction of peripheral blood or bone marrow samples should be diagnostic of CML in such cases (4,5).

PATHOPHYSIOLOGY OF ESSENTIAL THROMBOCYTHEMIA

Clonality

Like the other MPDs, ET was originally proven to be a clonal disorder that arises in a single pluripotent hematopoietic bone marrow stem cell in women who were coincidentally heterozygous for glucose-6-phosphate dehydrogenase (G6PD) deficiency (6). In these cases it was found that only one isoenzyme of G6PD was expressed in all blood cell lines while either isoenzyme was randomly expressed in other somatic cells. Subsequently, clonality has been confirmed in patients with MPDs using other assays involving X-linked polymorphic markers in female patients. The notion of clonality in all patients with ET has been recently challenged by Harrison, et al (7), who have pointed out some of the potential pitfalls of clonality assays using X-chromosome inactivation patterns unless suitable control cells (e.g., T lymphocytes) are used to exclude the possibility of a constitutively skewed or imbalanced pattern. Further studies are needed to define the molecular pathogenesis of ET and to address the possibilities that ET is a heterogeneous disease or that non-clonal ET can progress to clonal disease (8), mechanisms which might provide insight into the reasons for the clinical variability of the disease.

Abnormal megakaryocytopoiesis

Although the various MPDs arise as similar defects in a pluripotent hematopoietic stem cell, the different hematological phenotypes they exhibit (i.e., erythrocytosis in polycythemia vera, leukocytosis in CML, thrombocytosis in ET) may be due, at least in part, to lineage-restricted hypersensitivity of progenitor cells to their respective growth factors. In the case of ET, circulating stem cells have been shown to be hypersensitive to the major megakaryocytic growth factor, thrombopoietin (TPO) (9).

Platelets and megakaryocytes can physiologically regulate plasma

TPO levels by binding the growth factor to its receptor, *c-mpl*, on their cell surfaces. Therefore, when the platelet count decreases, increased levels of plasma free TPO are available to stimulate bone marrow megakaryocytopoiesis. Conversely, when the platelet count increases, plasma free TPO levels are reduced and megakaryocytopoiesis is suppressed. This mechanism to modulate TPO levels is designed to maintain steady-state platelet formation under normal circumstances. Thus, in general, plasma TPO levels are inversely correlated with circulating platelet and marrow megakaryocyte mass (10). However, in ET this physiological feedback system is lost. Plasma TPO levels in ET are actually elevated or "inappropriately" normal, despite the presence of thrombocytosis and an increased megakaryocyte mass (11,12). The dysregulation of TPO in ET may be due to reduced or abnormal *c-mpl* expression on the surfaces of clonally defective platelets and megakaryocytes, resulting in impaired binding and clearance of free TPO from the circulation (13).

In summary, as shown in Figure 1, the pathophysiology of abnormal megakaryocytopoiesis in ET appears to involve (a) reduced binding of TPO by the abnormal platelets and megakaryocytes of this MPD, (b) inappropriately elevated plasma TPO levels, and (c) hypersensitivity of megakaryocyte progenitors to thrombopoietic growth factors, specifically including TPO. It should be emphasized, however, that considerable further work will be required to elucidate these relationships.

Mechanisms of bleeding and thrombosis in essential thrombocythemia

While thrombotic and bleeding problems are generally not caused by reactive or secondary thrombocytosis states, even when the platelet count elevation is extreme, these hemostatic complications are the major causes of morbidity and mortality in ET. They are also important clinical problems encountered in the other MPDs.

When bleeding occurs in ET, it tends to be of the "platelet type": hemorrhage that occurs spontaneously or immediately after trauma, and typically involves superficial surfaces, such as skin and mucus membranes (e.g., gastrointestinal, genitourinary or oropharyngeal sites). Although about 10 to 25 percent of all thrombotic episodes are deep venous thrombosis and pulmonary embolism, arterial thrombotic complications occur more frequently than venous thrombosis in ET (14). This is not surprising in light of the predominantly platelet composition of arterial thrombi. In a recently published series of 187 consecutive patients with ET followed at a single institution, 50 percent had at least one thrombotic episode within 9 years of diagnosis

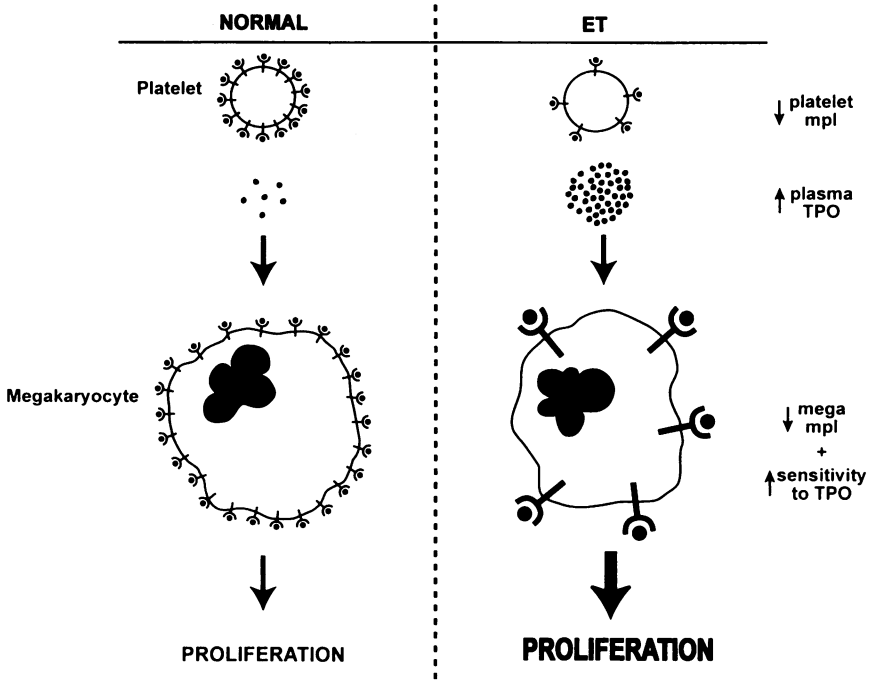

FIG. 1. Current working model of the abnormal regulation of megakaryocytopoiesis in essential thrombocythemia (ET), based on several lines of investigation described in the text. Despite the elevated platelet count, free plasma thrombopoietin (TPO) levels are increased in patients with ET due to loss of TPO receptors (mpl) on the surfaces of their defective platelets. Bone marrow megakaryocytes likewise have loss of expression of mpl, but exhibit increased sensitivity to the megakaryocyte-stimulating activity of TPO.

(15). Apparently paradoxically, both bleeding and thrombosis can complicate the natural history of an individual with ET or other MPDs.

We and many other groups have investigated the mechanisms of bleeding and thrombosis in the MPDs. As yet, no clear and comprehensive pathophysiological picture has emerged, but the following factors (Table 2) have been implicated: (a) thrombocytosis, (b) qualitative platelet abnormalities, and (c) other mechanisms (e.g., abnormalities of leukocytes or the vessel wall).

Thrombocytosis

In the related MPD, polycythemia vera, a clear relationship exists between the degree of hematocrit elevation and the risk of thrombotic complications. However, even in this disease, the erythrocytosis itself cannot be the sole determinant of thrombotic risk since individuals

TABLE 2
Possible Causes of Bleeding and Thrombosis in Essential Thrombocythemia and Other Myeloproliferative Disorders

1. Thrombocytosis
2. Qualitative platelet abnormalities
3. Abnormalities of other bone marrow stem cell-derived cells
   - Leukocytes
   - Endothelial cells (?)

with secondary erythrocytosis (e.g., lung disease, high altitude, benign or malignant renal disorders) generally do not suffer hemostatic complications with comparable degrees of polycythemia. In ET, the role of thrombocytosis in the pathogenesis of bleeding and thrombosis is much more uncertain. Nevertheless, in the only prospective clinical study published to date, it was found that platelet cytoreduction in a selected group of high-risk patients with ET significantly reduced the incidence of thrombotic episodes (16). Furthermore, interestingly, there is also good evidence that extreme thrombocytosis in ET (platelets $>2{,}000{,}000/\mu L$) is associated with an increased risk of bleeding (17). However, most retrospective studies have failed to establish a clear and consistent correlation between the degree of thrombocytosis and the occurrence of hemostatic complications in ET or the other MPDs (18,19).

Qualitative platelet abnormalities

Many different types of qualitative platelet abnormalities have been described in patients with ET and the other MPDs. These include striking morphological changes; decreased or increased platelet surface expression of receptors that may affect platelet function; abnormalities of platelet secretion and arachidonic acid metabolism; and an acquired form of von Willebrand's disease, in which the larger multimers of von Willebrand factor may be depleted form plasma by their adsorption onto the surfaces of abnormal ET platelets. Unfortunately, none of these qualitative platelet defects in ET has been clearly linked to the clinical bleeding or thrombotic tendency of this disease (19). There are several possible explanations for this disappointing lack of clinical correlations: more than one abnormality may coexist in the same population of circulating platelets, functionally offsetting each other; or platelet defects may change over time as part of the natural history of the disease and are therefore not recognized at the time of a specific bleeding or thrombotic event. Little is known about sequential qualitative platelet abnormalities as a function of disease progression in the MPDs. While quantitative and qualitative platelet abnormali-

ties are likely to contribute to the pathogenesis of hemostatic complications in ET, the possibility must be considered that other, as yet unrecognized, hemostatic defects are operative in these patients.

Abnormalities of other bone marrow stem cell-derived cell types

Qualitative abnormalities of leukocytes, which are involved in the hematopoietic stem cell defect of ET and the other MPDs, have been recently considered to contribute to the thrombotic tendency of these patients (20). The hemostatic complications of ET might also be due to primary abnormalities of endothelial cells. Indeed, the valvular lesions of nonbacterial thrombotic endocarditis, which may be sources of systemic thromboembolism in some of these patients, are frequently found by echocardiography in ET and the other MPDs (21).

The concept of stem cell plasticity has shed light on a strong relationship between hematopoietic stem cells and endothelial cells (22). Endothelial progenitor cells can be isolated from peripheral blood and bone marrow, and they are incorporated into sites of neovascularization *in vivo* (23). Stem cells in the bone marrow can contribute to cardiac repair and neovascularization after ischemic injury (24). Circulating endothelial progenitors are transplantable and capable of long-term repopulation of human allogeneic bone marrow transplant recipients (25). The emerging role of bone marrow-derived stem cells in vasculogenesis (26) suggests the possibility that endothelial dysfunction might contribute to the clinical bleeding and thrombotic complications of ET and the other MPDs. Therefore, while not yet tested, it is intriguing to hypothesize that the hemostatic complications of these stem cell disorders may be due, at least in part, to the repopulation of vascular intimal surfaces by functionally defective, bone marrow-derived endothelial cells.

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DISCUSSION

Billings, Baton Rouge: Andy that was very interesting. In the treatment of the pregnant woman with essential thrombocythemia, what are your thoughts? The reason I ask this question is for years, because of the mutogenesis, teratogenesis problem, I have used observation alone when hydroxyurea was the only therapy. Over the last couple of years, however, we have a new agent, anegrelide, which, of course, has no track record. With spontaneous abortion being a problem in essential thrombocytopemia, there are a number of women who would like to sustain the pregnancy until term. What are your thoughts?

Schafer, Houston: This is very important problem, of course, and there is no straightforward answer. There have been some large observational series, not randomized studies, mainly from the Mayo Clinic, which suggest that neither control of the platelet count nor antiplatelet therapy significantly alters the national history of the problem of spontaneous abortions. Of course the issue of trying to keep the platelet count normal in pregnant women is a very difficult one. As you mentioned, hydroxyurea is potentially teratogenetic. Anagrelide, which is a newer agent, which has been approved by the FDA, relatively selectively lowers the platelet count by inhibiting megakaryocyte proliferation but it does cross the placenta. Therefore, there is some concern, although not yet proven, that it might also be teratogenetic. Most of us, if it is needed, will treat women who are pregnant with an elevated platelet count with interferon, which appears to be the safest of choices. However, it is also accompanied by very significant side effects. So there is really no entirely satisfactory treatment for the elevated platelet count in pregnant women with essential thrombocythemia. At the same time, there are no convincing data at this point that suggests that control of thrombocytosis is in fact an important factor in producing successful pregnancies in such patients.

Schreiner, Great Falls: I enjoyed that very much. Do you happen to know of any clinically significant thrombocytosis following the thrombocytopenias that we see of varying degrees in bionics, that is the interface between the human biology and artificial organs? For those of you that haven't heard bionics, there is now a section on bionics in the Smithsonian Institute with a new office and curator and a place for putting the history of artificial organs in the Smithsonian. So you'll learn a lot more on bionics, it's the new word. When we first did perfusion of a resin, the reason why hemoperfusion was not used in humans because of the regular production of thrombocytopenia. We found that in prolonged perfusions, the platelets tie up to resin but can be physically dislodged afterwards, and when they did some of the patients actually got high platelet counts because they were making new platelets, or releasing new platelets during the time thrombocytopenia was acting as a stimulus for platelet formation and then adding the sequestered platelets. So one wonders whether, with a lot of plastics and valves being

put in whether cessation of this type of thrombocytopenia could lead to a clinically significant thrombocytosis. Have you ever heard of any?

Schafer: This is an interesting issue. There is, of course, the phenomenon of rebound thrombocytosis, which occurs after transient mylosuppression with thrombocytopenia or after successful treatment of ITP. We don't think this is a clinically significant issue with artificial surfaces, in which there is the general phenomenon of thrombocytopenia due to consumption of platelets by the artificial surface. However, I don't think it's clear whether or not actually removing those surfaces results in clinically significant thrombocytosis.

Kitchens, Gainesville: Andy that was very scholarly. Cardiovascular disease is very common, and both you and I spend a lot of time at the VA where smoking is even more common, and hypertension untreated is extraordinarily common, and I find the platelet is in the wrong place at the wrong time as an innocent bystander. When one of these people inevitably has some type of ischemic event, and they're also found serendipitously to have 800,000–900,000 or a million platelets, doctors want to blast these platelets back to where they came from. And I think Mae West was correct. I think there's a lot of guilt by association, and I think that this process, thrombocytosis (and I am not going to glorify it by calling most of thrombocytosis that I see to be the real model of myeloproliferative disease, ET) is the most over treated, most feared process that doesn't need to be feared most of the time. We see so many of these people. The first thing I do is look at their platelet count in the computerized VA record and they've had a million platelets for 6–8 years.

Schafer: I agree with you that we are sometimes overly concerned about treating asymptomatic patients who have elevated platelet counts. I think that perhaps even more important than worrying about essential thrombocythemia in those cases, which is really largely a diagnosis of exclusion, is the concern that many of us have that the thrombocythemia is actually caused by some occult process rather than essential thrombocythemia. In other words, in some of these cases are we are missing an occult malignancy for example. So, I think you are right, in many cases the concern should relate more to discovering the underlying cause than the thrombocytosis itself.

Davis, Charlottesville: You mentioned erythromelalgia, which goes back to the War of Secession; there was a description of this strange syndrome after war injuries. We still don't know what causes it. It may well be a syndrome related to thrombocytosis, and, as you know, some patients have responded to plain aspirin but not all. Do you think thrombocytosis is behind a lot of these cases, especially the ones that do respond to aspirin?

Schafer: Not necessarily, because there are certainly patients with classic erythromelalgia who do not have thrombocytosis, and in fact don't even have a myeloprolifative disorder. Patients with lupus, for example, may have erythromelalgia without thrombocytosis. However, there is some information for platelet kinetics in this disorder, which indicates that there is rapid increase platelet turnover in erythromelalgia, and that the increased platelet turnover can actually be corrected rapidly by using an antiplatelet agent like aspirin, while at the same time the symptoms resolve. So, there is some evidence that platelet turnover is an important factor that contributes to this syndrome.