Refresher Course

Essential Thrombocythemia

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INTRODUCTION

Essential thrombocythemia is a rare disease, but its clinical presentations are found commonly in general practice, e.g. cerebrovascular disease, ischemic heart disease, deep vein thrombosis, ischemic gangrene of toes and fingers, and bleeding disorders, etc. Provisional diagnosis can be done by routine blood cell count. This paper reviews the literature regarding the incidence, clinical feature, therapeutic options, and prognosis.

Literature Review

Essential thrombocythemia (ET) was first described by Ebstien and Goedel in 1934. Dameshex in 1951 suspected that ET might be a myeloproliferative disease. However, ET has been proven to be clonal myeloproliferative disease of a multipotent stem cell of unknown etiology, characterized by a persistently increased platelet count above $600x10^3$/μl, excessive proliferation of megakaryocyte in the bone marrow, a normal erythrocyte mass, stainable iron in the marrow or failure of a trial of iron therapy (1 g/dl) to promote a rise in hemoglobin after 1 month of treatment, absence of the Philadelphia chromosome and absence of prominent bone marrow fibrosis, and no known cause of reactive thrombocytosis.[1,2] Typically essential thrombocythemia occurs in adult in the sixth or seventh decade of life at initial diagnosis. Both sexes are equally affected, but in younger patients (less than 40 years of age) females appear to predominate.[2] There are some reports of familial essential thrombocythemia in which genetic transmission may occur.[3] Familial essential thrombocythemia is inherited in an autosomal dominant fashion and is associated with the megakaryocyte thrombopoietin receptor (cMpl) gene.[3] The annual incidence of ET in some cities has been reported as 1.5-2.38 per 100,000 of the population.[2,4] ET is a condition of unknown etiology, but behavioral exposure to hair dyes, living in the tuff house and working as an electrician are significant risk factors associated with the development of ET.[5] Some studies have shown that 5% of ET patients have abnormal chromosomes but can not identify a specific gene.[2]

Pathogenesis

1. The pathogenesis of ET has now been established as a clonal myeloproliferative disorder of a multipotent hemopoitic stem cell and it is a heterogeneous disorder.[1,6,7] No specific karyotypic abnormality has been demonstrated using an X chromosome inactivation pattern or by polymorphism in the human androgen receptor genes.[7,8] Both showed that a transforming event might occur at different point in the pathway.

2. In a pathogenetic study of ET, alteration and cellular transformation have been demonstrated in less than 5% of patients. Multiple abnormalities and no dominant pattern emerged.[2,9] Trisomy 8 was found to be the most common ploidy associated with ET, but some reports have not shown an increased frequency of patients with trisomy 8 and 9.[1,10] The bcr/abc transcripts were present in 21.4% of patients with ET and 12/25 Philadelphia chromosome negative ET patients.[11,12] The phenotype is expressed predominantly in the megakaryotype hemopoitic platelet line. A mutation in the thrombopoietin (TPO) gene or the cMpl gene may constitute the pathogenetic event leading to familial ET, which is inherited in an autosomal dominant manner, but this is not detected in nonfamilial'ET, therefore it is unlikely to contribute to the pathogenesis of ET.[3,13] Fourty seven percent of ET found in children is familial.[14]

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3. Regarding pathobiology of ET, the thrombopoietin cMpl system has been suggested as anticipating in the pathophysiology of ET but may not be directly linked to the pathogenesis of sporadic ET.[15] A normal or even slightly elevated TPO level in ET despite an increase megakaryocyte/platelet mass suggests an abnormal feedback mechanism in the regulation of TPO production and consumption or impairment of uptake and catabolism of TPO, due to low cMpl expression, and not as a result of any defect in the structure of TPO or cMpl.[15-19] Protooncogene cMpl is involved in spontaneous megakaryopoiesis in myeloproliferative disease. Platelet cMpl expression is dysregulated in the patient with ET but this is not of diagnostic value.[19] Some reports show that autonomous megakaryocyte growth in ET is neither related to a cMpl mutation nor to an autocrine stimulation by a cMpl ligand and some suggest that the signal pathway mediated by cMpl after binding to TPO may be impaired in ET.[20]

4. In thrombokinetic study, platelet production markedly increases autonomously, and is not regulated by cytokines. The possible mechanisms of thrombocytosis are:-

4.1 Spontaneous growth of megakaryocytes and/or erythroid colony formation by progenitors from the bone marrow and/or peripheral blood.[1]

4.2 Increased sensitivity to colony stimulating activity, e.g. IL-3.[1]

4.3 Decreased inhibition by platelet inhibiting factor, e.g. TGF-β_1.[1]

5. The mechanism of bleeding and thrombosis in ET may be related to qualitative and quantitative platelet changes (overproduction of poorly functioning platelets), and normal platelet survival.[2] The mechanisms of thrombogenesis and hemorrhage are not completely clear, because reduction or normalization of the platelet count does not completely eliminate the risk of thrombohemorrhage.[1] A possible multifactorial mechanism is:-

5.1 Abnormal platelets

1) Hypoaggregation (decreased response to ADP, collagen, adrenalin mediated aggregation).[1]

2) On the other hand, some reports show hyperaggregability or spontaneous aggregation (elevated β-thromboglobulin).[1]

3) Platelet membrane defect. Decrease concentration of glycoprotein Ib and IIb/IIIa complex, and glycoprotein Ia-IIa leading to a prolonged bleeding time.[1] An abnormal membrane may account for enhanced thrombin generation (increase β-thromboglobulin, thromboxane generation) which may lead to a relatively high thrombotic risk.[2]

4) Platelet survival is reduced in thrombotic ET.[2]

There is no currently available test of platelet function to predict the risk of occurrence of thrombotic or hemorrhagic complication. However the role of platelet activation in the pathogenesis of ischemic erythromelalgia has been established and a correlation between hemorrhagic complication and platelet counts over $10^6/\mu l$ has been established.

5.2 A possible mechanism linking the high grade thrombocytosis to bleeding is a reduction of von Willebrand factor[1], caused by

1) Consumption of von Willebrand factor and clot fragility due to the mechanical effect of a high platelet count or to inhibition of fibrin polymerization by platelet glycoprotein Ib.

2) Increased removal of large von Willebrand factor multimers from plasma.

5.3 Hypercoagulation. In vivo, leukocyte activation (demonstrated by increased thrombin-antithrombin complex, prothrombin fragment 1, 2 and D-dimer) occurs in association with coagulation system activation.[21]

5.4 Reduction of natural anticoagulants (antithrombin III, protein-C, protein-S level and activated protein-C resistance) in thrombotic ET patients, suggest that the deficiency is acquired, and may be responsible for low grade activation of coagulation.[1]

5.5 Reduction in fibrinolytic activity is partly caused by a higher concentration of plasmatic plasminogen activator inhibitor-1(PAI-1) as well as releasing of more active platelet PAI-1, resulting in thrombogenesis in ET patients.[22,23]

5.6 Subclinical vascular endothelial damage as demonstrated by significantly increased serum thrombomodulin and von Willebrand factor antigen.[21,24] But some disagree with this and report no evidence of vascular endothelial damage.[1]

Clinical Manifestations

The incidence of thrombohemorrhagic complications in myeloproliferative disease is about 41%, hemorrhage 33% and thrombosis 19%. The presenting symptoms of ET patients are quite variable; from asymptomatic (33-84%), vasomotor symptoms and thrombohemorrhagic manifestations or a combination[25,26] with an inherent tendency to undergo leukemic transformation or an indolent clinical course.[27] In those who are primarily asymptomatic, the rate of increase in symptoms is about 7% per year but this is not directly related to platelet count.[28]

The clinical presentations are as follows:-

1. Occlusive vascular manifestations predominate, endangering older patients and those with a prior history of a thrombotic episode.[1] In ET patients, thrombosis is found more than hemorrhage and arterial thrombosis is more common than venous thrombosis.

1.1 Disturbance of microcirculation (or vasomotor or functional symptoms) is the most common initial presenting symptoms, usually involving the toes and fingers (erythromelalgia).[2] Erythromelalgia refers to the syndrome of redness or dusky congestion of swollen extremities with burning pain, usually asymmetrical, enhanced by warmth which leads to acrocyanosis and distal gangrene with intact arterial pulses.[1] In pregnant women with ET, it is associated with adverse placental outcome and this is likely to be due to thrombosis of the uteroplacental circulation.

1.2 Thrombosis of the medium and large arteries in ET patients occurs commonly. Digital gangrene or gangrene of the extremities due to occlusion of peripheral arteries has been found in 30%, ischemic heart disease or myocardial infarction has been found in 18% with multivessel coronary artery occlusion.[29] Cerebral artery thrombosis leads to neurological presentation with headache, paresthesia, visual disturbance, dysarthria, dysphonia, seizure, hemiparesis, etc. Twenty two percent of patients with ET first present with a stroke and 0.54% of all patients with cerebrovascular disease have ET.[30] Renal artery occlusion is found in about 10% of those presenting with acute renal failure. A large aortic thrombus has been reported. Some reports showed that in ET patients with arterial occlusion, 52-75% had other atherosclerotic risk factors such as old age (over 40 yrs), hypertension, DM, dyslipidemia and especially cigarette smoking.[1,29]

1.3 Venous thrombosis is found less commonly in ET patients.[1] The presenting symptoms depend on the site of the thrombotic vein, e.g. deep vein thrombosis of the leg, pelvis, splenoportal vein, hepatic vein, splenic vein, other splanchnic veins or central retinal vein occlusion with neovascular glaucoma. Thrombosis of the hepatic vein leads to clinical presentation of Budd Chiari syndrome.[2]

2. Hemorrhagic events are less common than thrombosis and are frequently found in ET patients with platelet counts greater than $10^6/\mu l$.[2] However, others have found no correlation between risk of hemorrhage and platelet count.[1] Hemorrhage is found as an initial manifestation in 37%, a subsequent manifestation in 14%, with mainly minor bleeding, e.g. ecchymosis, intramuscular hematoma, gingivorrhagia, epistaxis, gastrointestinal bleeding and menorrhagia. Splenomegaly has been found in 26-48% of ET patients. Hepatomegaly has been found in about 4% of ET patients.[2]

In pregnant women with ET, a successful pregnancy is achieved in only 50%, with an increased incidence of first trimester miscarriage, ectopic pregnancy, spontaneous abortion, abruptio placenta in 43-49%, and an increased incidence of intrauterine growth retardation and stillbirth. This is likely to be due to arterial or venous thrombosis of the uteroplacental circulation.[2,25,31,32]

Laboratory Findings in ET Patients

1. **Peripheral blood cell examination.** The platelet count may exceed $600x10^3/\mu l$ with abnormal morphology in 80%, with abnormal size, shape and structure, often with megakaryocyte fragments. Abnormal platelet function is found in 83% of ET patients. Mild anemia may be found but most patients have a normal hemoglobin concentration, and a leukocytosis if present is usually less than $20,000/\mu l$.[2,16,21]

2. **Bone marrow examination**. There is usually marked megakaryocytic hyperplasia with

clusters. The megakaryocytes are large, with bizarre megakaryocytic nuclear pleomorphism (multilobulated nuclei).[33] The presence of pathologic megakaryocytosis in the bone marrow is a more reliable diagnostic criterion than a definite platelet limit.[2,28]

3. **Other investigations**. Normal serum ferritin level. Bleeding time is prolonged in 10-20% of ET patients with hemorrhagic complication. Platelet aggregation study is frequently abnormal, with impaired aggregation in response to epinephrine, ADP and collagen (55%).[2] There is usually absence of the Philadelphia chromosome. Measurement of non-specific markers of infection or inflammation, e.g. acute phase reactants such as C-reactive protein concentration, erythrocyte sedimentary rate and plasma fibrinogen, suggest a diagnosis of reactive thrombocytosis rather than ET. In spontaneous colony formation studies, megakaryocyte colony growth may distinguish ET from reactive thrombocytosis, but not from other chronic myeloproliferative disorders (PV, AMM, CML). Cytogenetic studies in young ET patients may be abnormal. There is a reduction in the expresstion of the megakaryocyte thrombopoietin receptor (c-Mpl) both in circulating platelets and bone marrow.[19,20] About 25% of ET patients show elevated uric acid levels and pseudohyperkalemia.[2] Some ET patients with bleeding complications may demonstrate a decrease in von Willebrand factor, ristocetin cofactor activity, collagen binding activity, and large von Willebrand factor multimers, and also a decrease in natural anticoagulants including protein-C, protein-S, antithrombin III, and resistance to activated protein-C. This may contribute to the thrombotic tendency seen in some ET patients. Plasma erythropoietin (EPO) concentration is low in PV patients, but in ET patients the EPO concentration is lower than the reference value by about 50%.

Using gamma camera scintigraphy, splenomegaly has been detected in at least 50% of newly diagnosed ET patients.[34]

Magnetic resonance imaging is a useful tool for the differential diagnosis of ET and myelofibrosis. ET does not show a modified MR signal from the dorsal vertebrae, whereas in myelofibrosis the signal is markedly reduced with a very small degree of reconversion (fatty or absent). In myelofibrosis there is a marked degree of reconversion.[35]

Diagnosis

In patients whose platelet count is above 10^6 /μl, reactive thrombocythemia accounts for 82%, a myeloproliferative disorder for 14%, and 4% is of uncertain etiology. Of the myeloproliferative disorders, it is found that 42% have chronic myeloid leukemia, 29% have essential thrombocythe-mia, 13% have polycythemia vera, 5% have myelofibrosis and 11% are unclassified.[1] As noted above, ET is still diagnosed after excluding all causes of reactive thrombocytosis as well as other chronic myeloproliferative disorders. However the criteria for diagnosis of ET has been set by the Polycythemia Vera Study Group[1] as:-

1. A platelet count > 600 x 10^3/μl.
2. A hemoglobin less than or equal to 13 g/dl or a normal red cell mass. (male < 36 ml/kg, female < 32 ml/kg)
3. Stainable iron in the bone marrow or failure of an iron supplement trial (< 1g/dl rise in hemoglobin after 1 month of iron therapy).
4. No Philadelphia chromosome.
5. Collagen fibrosis of marrow.
   5.1 Absence or
   5.2 < 1/3 biopsy area without either splenomegaly or leukoerythroblastic reaction.
6. No known cause of reactive thrombocytosis.

The diagnosis of ET can be strongly supported by :-

1. Abnormal platelet morphology and function.
2. Bone marrow examination, i.e. clinicopathology, histochemistry or bone marrow biopsy. The initial stage of the disease process requires sequential examination to reach a correct diagnosis.[36]
3. Positive abnormalities found in in vitro growth of hemopoietic progenitors and spontaneous megakaryocyte colony formation and clonality assay.[2,7]
4. High megakaryocyte endomitotic index.[37]
5. Clinical symptoms and signs. Some reports diagnose ET by clinical symptoms in 79%.[28]
6. Measurement of serum TPO level may be useful in the future in the diagnosis of ET.

Some reports show differential criteria for ET and RT.[1]

Treatment

Treatment strategies in essential thrombocythemia remain controversial, with regard to using platelet cytoreductive agents because the life expectancy of an ET patient is generally normal and because of the leukemogenic potential of long term use of these agents.[38] But as we know, the incidence of major bleeding is rare and seems to be related to platelet counts above $10^6/\mu l$. The risk of major thrombosis is higher in ET patients age more than 60 years and with previous occlusive events. The physicians must balance the prevention of bleeding and/or thrombotic complications with the risks of drug side effects and toxicity. In general, low risk and young ET patients are not treated and treatment is reserved for symptomatic ET patients who have a platelet count above or equal to $1,500x10^3/\mu l$, and a previous history of thrombosis/ hemorrhage, with a platelet lowering agent to achieve a target platelet count of $<350x10^3/\mu l$ without developing leukopenia.[26,28,39-42] The recent availability of non-mutagenic drugs such as interferon and anagrelide, gives the opportunity for long term treatment of ET patients.[26,40] Reducing the number of platelets to below $450x10^3/\mu l$ as a therapeutic target will reduce the incidence of thrombohemorrhagic problems. In addition, the physician should consider the cost effectiveness of treatment.

1. Platelet cytoreductive agents

1.1 Alkylating agents and radiophosphorus ^{32}P

Since 1970, most drugs used in the treatment of ET such as alkylating agents and radiophosphorus were carcinogenic themselves, with acute leukemia occurring in 10.3% of patients treated with ^{32}P and 31% of those treated with alkylating agents. Their use may still be warranted.[26,43]

1.2 Hydroxyurea

Hydroxyurea impairs DNA synthesis by inhibiting ribonucleotide reductase. It is currently the most common drug used initially. It is less leukemogenic (approximate 3.5-9%), with minimal side effect in the high risk elderly patient. However, prolonged use of hydroxyurea in ET patients may have to be reconsidered especially in asymptomatic cases.[1,44]

1.3 Recombinant interferon-alpha

Interferon exerts a direct antiproliferative effect on megakaryocyte. It is a non-mutagenic and non-leukemogenic agent; but its cost, need for parenteral use, side effects and toxicity have prevented its widespread use as first line therapy. It has a response rate of approximately 90%. Interferon-alpha is a useful drug for symptomatic pregnant ET patients because it does not cross the placenta.[2,45] An initial dose of 3 million units daily can rapidly decreases the platelet count within 2 months.The average dose is about 3 million units per day, with a maintenance dose of 3 million units once, twice or three times a week. A rise in the platelet count during maintenance therapy is usually caused by development of neutralizing antibody to interferon. Side effects are flu-like symptoms, fever, bone and muscle pain, fatigue, lethargy, and depression. Long-term use may result in weight loss, alopecia, autoimmune thyroiditis leading to hypothyroidism, and autoimmune hemolytic anemia. We consider that interferon-alpha is a reasonable alternative treatment for symptomatic high risk young ET patients.

1.4 Anagrelide

Anagrelide is an orally active imidazoquinazoline with anti-cyclicAMP phosphodiesterase activity which inhibits platelet aggregation. Its mechanism is unknown but it is believed that it may inhibit or interfere with megakaryocyte maturation.[46] Anagrelide has potent platelet reducing activity with little effect on white blood cell count. More than 90% of ET patients respond to anagrelide with a maintenance dose of approximately 2-2.5 mg/day.[46-48] Significant side effects of anagrelide are related to the drug's direct vasodilating and positive inotropic effects, including fluid retention which may even produce frank congestive heart failure, palpitations, tachycardia, postural hypotension, headache, dizziness, nausea, diarrhea, abdominal pain and cough, which necessitate its discontinuation in 10-16%.[43,47] Mild to moderate anemia may occur with long term use.[48] Anagrelide is efficacious and safe in ET patients. Nevertheless the mutagenic risk of anagrelide has not been investigated with long term follow up studies.[40]

2. Antiplatelet therapy

Low dose aspirin has been shown to substantially reduce raised thromboxane A2 production from platelets. Aspirin minimizes the vascular occlusive risk when combined with cytoreduction. Aspirin in a dose of 300 mg/day, reducing to a maintenance dose of 75 mg/day, is use as prophylactic therapy for prevention of thrombosis.[1,49] Antiplatelet agents such as low dose aspirin (100 mg/day) and dipyridamole are effective in the treatment of microvascular arterial complications of the extremities and prevent recurrent thrombotic episode in ET patients, especially those with cerebrovascular or coronary ischemia and common atherosclerotic risk factors.[1,29,50] Aspirin is probably not indicated in asymptomatic ET patients because of uncertain benefit in primary prevention and is contraindicated in patients with previous episodes of bleeding particularly from the GI tract, or a very high platelet count (over 1,500 $x10^3/\mu l$) which may lead to acquired von Willebrand factor deficiency.[51] In symptomatic pregnant ET patients, low dose aspirin has been used in the first and second trimesters. It has been recommended that this should be discontinued at least 1 week before delivery.[52]

3. Anticoagulants

Anticoagulants such as heparin and warfarin may be useful in some cases of symptomatic ET with a hypercoagulable state. But prophylactic therapy for post-operative deep vein thrombosis should be avoided.[43,99]

The recommendation for the treatment of ET patients is summarized in Table 1.[43]

Table 1. Relative indication and contraindication for cytoreductive and antiplatelet therapy in essential thrombocythemia.

Therapy	Asymptomatic				Symptomatic		
	Age < 60 yr. no associated cardiovasc. risk	Platelets > 2 x 10^6/ mm^3	Associated cardiovasc. risks	Age > 60 yr.	Digital or cerebrovasc. ischemia	Previous thrombosis	Previous bleeding
Cytoreduction	0	±	±	±	+	+	+
Aspirin	0	0	+	+	+	+	-

+ indicated; ± possibly indicated; 0 not indicated; - contraindicated.

4. Plateletpheresis

Plateletpheresis has a major role as an emergency procedure in ET patients with extreme thrombocythemia with severe complications due to massive bleeding or thrombosis (e.g. pulmonary emboli, CVA, acute MI, peripheral arterial occlusion of extremities with gangrene), and pregnancy. This procedure is useful, safe, and produces immediate clinical improvement due to the rapid reduction of circulating platelets by > 50%. Platelet counts usually rise after discontinuing plateletpheresis within 24-48 hours. So the treatment should be combined with platelet lowering agents.[2,54]

Chronic plateletpheresis is not the treatment of choice in ET patients. Plateletpheresis is expensive, inconvenient, and disturbs the normal life style of the patients. Therefore this procedure should be considered when other treatments fail or there is a contraindication to conventional treatment.

5. Transplantation

Hemopoietic stem cell transplantation is for advanced ET patients with advanced disease requiring curative treatment.[55]

Prognosis

The prognosis of ET patients is generally good with nearly normal life expectancy, especially in untreated asymptomatic patients.[1,2,26] In symptomatic ET patients with a relatively benign clinical course, there is still a high rate of morbidity which affects their quality of life. There is no relationship between the clinical, initial platelet count, and pathologic findings on bone marrow biopsy; and none of the histologic and clinical parameters were predictive of survival or the occurrence of major clinical events.[28,55] Survival is mainly influenced by the rate of ET related complications during follow up and blastic transformation. The three major factors associated with blastic transformation are cytogenetic abnormalities, myelofibrotic features, and the use of cytotoxic agents.[40] However, appropriate treatments have been shown to reduce complication and significantly extend the life span of patients.

SUMMARY

Essential thrombocythemia is a rare and probably under-recognized until the disease is advanced and the patients have disabilities which affect their quality of life. Physicians should be aware of the need to detection ET early, especially in symptomatic patients so that appropriate treatment to prevent recurrence of thrombohemorrhagic complication may be given.

REFERENCES

1. Levine SP. Thrombocytosis. In: Lee GR, Foerter J, Lukens J, Paraskevas F, Greer JP, Rodgers GM, eds.Wintrobe's Clinical Hematology. 10th ed. Williams & Wilkins, A Waverly Company, 1999: 1648-60.
2. Hoffman R. Primary thrombocythemia. In: Hoffman R, Benz EJ, Sanford J Jr, Furie SB, Cohen HJ, Silberstein LE, McGlave, eds. Hematology 3rd ed. Basic Principles and Practice. Churchill Livinstone, New York, 2001: 1188-204.
3. Kondo T, Okabe M, Sanada M, et al. Familial essential thrombocythemia associated with one base deletion in the 5′ untranslated region of the thrombopoietin gene. Blood 1998; **92:** 1091-96.
4. Kutti J, Ridell B. Epidemiology of the myeloproliferative disorders: essential thrombocythemia, polycythemia vera and idiopathic myelofibrosis. Pathol Biol (Paris) 2001; **49:** 164-66.
5. Mele A, Visani G, Pulsoni A, et al. Risk factors for essential thrombocythemia :A case control study. Italian Leukemia Study Group. Cancer 1996; **77:** 2157-61.
6. Harrison CN, Gale RE, Maclrin SJ, Linch DC. A large proportion of patients with a diagnosis of essential thrombocythemia do not have a clonal disorder and may be at lower risk of thrombotic complications. Blood 1999; **93:** 417-24.
7. Shih LY, Lin TL, Dunn P, et al. Clonality analysis using X- chromosome inactivation patterns by HUMARA-PCR assay in female control patients with idiopathic thrombocytosis in Taiwan. Exp Hematol 2001; **29:** 202-8.
8. Tefferi A. Recent progress in the pathogenesis and management of essential thrombocythemia. Leuk Res 2001; **25:** 369-77.
9. Tefferi A. Pathogenetic mechanisms in chronic myeloproliferative disorders: polycythemia vera, essential thrombocythemia, agonogenic myeloid metaplasia, and chronic myelogenous leukemia. Semin Hematol 1999; **36(Suppl 2):** 3-8.
10. Swolin B, Safai KS, Anghem E, Kutti J. No increased frequency of trisomies 8 and 9 by fluorescence in situ hybridization in untreated patients with essential thrombocythemia. Cancer Genet Cytogenet 2001; **126:** 56-59.
11. Yamada H, Murakami T, Kaneda T, et al. Clinical significance of major and minor bcr/abl chimeric transcripts in essential thrombocythemia. Jpn J Clin Oncol 2000; **30:** 472-77.
12. Aviram A, Blickstein D, Stark P, et al. Significance of bcr-abl transcripts in bone marrow aspirates of Philadelphia-negative essential thrombocythemia patients. Leuk Lymphoma 1999; **33:** 77-82.
13. Harrison CN, Gale RE, Wiestner AC, et al. The activating splice mutation in intron 3 of the thrombopoietin gene is not found in patients with nonfamilial essential thrombocythemia. Br J Haematol 1998; **102:** 1341-43.
14. Dror Y, Zipursky A, Blanchette VS. Essential thrombocythemia in children. J Pediatr Hematol Oncol 1999; **21:** 356-63.
15. Matsumura I, Horikawa Y, Kanakura Y. Functional roles of thrombopoietin-c-mpl system in essential thrombocythemia. Leuk Lymphoma 1999; **32:** 351-58.
16. Li J, Xia Y, Kuter DJ. The platelet thrombopoietin receptor number and function are markedly decreased in patients with essential thrombocythemia. Br J Haematol 2000; **111:** 943-53.

17. Yoon SY, Li CY, Tefferi A. Megakaryocyte c-Mpl expression in chronic myeloproliferative disorders and the myelodysplastic syndrome: immunoperoxidase staining patterns and clinical correlates. Eur J Haematol 2000; **65:** 170.
18. Mesa RA, Li CY, Yoon SY, et al. Clinical correlates of megakaryocyte c-mpl expression in essential thrombocythemia: A clinicopathologic study of 169 patients. Blood 2000; **96:** 742a.
19. Harrison CN, Gale RE, Pezella F, et al. Platelet c-mpl expression is dysregulated in patients with essential thrombocythemia but this is not diagnostic value. Br J Haematol 1999; **107:** 139.
20. Taksin AL, Couedic JPL, Dusanter FI, et al. Autonomous megakaryocyte growth in essential thrombocythemia and idiopathic myelofibrosis is not related to a c-mpl mutation or to an autocrine stimulation by Mpl-L. Blood 1999; **93:** 125-39.
21. Falanga A, Marchetti M, Evagelista V, et al. Polymorphonuclear leukocyte activation and hemostasis in patients with essential thrombocythemia and polycythemia vera. Blood 2000; **96:** 4261-66.
22. Senno SL, Pechet L. Clinical implications of elevated PAI-1 revisited: multiple arterial thrombosis in a patient with essential thrombocythemia and elevated plasminogen activator-1 (PAI-1) levels: a case report and review of the literature. J Thromb Thrombolysis 1999; **8:** 105-12.
23. Rosc D, Kremplewska-Nalezyta E, Gadomska G, et al. Plasminogen activators (t-PA and u-PA) and plasminogen activators inhibitors (PAI-1 and PAI-2) in some myeloproliferative syndromes. Med Sci Monit 2000; **6:** 684-91.
24. Cobankara V, Oran B, Ozatli D, et al. Cytokines, endothelium and adhesive molecules in pathologic thrombopoiesis. Clin Appl Thromb Hemost 2001; **7:** 126-30.
25. Vignal CV, Lourenco DM, Noguti MA, et al. Hemorrhagic and thrombotic complications in patients with myeloproliferative diseases. Rev Paul Med 1997; **115:** 1575-79.
26. Barbui T, Finazzi G. Clinical parameters for determining when and when not to treat essential thrombocythemia. Semin Hematol 1999; **36(Suppl 2):** 14-18.
27. Tefferi A, Fonseca R, Pereira DL, Hoagland HC. A long term retrospective study of young women with essential thrombocythemia. Mayo Clin Proc 2001; **76:** 22-28.
28. Lengfelder E, Hochhaus A, Kronawitter U, et al. Should a platelet limit of $600x10^9$/l be used as a diagnostic criterion in essential thrombocythemia? An analysis of the natural course including early stages. Br J Haematol 1998; **100:** 15-23.
29. Rossi C, Randi ML, Zerbinati P, et al. Acute coronary disease in essential thrombocythemia and polycythemia vera. J Intern Med 1998; **244:** 49-53.
30. Randi ML, Fabris F, Cella G, et al. Cerebral vascular accidents in young patients with essential thrombocythemia : relation with other known cardiovascular risk factors. Angiology 1998; **49:** 477-81.
31. Wright CA, Tefferi A. A single institutional experience with 43 pregnancies in essential thrombocythemia. Eur J Haematol 2001; **66:** 152-59.
32. Schmidt JJ, Neumeister P, Kainer F, et al. Treatment of essential thrombocythemia during pregnancy: antiabortive effect of interferon-alpha? Ann Hematol 1998; **77:** 291-92.
33. Michiels JJ, Kutti J, Stark P, et al. Diagnosis, pathogenesis and treatment of the myeloproliferative disorders, essential thrombocythemia, polycythemia vera, essential megakaryocytic granulocytic metaplasia and myelofibrosis. Neth J Med 1999; **54:** 46-62.
34. Carneskoj J, Wadenvik H, Fjalling M, Kutt J. Assessment of spleen size using gamma camera scintigraphy in newly diagnosed patients with essential thrombocythemia and polycythaemia vera. Eur J Haematol 1996; **56:** 158-62.
35. Rozman C, Cervantes F, Rozman M, et al. Magnetic resonance imaging in myelofibrosis and essential thrombocythemia: contribution to differential diagnosis. Br J Haematol 1999; **104:** 574-80.
36. Thiele J, Kvasnicka HM. Clinicopathology and histochemistry on bone marrow biopsies in chronic myeloproliferative disorders-a clinical diagnosis and classification. Patho Biol 2001; **49:** 140-47.
37. Baatout S, Chatelain B, Staquet P, et al. endomitotic index of megakaryocytes measured by flow cytometry helps to diagnose hematological disorders with abnormal platelet counts. Anticancer Res 1998; **18:** 3347-53.
38. Barbui T, Finazzi G, Dupuy E, et al. Treatment strategies in essential thrombocythemia. A critical appraisal of various experiences in different centers. Leuk Lymphoma 1996; **22(Suppl 1):** 149-60.
39. Ruggeri M, Finazzi G, Tosetto A, et al. No treatment for low-risk thrombocythaemia: results from a prospective study. Br J Haematol 1998; **103:** 772-77.
40. Finazzi G, Barbui T. Treatment of essential thrombocythemia with special emphasis on leukemogenic risk. Ann Hematol 1999; **78:** 389-92.
41. Besses F, Cervantes F, Pereira A, et al. Major vascular complications in essential thrombocythemia: a study of the predictive factors in a series of 148 patients. Leukemia 1999; **13:** 150-54.
42. Briere J, Guilmin F. Management of patients with essential thrombocythemia:current concepts and perspectives. Pathol Biol 2001; **49:** 178-183.

43. Schafer AI. Management of thrombocythemia. In: Stossel TP, ed. Current review of hematology. Philadelphia:Rapid Science Publishers,1997: 341-46.
44. Sterkers Y, Preudhomme C, Lai JL, et al. Acute myeloid leukemia and myelodysplastic syndromes following essential thrombocythemia treated with hydroxyurea: high proportion of cases with 17p deletion. Blood 1998; **91:** 616-22.
45. Delage R, Demers C, Cantin G, Roy J. Treatment of essential thrombocythemia during pregnancy with interferon-alpha. Obstet Gynecol 1996; **87:** 814-17.
46. Tefferi A, Silverstein MN, Petitt RM, et al. Anagrelide as a new platelet-lowering agent in essential thrombocythemia: mechanism of action, efficacy, toxicity, current indications. Semin Thromb Hemost 1997; **23:** 379-83.
47. Petrides PE, Beykirch MK, Trapp OM. Anagrelide, a novel platelet lowering option in essential thrombocythemia:treatment experience in 48 patients in Germany. Eur J Haematol 1998; **61:** 71-76.
48. Storen EC, Tefferi A. Long-term use of anagrelide in young patients with essential thrombocythemia. Blood 2001; **97:** 863-66.
49. Willoughby S, Pearson TC. The use of aspirin in polycythaemia vera and primary thrombocythaemia. Blood Rev 1998; **12:**12-22.
50. Micheils JJ. Aspirin and platelet-lowering agents for the prevention of vascular complications in essential thrombocythemia. Clin Appl Thromb Hemost 1999; **5:** 247-51.
51. Cortelazzo S, Marchetti M, Orlando E, et al. Aspirin increases the bleeding side effects in essential thrombocythemia independent of the cyclooxygenase pathway: role of the lipoxygenase pathway. Am J Hematol 1998; **57:** 277-82.
52. Pineo GF, Blahey WB. Essential thrombocythemia and complications of pregnancy. Am J Hematol 1991; **36:** 221.
53. Ohto T, Shihara H, Miyauchi Y, Nakajima N. A case of coronary artery bypass surgery using left internal thoracic artery and right gastroepiploic artery for a patient with essential thrombocythemia. Nippon Kyobu Geka Gakkai Zasshi. 1998; **46:** 767-71.
54. Schiffer CA.Therapeutic cytopheresis. In : Wiernik PH, Canellos GP, Kyle RA, Schiffer CA, eds. Neoplastic diseases of the blood.New York:Churchill Livingstone Inc,1985: 1006-7.
55. Annaloro C, Lambertenghi Deliliers G, Oriani A, Pozzoli E. Prognostic significance of bone marrow biopsy in essential thrombocythemia. Haematologica 1999; **84:** 17-21.