Prognostic factors for thrombosis, myelofibrosis, and leukemia in essential thrombocythemia: a study of 605 patients

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ABSTRACT

Background
Essential thrombocythemia is a chronic myeloproliferative disorder; patients with this disorder have a propensity to develop thrombosis, myelofibrosis, and leukemia.

Design and Methods
We studied 605 patients with essential thrombocythemia (follow-up 4596 person-years) with the aim of defining prognostic factors for thrombosis, myelofibrosis, and leukemia during follow-up.

Results
Sixty-six patients (11%) developed thrombosis with a 10-year risk of 14%. Age >60 years (p<0.001) and a history of thrombosis (p=0.03) were independent risk factors for thrombosis. Progression to myelofibrosis occurred in 17 patients (2.8%) with a 10-year risk of 3.9%. Anemia at diagnosis of essential thrombocythemia was significantly correlated (p<0.001) with progression to myelofibrosis. Leukemia occurred in 14 patients (2.3%) at a median time of 11 years after the diagnosis of essential thrombocythemia; the risk was 2.6% at 10 years. Age >60 years (p=0.02) was significantly correlated with the development of leukemia. Cytotoxic treatment did not imply a higher risk of leukemia. At the time of the analysis, 64 of the 605 patients (10.6%) had died. The 10-year probability of survival was 88%, with a median survival of 22.3 years. Age >60 years (p<0.001) and history of thrombosis (p=0.001) were independent risk factors for survival.

Conclusions
The findings from this study on a large series of patients treated according to current clinical practice provide reassurance that essential thrombocythemia is an indolent disorder and affected patients have a long survival. The main risk is thrombosis, while myelofibrosis and leukemia are rare and late complications.

Key words: thrombocythemia, polycythemia, myelofibrosis, leukemia, prognosis.

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Introduction

Essential thrombocythemia (ET) is a chronic myeloproliferative disorder.[1] The initial phase of the disease is characterized by thrombocytosis and a high risk of vascular complications.[2] In the long term, patients with ET may develop myelofibrosis (post-ET myelofibrosis), or leukemia.[3] Post-ET myelofibrosis is considered a *bona fide* expected evolution of the disease, while the occurrence of leukemia might be related to cytotoxic therapy. The use of more than one cytotoxic agent in ET has been implicated in the occurrence of leukemia,[4] while the use of hydroxyurea alone or pipobroman alone seems not to be correlated with leukemic transformation.[3-5] As regards to survival, the life-expectancy of patients with ET was found to be not different from that of the general population.[3,6]

The identification of the *JAK2* (V617F) mutation offers new insights into the pathogenesis of chronic myeloproliferative disorders.[7] About half the patients with ET carry this mutation. Those patients with ET who are *JAK2* (V617F)-positive usually have higher hemoglobin levels[8-10] and female patients have a higher risk of pregnancy complications.[11] The mutation burden is generally low (median value 10%), meaning that hematopoiesis is sustained by wild type cells or cells heterozygous for the mutation.[12-14] Patients with *JAK2* (V617F)-positive ET may develop leukemia with *JAK2* (V617F)-negative leukemic blast cells.[15,16]

We studied a series of 605 ET patients consecutively diagnosed and followed at our institution. The aim was to identify prognostic factors for thrombosis, myelofibrosis, and leukemia.

Design and Methods

Patients

This study included 605 patients with ET. The diagnosis of ET was made in accordance with the criteria in use at the time of first observation.[1,17-19] Patients were followed from 1975 to 2008 at the Division of Hematology of the Fondazione Policlinico San Matteo, University of Pavia, Italy. In the present analysis we considered as thrombotic events: ischemic stroke, cerebral transient ischemic attack, acute myocardial infarction, peripheral arterial thrombosis, and venous thromboembolism including deep venous thrombosis of the extremities or of abdominal veins, and pulmonary embolism. The diagnosis of post-ET myelofibrosis was made on the basis of the International Working Group on Myelofibrosis Research and Treatment (IWG-MRT) criteria.[20] The diagnosis of acute leukemia was made according to World Heath Organization (WHO) criteria, using a 20% blast threshold for the diagnosis.[21] The morphological classification of blast cells was made according to French-American-British (FAB) criteria.[22] This study was approved by the institutional ethics committee of Pavia, and the procedures followed were in accordance with the Helsinki Declaration of 1975, as revised in 2000.

Statistical analysis

The Kaplan-Meier product-limit method was used to estimate univariate survival curves, and the log-rank test was adopted to compare the survival curves. Cox proportional hazards regression was used to carry out multivariate survival analyses. The following parameters (available for all patients) at diagnosis of ET were taken into consideration to find prognostic risk factors for thrombosis, myelofibrosis, leukemia, and survival in univariate and multivariate models: age, sex, platelet count, hemoglobin level, white blood cell count, spleen and liver size (considered as continuous numerical variables), age over 60 years, thrombocytosis (platelet count over 1000×10^9/L and over 1500×10^9/L), leukocytosis (white blood cell count over 8.7×10^9/L, 10×10^9/L and over 15×10^9/L), anemia (hemoglobin below 12.5 g/dL for females and below 13.5 g/dL for males), and history of thrombosis (before and at diagnosis). In addition, for the univariate analyses we considered cardiovascular risk factors, the presence of arterial hypertension, diabetes, smoking, and hypercholesterolemia (data available for 337 patients), lactate dehydrogenase concentration (data available for 279 patients), and $CD34^+$ cell count[14] (data available for 201 patients). Left censoring (delayed entry) techniques were adopted to allow for time-to-treatment when investigating risk of events from the time of starting treatment with cytotoxic agents. The effect of starting a cytotoxic treatment was investigated by defining treatment as a time-dependent co-variate. All analyses were performed using Statistica 7.1 (Statsoft Inc, Tulsa, OK, USA), Stata 9 (StataCorp LP, College Station, TX, USA) software, and Microsoft Excel.

Results

Disease information prior to diagnosis and at diagnosis of essential thrombocythemia

To investigate whether a latent phase exists before the diagnosis of ET, we asked 212 consecutive patients to retrieve blood cell counts if performed before diagnosis. Data on platelet count were obtained for 115 (54%) of these 212 patients. A platelet count exceeding 400×10^9/L was detected in 103 patients (89%). The time elapsed between this evaluation and the diagnosis of ET ranged from 6 to 183 months (median, 31 months).

The clinical features at diagnosis of ET are summarized in Table 1. Data from cytogenetic analyses were available for 220 patients (>20 metaphases): seven had an abnormal karyotype and did not develop hematologic malignancies. The median follow-up of the whole cohort was 5.6 years (range, 0-26 years) with a total follow-up of 4596 person-years. Treatment depended on the physicians' clinical judgment (Table 1). The Mann-Whitney U test showed that patients treated with antiplatelet agents alone had a significantly shorter follow-up than those treated with cytotoxic agents ($p<0.001$). Among patients receiving cytotoxic agents, the follow-up of those treated with hydroxyurea alone was significantly shorter than that of those

Table 1. Summary of demographic and hematologic characteristics at diagnosis and of treatments in 605 patients with essential thrombocythemia.

Characteristic	
N. of patients	605
Age at diagnosis, years, median (range)	50 (16-90)
Sex (male/female)	224/381
History of thrombosis (before and at diagnosis) (%)	90 (14.8)
Leukocyte count,×10^9/L, median (range)	8.9 (4.2-22.1)
Leukocyte count ≥10×10^9/L (%)	218 (36)
Leukocyte count ≥15×10^9/L (%)	34 (5.6)
Hemoglobin level, g/dL, median (range)	14.2 (10.1-17)
Anemia (Hb <12.5 g/dL in females, <13.5 g/dL in males) (%)	50 (8.2)
Platelet count, ×10^9/L, median (range)	811 (490-3600)
Platelet count ≥1000×10^9/L (%)	169 (27.9)
Platelet count ≥1500×10^9/L (%)	42 (7)
Splenomegaly (%)	65 (10.7)
Hepatomegaly (%)	78 (12.8)
LDH, mU/mL, median (range)	386 (60-1860)
Circulating CD34-positive cells /μL	3.8 (0-13.1)
Cytotoxic agents, n. of pts (%);	406 (67);
median follow-up, years (range)	7.6 (0-26)
hydroxyurea alone, n. of pts (%);	147 (36);
median follow-up, years (range)	4.6 (0-25)
PB alone, n. of pts (%);	154 (38);
median follow-up, years (range)	8.6 (0.6-25)
More than one 1 agent, n. of pts (%);	105 (26);
median follow-up, years (range)	12.4 (0.2-26)
Antiplatelet drug only, n. of pts (%);	199 (33);
median follow-up, years (range)	2.8 (0-21)

LDH: lactate dehydrogenase; PB: pipobroman; HU: hydroxyurea; pts: patients.

Table 2. Thrombosis, myelofibrosis and leukemia during follow-up in 605 patients with essential thrombocythemia.

Events	*Incidence, x 1000 person-years*	*10-year risk*	*Risk factors*
Thrombosis	15.3	14%	Age >60 years, prior thrombosis
HU-treated	17.1		
PB-treated	21.5		
Treated with >1 agent	13.1		
Myelofibrosis	3.7	3.9%	Anemia
HU-treated	2.3		
PB-treated	1.9		
Treated with >1 agent	8.8		
Leukemia	3	2.6%	Age >60 years
HU-treated	5.7		
PB-treated	2.6		
Treated with >1 agent	3.9		

PB: pipobroman; HU: hydroxyurea.

treated with pipobroman alone (p<0.001) or with more than one cytotoxic agent (p<0.001).

Prognostic risk factors for thrombosis

During follow-up, 66 (11%) patients developed thrombosis including: ischemic stroke (n=17, 26%), transient ischemic attacks (n=18, 27%), acute myocardial infarction (n=12, 18%), peripheral arterial thrombosis (n=4, 6%) and deep vein thrombosis of the extremities (n=9, 14%) and of abdominal veins (n=6, 9%). The cumulative risk of thrombosis during follow-up was 5% at 5 years, and 14% at 10 years (Figure 1; Table 2). Univariate survival analysis showed that age (p=0.001), age over 60 years (p<0.001), and platelet count under 1000×10^9/L (p= 0.04) were significant risk factors for thrombosis. Leukocytosis *per se* was not a risk factor for thrombosis during follow-up or at diagnosis. In a multivariate Cox proportional hazard regression analysis, age over 60 years (p<0.001) and a history of thrombosis (p=0.03) at diagnosis retained a significant impact on thrombosis during follow-up. When adjusting for exposure to cytotoxic agents as a time-dependent co-variate in a multivariate Cox proportional hazard regression analysis, the impact of age and history of thrombosis on thrombosis was not modified. It is of interest that treatment with cytotoxic agents had a protective effect on the risk of thrombosis (p=0.03).

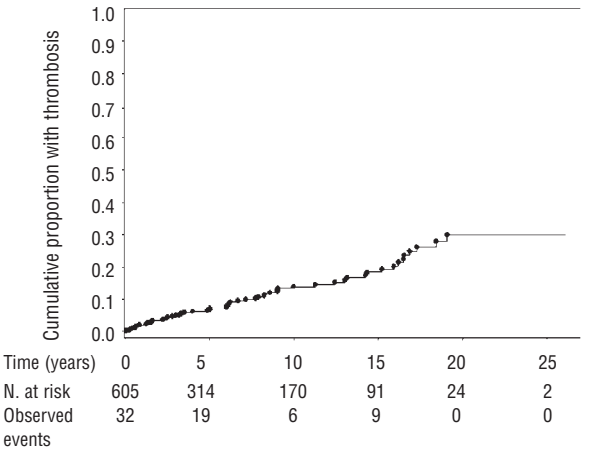

Figure 1. Risk of thrombosis in 605 patients with essential thrombocythemia. At the bottom: observation time, number of patients at risk in each 5-year time band, and number of thromboses observed within each period.

Prognostic risk factors for post-essential thrombocythemia myelofibrosis and leukemia

Disease duration was significantly longer in patients who progressed to develop post-ET myelofibrosis (p<0.001) and leukemia (p<0.001) than in those who did not. A progression to myelofibrosis occurred in 17 (2.8%) of the 605 patients at a median follow-up of 9.1 years (range, 3.7-18.7) after the diagnosis of ET. The cumulative risk of post-ET myelofibrosis increased over time and was 0.3% at 5 years and 3.9% at 10 years (Figure 2; Table 2). The patient who developed post-ET myelofibrosis after 3.7 years had had a splenectomy 4.3 years before the diagnosis of ET with a pre-surgery platelet count of 510×10^9/L. Univariate Kaplan-Meier survival analysis showed that anemia was a significant risk factor for post-ET myelofibrosis (p<0.001). Four (8%) of the 50 patients with anemia had grade 1 myelofibrosis[23] at diagnosis: one developed post-ET myelofibrosis 18 years later.

Leukemia occurred in 14 (2.3%) of 605 patients at a

median time of 11 years (range 6.6-25 years) after the diagnosis of ET. The cumulative risk of leukemic transformation was 2.6% at 10 years and 5.3% at 15 years (Figure 3). Leukemia was of myeloid origin in all cases (12 M0-M1 and two M7). Concerning risk factors for leukemia at diagnosis of ET, univariate Kaplan-Meier survival analysis showed that age (p=0.001) and age over 60 years (p=0.02) were risk factors for leukemic transformation. Of the 14 patients with leukemia, five had received hydroxyurea alone, four pipobroman alone, and five more than one cytotoxic agent. A univariate Kaplan Meier survival model demonstrated that patients treated with cytotoxic agents did not have a significantly different risk of leukemic transformation than that of patients treated with antiplatelet agents alone. To investigate the potential role of the different cytotoxic agents on leukemic transformation, we restricted the analysis to patients who received cytotoxic agents. Hence, we built a Cox regression model accounting for left censoring of the time elapsed between the diagnosis of ET and the start of cytotoxic therapy, using the type of agent and duration of treatment as co-variates. We did not find significant differences in leukemic transformation between patients treated with hydroxyurea or pipobroman when adjusting for time to treatment and treatment duration.

Prognostic risk factors for survival

At the time of the analysis, 64 (10.6%) patients had died. The rate of patients lost to follow-up was 4.5%. The probability of survival was 97% at 5 years, 88% at 10 years and 79% at 15 years, with a median survival of 22.3 years. Univariate Kaplan-Meier survival analysis showed that male sex (p=0.014), age over 60 years (p<0.001), anemia (p<0.01), lactate-dehydrogenase concentration above the upper limit of normal (p=0.01), a history of thrombosis (p=0.023), and age (p<0.001) were risk factors for survival. In a multivariate Cox proportional hazard regression analysis, only age over 60 years (p<0.001) and a history of thrombosis (p=0.001) retained a significant effect on survival. This result was not modified when adjusting for exposure to cytotoxic agents as a time-dependent covariate.

Discussion

We analyzed a series of 605 consecutive patients with ET treated according to current clinical practice. The aim of the study was to define prognostic factors for thrombosis, myelofibrosis, and leukemia.

To investigate whether a latent phase of thrombocytosis exists before the diagnosis of ET, we evaluated 115 patients for whom blood cell counts before diagnosis were available. Of these, 89% had platelet counts exceeding normal values, providing evidence of a latent phase pre-diagnosis lasting a median of 2.5 years. At diagnosis, leukocytosis was infrequent (5.6% had leukocyte counts over 15×10^9/L), as was anemia (8.4% had hemoglobin levels below normal, and none

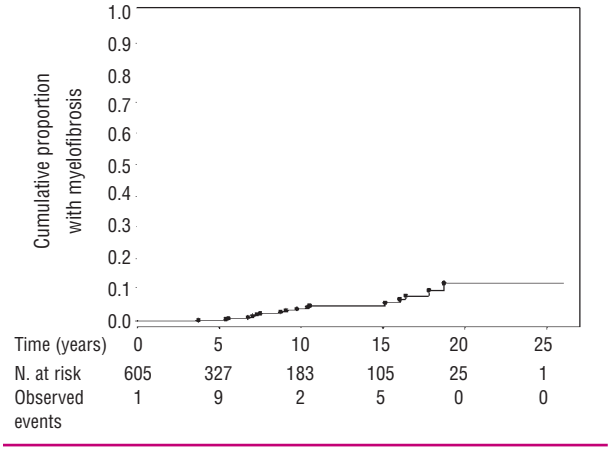

Figure 2. Risk of myelofibrosis in 605 patients with essential thrombocythemia. At the bottom: observation time, number of patients at risk in each 5-year time band, and number of cases of progression to myelofibrosis observed within each period.

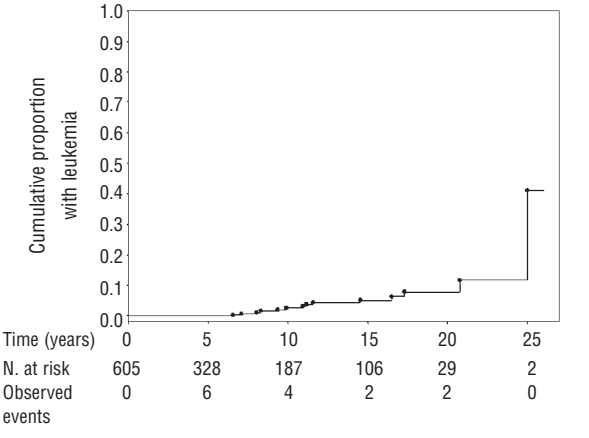

Figure 3. Risk of leukemia in 605 patients with essential thrombocythemia. At the bottom: observation time, number of patients at risk in each 5-year time band, and number of cases of leukemic transformation observed within each period.

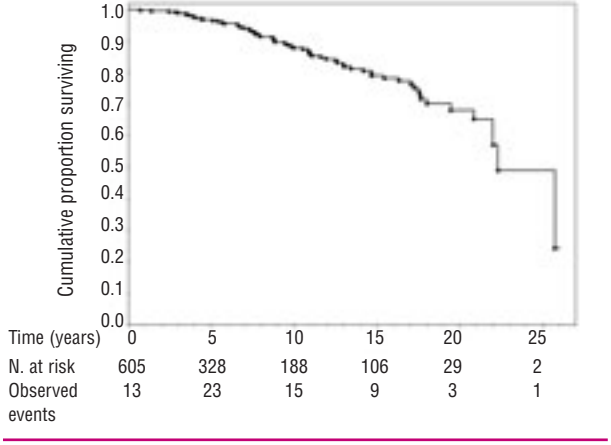

Figure 4. Survival of 605 patients with essential thrombocythemia. At the bottom: observation time, number of patients at risk in each 5-year time band, number of deaths observed within each period.

below 11.5 g/dL). Only 10% had an increased spleen size. Circulating CD34+ cell counts were within the normal range in all patients. Concerning vascular complications during follow-up, we found that patients over 60 years old or those with a history of thrombosis had a significantly higher risk of thrombotic complications. This is in keeping with prior reports.[24-26] In our series, the presence of cardiovascular risk factors such as arterial hypertension, diabetes, smoking, and hypercholesterolemia, did not increase the risk of thrombosis. Many studies have evaluated these risk factors with conflicting results.[5,24-30] Retrospective studies are unlikely to provide a definitive conclusion on this topic, probably because the risk may be reduced by specific treatments or life-style modification. In fact, appropriate management of these reversible risk factors is an integral part of treatment of ET.[2] Recently, leukocytosis at diagnosis has been reported as an emerging risk factor for thrombosis in ET.[24,31,32] We did not find a correlation between leukocytosis and thrombosis during follow-up. This is in keeping with the results of Tefferi's study.[33] The relationship between leukocyte count at diagnosis and subsequent thrombosis is confounded by treatment effects on both variables. As aspirin may, *per se*, reduce leukocyte-platelet interactions in ET[34] and was shown to control thrombosis in polycythemia vera,[35] this treatment may reduce thrombosis in ET. All the patients in this study received antiplatelet therapy, unless contraindicated.

Criteria for the diagnosis of post-ET myelofibrosis, combining clinical and histopathological parameters, have been defined recently.[20] We found that post-ET myelofibrosis occurs rarely, with a risk of 6% at 15 years. This complication is much less frequent in ET than in patients with polycythemia vera[36] and it is a late event during the course of the disease.[4,6,37-39] We identified anemia as the only risk factor for post-ET myelofibrosis, which agrees with results of a prior study.[6] We did not find a correlation between the use of cytotoxic agents and post-ET myelofibrosis, suggesting that myelofibrosis, although rare, is a natural evolution of ET.[37]

The cumulative risk of leukemia was 2.6% at 10 years and 5.3% at 15 years. Studying parameters at diagnosis of ET as risk factors for leukemic transformation, Gangat *et al*.[5] defined a prognostic model that includes anemia and thrombocytosis as risk factors. Prognostication was performed on 20 patients with leukemic transformation. In our series, we found that age over 60 years old was the single risk factor for leukemic transformation. Advanced age has also been reported as a risk factor for leukemic transformation in patients with polycythemia vera.[40] Concerning the potential role of cytotoxic agents in the development of leukemia, prior studies reported that hydroxy-urea may be leukemogenic,[19,41] and that the use of more than one cytotoxic agent may increase the risk of leukemic transformation.[4,42] However, leukemia may develop in patients who have never received cytotoxic drugs,[43] and large studies did not show that cytotoxic agents were involved in leukemic transformation.[3-5] Our results indicate that the risk of leukemia increases over time and that patients treated with cytotoxic agents do not have a significantly different risk of leukemic transformation compared to that of patients receiving antiplatelet agents alone. Furthermore, we did not find significant differences in the rates of leukemic transformation between patients treated with hydroyurea and those treated with pipobroman, also when a Cox regression model accounting for left censoring of the time elapsed between diagnosis of ET and start of cytotoxic therapy was applied.

Concerning survival, this study shows that ET is a very chronic disorder with affected patients having a median survival exceeding 22 years. In addition, the life-expectancy of patients with ET does not differ from that of the general population.[3,44] By multivariate analysis, we found that age over 60 years old and a history of thrombosis at diagnosis significantly affect survival. The impact of age[5,24,30,45,46] as well as thrombosis[3,5,47] on survival has been reported in other studies.

In conclusion, the findings of this study on a large series of patients treated according to current clinical practice provide reassurance that ET is an indolent disorder and affected patients live for a long time. The main risk is thrombosis, while myelofibrosis and leukemia are rare and late complications. Age over 60 years old and a history of thrombosis are risk factors for thrombotic complications. Anemia at diagnosis is a risk factor for myelofibrosis, while age is a risk factor for leukemia.

Authorship and Disclosures

FP conceived the study, collected, analyzed, and interpreted the data, and wrote the paper; ER collected, analyzed and interpreted data, and gave final approval; LA collected data, and gave final approval; EB performed bone marrow evaluation, and gave final approval; CE collected data, and gave final approval; DP performed molecular studies, and gave final approval; SB performed molecular studies, and gave final approval; CA collected data, and gave final approval; MV collected data, and gave final approval; EB collected data, and gave final approval; CP performed statistical analysis, and gave final approval; ML conceived the study, collected data, wrote the paper, and gave final approval. The authors reported no potential conflicts of interest.

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