Recurrent thrombosis in patients with polycythemia vera and essential thrombocythemia: incidence, risk factors, and effect of treatments

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ABSTRACT

Background
Prior thrombosis is a well-established risk factor for re-thrombosis in polycythemia vera and essential thrombocythemia but scarce data are available on the rate of re-thrombosis and the optimal strategy for prevention of recurrence.

Design and Methods
We retrospectively estimated the rate of recurrence in a multicenter cohort of 494 patients (polycythemia vera/essential thrombocythemia 235/259) with previous arterial (67.6%) or venous thrombosis (31%) or both (1.4%). First thrombosis was cerebrovascular disease in 191 cases, acute coronary syndrome in 106, peripheral arterial thrombosis in 44, and venous thromboembolism in 160. Microcirculatory events were not computed.

Results
Thrombosis recurred in 166 patients (33.6%), with an incidence of 7.6% patient-years. Sex, diagnosis (polycythemia vera or essential thrombocythemia), and presence of vascular risk factors did not predict recurrence, whereas age >60 years did (multivariable hazard ratio [HR], 1.67; 95% confidence interval [CI] 1.19-2.32). Increased leukocyte count at the time of the first thrombosis was a risk factor for recurrence in patients <60 years old (HR 3.55; 95% CI 1.02-12.25). Cytoreduction halved the risk in the overall cohort (HR 0.53; 95% CI 0.38-0.73) and the combination with antiplatelet agents or oral anticoagulants was more effective than administration of single drugs. Significant prevention of rethrombosis was independently achieved in patients with venous thromboembolism by both oral anticoagulants (HR 0.32; 95% CI 0.15-0.64) and antiplatelet agents (HR 0.42; 95% CI 0.22-0.77), in those with acute coronary syndrome by cytoreduction (HR 0.30; 95% CI 0.13-0.68), and in those with cerebrovascular disease by antiplatelet agents (HR 0.33; 95% CI 0.16-0.66). The overall incidence of major bleeding was 0.9% patient-years and rose to 2.8% in patients receiving both antiplatelet and anti-vitamin K agents.

Conclusions
In patients with polycythemia vera and essential thrombocythemia, cytoreduction protects against recurrent thrombosis, particularly after acute coronary syndrome. The contemporary use of oral anticoagulants (after venous thromboembolism) or antiplatelet agents (after cerebrovascular disease or venous thromboembolism) further improves the protective effect. Such findings call for prospective studies aimed at investigating whether strategies tailored according to the type of first thrombosis could improve prevention of recurrences.

Key words: polycythemia vera, essential thrombocythemia, recurrent thrombosis, cytoreductive treatment, antiplatelet treatment, oral anticoagulant treatment.

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The online version of this article contains a supplemental appendix.

Introduction

Polycythemia vera and essential thrombocythemia are typically complicated by thrombosis, with a rate of major thrombosis as high as about 50%.[1-7] Advanced age and a prior history of thrombosis are the two most important risk factors for vascular complications; hypercholesterolemia, hypertension, smoking, and diabetes have been recognized as predictors of thrombosis by some studies.[1-7] More recently, leukocytosis has been reported as an independent risk factor for thrombosis in both polycythemia vera and essential thrombocythemia.[7-9] The additional value of the *JAK2* V617F mutation as a risk factor for thrombosis is currently being investigated.[10,11]

The incidence of recurrence after a first thrombotic event is difficult to establish, since the published cohorts of patients are composed of both asymptomatic individuals and patients who have had a previous thrombosis.[1-7] In a large prospective cohort of 1,638 patients with polycythemia vera the incidence of recurrent thrombosis was 5% patient-years among individuals <65 years and 10.9% patient-years among those >65 years old.[3] No data from large cohorts of patients with essential thrombocythemia are available. In one study the incidence of re-thrombosis was 31.4% patient-years;[4] in another series of 85 patients with essential thrombocythemia the probability of recurrent thrombosis was 42.6% at 6 years.[5] Very few controlled data are available on factors that influence the rate of recurrent thrombosis. In a retrospective study of 304 patients with polycythemia vera and essential thrombocythemia, the rate of recurrent venous thrombosis was 15%, all cases having occurred in the absence of anticoagulation.[12] In one report it was claimed that aspirin alone or cytoreduction were equally effective in preventing recurrence in 60 patients with essential thrombocythemia and previous thrombosis.[13] In very small retrospective investigations, oral anticoagulant treatment failed to prevent recurrent arterial or venous thrombosis in between 25% and 40% of cases.[14,15] In conclusion, no firm recommendations concerning optimal antithrombotic prophylaxis after a first arterial or venous thrombotic event are available.[16-18]

We conducted a multicenter retrospective cohort study aimed at investigating the rate of recurrent thrombosis among patients with polycythemia vera and essential thrombocythemia, the risk factors for recurrence, and the efficacy and safety of different antithrombotic and/or cytoreductive strategies in preventing re-thrombosis.

Design and Methods

Study patients

In the frame of the hematology centers belonging to GIMEMA (*Gruppo Italiano Malattie Ematologiche dell'Adulto*) a retrospective study was conducted among patients with a diagnosis of polycythemia vera or essential thrombocythemia according to either the Polycythemia Vera Study Group or World Health Organization criteria[19-21] and a previous history of major thrombosis. The participating centers were asked to fill in a form concerning their patients with polycythemia vera and essential thrombocythemia consecutively diagnosed from January 1985 to December 2005 who had suffered from at least one major thrombotic event related to the hematologic disease (index event). Patients who died or who were lost to follow-up also had to be recruited; the date and cause of death or the date of the last visit to the center were recorded for these cases.

The thrombotic events had to be recorded only if they occurred after referral to the specialized hematology centers or in the 2 years preceding the diagnosis, according to the knowledge that 75% of thromboses heralding polycythemia vera happen in this interval of time.[2] The latter patients were, however, labeled as having had thrombosis as the inaugural manifestation of their disease. Events having occurred more than 2 years prior to the diagnosis of the hematologic disease were labeled as remote thrombosis. Other information required concerned the type of index vascular event, the concomitant risk factors for thrombosis, the treatment (both cytoreductive and antithrombotic) given after the index thrombosis and its duration, and the occurrence of recurrent major thrombosis or major bleeding after the index thrombotic event. Vascular risk factors included hypercholesterolemia, hypertension, smoking, diabetes, and chronic atrial fibrillation. Information concerning thrombophilia was also required: the thrombophilic alterations recorded were deficiencies of antithrombin, protein C, and protein S, and the presence of factor V Leiden, prothrombin G20210A, fasting hyperhomocysteinemia, lupus anticoagulant, and antiphospholipid antibodies.[17]

Definition of the events

The major thrombotic events of interest were ischemic stroke, transient ischemic attacks, acute myocardial infarction, unstable angina pectoris, peripheral arterial thrombosis, retinal artery or vein occlusion, deep venous thrombosis (including thrombosis of cerebral and splanchnic veins) and pulmonary embolism. Acute coronary syndrome encompassed acute myocardial infarction as well as unstable angina pectoris. Cerebrovascular disease encompassed ischemic stroke as well as transient ischemic attacks. Splanchnic venous thrombosis included occlusion of hepatic, portal, mesenteric, and splenic veins. The diagnosis of first or recurrent major thrombotic event was accepted only if objectively proven according to previously published criteria[22] or in the presence of unequivocal medical documentation recording the occurrence of angina or a transient ischemic attack. Superficial vein thromboses, diagnosed by objective ultrasound methods, were also analyzed. Fatal events, documented by autopsy, were included in the analysis of outcome

events. Microcirculatory events (including vascular headaches, dizziness, visual disturbances, burning pain sensation in the palms of the hands and soles of the feet, distal paresthesia and acrocyanosis) were not analyzed as events of interest. Bleeding was defined according to Palareti *et al.*[23] Bleeding was defined as major when it was intracranial, ocular (with blindness), articular, or retroperitoneal; if surgery or angiographic intervention was required to stop the bleeding; and if the bleeding led to a reduction of 2 g/dL or more in the hemoglobin concentration and/or necessitated transfusion of two or more blood units.

Methods

Differences in proportions were estimated by the Fisher's exact test (statistical significance defined as $p<0.05$). The relative risk with 95% confidence interval (CI) was calculated using a 2x2 contigency table.

The interval from the index thrombosis to a recurrence (uncensored observations) or to death or the last visit to the center (censored observations) was analyzed in order to estimate the probability of recurrence as a function of time, according to the method of Kaplan and Meier. The probability of recurrence was compared between groups by the log-rank test (statistical significance for $p<0.05$) and the relative risk of recurrence was estimated as a hazard ratio (HR) with the use of a Cox proportional hazards regression model. The HR was adjusted taking recurrence as the dependent variable and with covariates being gender, diagnosis (polycythemia vera or essential thrombocythemia), age at the time of the index thrombotic event (less than or more than 60 years old), presence of one or more vascular risk factors, history of remote thrombosis, type of index thrombosis (arterial or venous), and type of treatment after thrombosis: antithrombotic prophylaxis with antiplatelet agents or long-term oral anticoagulation, any type of pharmacological cytoreductive treatment, phlebotomy. Statistical analyses were performed using GraphPad PRISM 3.0 software (GraphPad Software, Inc., San Diego, CA, USA) for univariable methods and GB-STAT V6.5 (Dynamic Mycrosystems, Silver Spring, MD, USA) for multivariable methods.

Results

Study patients

The initial cohort consisted of 3,139 patients with polycythemia vera (n=1,151) or essential thrombocythemia (n=1,988); 494 of them (235 with polycythemia vera and 259 with essential thrombocythemia) had a history of thrombosis, with the rate being 20.4% in patients with polycythemia vera and 13.0% in those with essential thrombocythemia. The baseline clinical features of the patients with thrombosis are shown in Table 1. A detailed description of the patients stratified according to diagnosis and of the index thrombotic events is reported in *Online Supplementary Tables S1* and *S2*. Male patients were more represented in polycythemia vera than in essential thrombocythemia. As regards the other characteristics, the two groups were similar in age and in the proportion of patients having one or more vascular risk factors or a history of remote thrombosis (*Online Supplementary Table S1*). The prevalence of vascular risk factors in the investigational cohort was roughly similar to that in overall Italian patients with ischemic stroke or myocardial infarction as regards smoking habit, hypertension, and hypercholesterolemia, whereas the prevalence of diabetes or atrial fibrillation was about half that in the general surveys.[24-26] Inherited or acquired thrombophilia was investigated in only 142 patients, being present in 29.5% of them with the positive results balanced between patients with polycythemia vera or essential thrombocythemia. Mild hyperhomocysteinemia was found in eight patients, antiphosholipid antibodies in six, inherited thrombophilia in 20, and a combination of inherited thrombophilia and antiphospholid antibodies or hyperhomocysteinemia in eight. Thrombosis was the inaugural manifestation in 56.8% of the patients, with no difference between those with polycythemia vera or essential thrombocythemia, and occurred in the remaining patients at a median interval after diagnosis of 4 years (range, 0.5–20).

About two thirds of index thromboses were arterial, being acute coronary syndrome in 21.4% of the patients and cerebrovascular disease in 38.6%. The first thrombotic event was venous in 32.3% of the patients, in 8.5% of them involving cerebral or abdominal vessels. No difference in any rate of first clinical manifestations was noticed between patients with polycythemia vera or essential thrombocythemia, except for a higher rate of venous thrombosis of the limbs in patients with polycythemia vera (*Online Supplementary Table S2*).

Treatment after the index thrombotic event

After thrombosis the large majority of patients received antiplatelet agents and/or cytoreduction (Table 1). Three hundred and sixty-two patients (73.2%) received antiplatelet agents: in almost all of them (334, 92.3%) aspirin was given, associated with another antiplatelet agent or with oral anticoagulants in 17 and 11 cases, respectively. Among the patients receiving indefinite treatment with antiplatelet agents, 21 had been given previous short-term treatment with oral anticoagulants (<1 year) and 33 were administered low-molecular weight heparin for a period no longer than 3 months. Ninety patients (18.2%) were prescribed long-term treatment (>1 year) with anti-vitamin K agents. Out of the 351 patients receiving pharmacological cytoreduction, 271 (77.2%) were prescribed hydroxyurea. Other cytoreductive agents used were pipobroman (7.4%), busulphan (3.9%), interferon (3.2%), and anagrelide (3.2%); 18 patients (5.1%) received hydroxyurea or pipobroman or

busulphan during different periods. Cytoreduction was not prescribed for the remaining patients because in those with polycythemia vera phlebotomy was considered by the care physicians to be effective in controlling the hematocrit value or because in those with essential thrombocythemia the platelet count was lower than 1000×10^9/L. However, also 74% of the patients starting cytoreduction (60% of those with polycythemia vera and 86% of those with essential thrombocythemia) had a hematocrit < 0.50 or a platelet count < 1000×10^9/L and were prescribed antiproliferative drugs only because of the history of thrombosis.

Overall, 311 patients (62.9%) received a combined treatment including a cytoreductive agent plus an antithrombotic drug (*Online Supplementary Table S3*). Only nine patients (1.8% of the entire cohort) did not receive any treatment after the first thrombosis and an additional four patients were treated only by phlebotomy (*Online Supplementary Table S3*).

Incidence of recurrent thrombosis

The recurrent events recorded in the patients are reported in Table 2. We computed a total observation time of 2,952 years after the first thrombosis (median 5.3); the observation time exceeded 5 years in 257 patients (52%) and 10 years in 84 (17%). One hundred and sixty-six patients (33.6%) had a first recurrence with an incidence of events of 5.6% patient-years, (4.3% patients-years among patients < 60 years old and 6.8% patients-years among those > 60 years old). The cumulative probability of recurrence was 17.7% (95% CI 14.2-21.2) at 2 years after the first thrombosis, 30.8% (95% CI 26.1-35.4) at 5 years, and 49.9% (95% CI 43.0-56.8) at 10 years. The first recurrent thrombosis involved arterial vessels in 60.8% of cases and venous vessels in 39.7% of cases. A more detailed description of the recurrent thrombotic events is reported in *Online Supplementary Table S4*. In 39 patients (19 with polycythemia vera and 20 with essential thrombocythemia) we recorded 58 additional recurrences after the first rethrombosis; these additional recurrence were arterial in 34 cases and venous in 24. Thus, the overall incidence of recurrent events was 7.6% patient-years (4.6% for arterial recurrences and 3.0% for venous recurrences). The overall incidence of recurrence was 5.9% patient-years among patients < 60 years old at the time of the index thrombosis and 8.9% among those > 60 years. Recurrent thrombosis was fatal in two female patients with polycythemia vera, one of whom had an ischemic stroke at the age of 67 and the other a pulmonary embolism at the age of 77.

Risk factors for recurrent thrombosis

Gender, diagnosis of polycythemia vera or essential thrombocythemia, and the presence of vascular risk factors or a history of remote thrombosis did not affect the probability of recurrence (details are reported in *Online Supplementary Table S5*). Age >60 years at the index event was associated with a significant increase in risk of recurrence in comparison with the risk in younger patients after adjustment for gender, diagnosis, presence of vascular risk factors, history of remote thrombosis, type of first thrombosis, and antithrombotic or cytoreductive treatment (multivariable HR 1.67; 95% CI 1.19-2.32) (*Online Supplementary Table S5*). The type of index event (arterial or venous thrombosis) did not influence the probability of recurrence; however, after adjustment for covariates, the risk of arterial or venous recurrence was significantly higher in patients having had a first arterial thrombosis (multivariable HR 5.75, 95% CI 1.66-19.86) or a first venous thrombosis (multivariable HR 4.23; 95% CI 2.54-7.06), respectively. When the index thrombosis occurred,

Table 1. Baseline characteristics of the patients.

Diagnosis (PV/ET) – n.	235/259
Sex (Male/Female) – n.	239/255
Median age at diagnosis – years (range)	62 (18-88)
Median age at thrombosis – years (range)	64 (20-90)
First arterial index thrombosis – n. (%)	341 (69.0)*
First venous index thrombosis – n. (%)	160 (32.3)*
Presence of at least one vascular risk factor – n. (%)	289 (58.5)
smoking	71 (14.3)
hypertension	230 (46.5)
hypercholesterolemia	62 (12.5)
diabetes mellitus – no. (%)	41 (8.2)
chronic atrial fibrillation – n. (%)	30 (6.0)
Presence of inherited or acquired [thrombophilia – n./n. tested (%)	42/142 (29.5)
History of remote thrombosis – n. (%)	51 (10.3)
Thrombosis at diagnosis of PV and ET [or during the preceding 2 years n. (%)	281 (56.8)
First thrombosis after diagnosis [(index thrombotic event) – no. (%)	213 (43.2)
Patients receiving one or more [treatment after the index thrombosis:	
antiplatelet agents–n. (%)	362 (73.2)
long-term oral anticoagulation – n. (%)	90 (18.2)
phlebotomy–n. (%)	155 (31.3)
any pharmacological cytoreductive treatment - n. (%)	351 (71.0)

*PV: polycythemia vera; ET: essential thrombocythemia. *In 5 patients with PV and 2 with ET the first event involved both arterial and venous vessels. #Cytoreductive treatment included hydroxyurea, pipobroman, busulphan, interferon, and anagrelide.*

Table 2. Recurrences after the index thrombotic event in the overall cohort of patients.

Total observation time – years	2,952
Median observation time – years (range)	5.3 (0.1-26.2)
Overall first recurrences – n. (% of patients)	166 (33.6)
Incidence of first recurrences (% patient-years)	5.6
Recurrent arterial thrombosis – n. (% of recurrences)	101 (60.8)*
Recurrent venous thrombosis – n. (% of recurrences)	66 (39.7)*
Major bleeding after the index thrombosis – n. (% of patients)	27 (5.4)
Incidence of major bleeding after the index thrombosis [(% patient-years)	0.9

In one patient with polycythemia vera, recurrence involved both arterial and venous vessels.

354 patients were not receiving cytoreduction and the leukocyte count at that time was available for 253 of them (71.4%), with a median value of 10.2×10^9/L (range, 3.1-24.9). Among the patients with a leukocyte count in the highest quartile (> 12.4×10^9/L) the rate of recurrences was not significantly increased with respect to that among patients with a leukocyte count in the lowest quartile (<7.81×10^9/L) (36.5% versus 26.9%; multivariable HR 1.60, 95% CI 0.79-3.23); however, in patients <60 years old (n=109) the rate of recurrences was higher in those with a leukocyte count in the highest quartile with respect to the rate in those with a leukocyte count in the lowest quartile (44.4% versus 18.5%; multivariable HR 3.55, 95%CI 1.02-12.25, after adjustment for age and the covariates listed earlier).

The rate of recurrences was higher in patients with thrombophilia than in those without (42.8% versus 25.0%; multivariable HR 1.91, 95% CI 1.01-3.60, after adjustment for covariates). Analysis by age group showed a higher risk of recurrence associated with thrombophilia only in patients < 60 years old (n= 82, 37.0% versus 18.1%; multivariable HR 2.68, 95% CI 1.01-7.16).

Effect of different therapeutic strategies on re-thrombosis

The efficacy of antithrombotic or cytoreductive treatment was estimated in the overall cohort after adjustment for gender, diagnosis, age, presence of vascular risk factors, history of remote thrombosis, and type of first thrombosis; results are summarized in *Online Supplementary Table S5*. A significant reduction of the risk for recurrence was achieved only by cytoreductive treatment (multivariable HR 0.53, 95% CI 0.38-0.73), whereas the decrease in risk independently associated with antiplatelet agents or phlebotomy only bordered statistical significance (multivariable HR 0.72, 95% CI 0.50-1.02, and HR 0.72, 95% CI 0.47-1.09, respectively). The reduction of the risk of recurrence associated with cytoreduction was fully confirmed after exclusion of the patients receiving cytoreduction not only for their history of thrombosis but also because of hematocrit values not controlled by phlebotomies or because of platelet counts exceeding $1{,}000\times10^9$/L (26% of those with cytoreduction), producing a multivariable HR of 0.51 (95% CI 0.36-0.74).

Combined treatments were more effective than single agent strategies at preventing re-thrombosis. Details of the different combined treatments and the respective rates of recurrences are reported in *Online Supplementary Table S2*. The use of a cytoreductive drug together with an antiplatelet agent reduced the risk of recurrence in comparison with both cytoreduction alone (univariable HR 0.56, 95% CI 0.24-0.85) and antiplatelet agents alone (univariable HR 0.67, 95% CI 0.41-0.99). Similarly, the use of cytoreduction together with oral anticoagulation

Table 3. Risk factors for first recurrent thrombosis of patients with arterial or venous index thrombosis according to the baseline characteristics (multivariable analysis).

	First arterial thrombosis (n= 341)		*Acute coronary syndrome (n= 106)*		*Cerebrovascular disease (n= 191)*		*First venous thrombosis (n= 160)*	
	Hazard ratio (95% CI)	*p*	*Hazard ratio (95% CI)*	*p*	*Hazard ratio (95% CI)*	*p*	*Hazard ratio (95% CI)*	*p*
Sex (male vs. female)	1.02 (0.68-1.51)	0.89	1.17 (0.51-2.66)	0.70	0.86 (0.49-1.48)	0.61	1.21 (0.67-2.16)	0.51
Diagnosis (PV vs ET)	1.33 (0.77-2.27)	0.29	1.29 (0.44-3.73)	0.63	1.07 (0.52-2.18)	0.84	0.91 (0.48-1.69)	0.78
Age at thrombosis [(>60 yrs vs < 60 yrs)	1.34 (0.88-2.02)	0.15	1.54 (0.70-3.37)	0.27	2.09 (1.12-3.86)	0.01	2.26 (1.30-3.91)	0.003
One or more vascular risk [factors (presence vs absence)*	0.99 (0.80-1.21)	0.93	0.81 (0.38-1.69)	0.58	0.99 (0.96-1.01)	0.99	0.91 (0.52-1.58)	0.76
History of thrombosis prior [to diagnosis of PV or ET (presence *vs.* absence)§	1.14 (0.64-2.02)	0.65	2.10 (0.93-4.72)	0.07	0.33 (0.07-1.40)	0.14	0.72 (0.28-1.84)	0.49
Treatment after the index thrombotic event								
[antiplatelet agents	0.67 (0.41-1.08)	0.10	0.86 (0.28-2.57)	0.79	0.33 (0.16-0.66)	0.002	0.42 (0.22-0.77)	0.006
long-term oral anticoagulation	1.01 (0.93-1.09)	0.73	1.02 (0.88-1.17)	0.73	0.82 (0.27-2.45)	0.72	0.32 (0.15-0.64)	0.001
phlebotomy	0.76 (0.43-1.31)	0.33	0.58 (0.19-1.73)	0.33	0.85 (0.40-1.79)	0.68	0.72 (0.35-1.47)	0.38
any pharmacological [cytoreductive treatment#	0.47 (0.31-0.70)	0.0003	0.30 (0.13-0.68)	0.004	0.68 (0.37-1.22)	0.20	0.66 (0.38-1.13)	0.14

*PV, polycythemia vera; ET, essential thrombocythemia; *smoking, hypertension, hypercholesterolemia, diabetes mellitus, chronic atrial fibrillation; §history of thrombosis prior to 2 years before the diagnosis of PV or ET; #cytoreductive treatment included hydroxyurea, pipobroman, busulphan, interferon, and anagrelide*

significantly reduced the risk of recurrence in comparison with both cytoreduction alone (univariable HR 0.37, 95% CI 0.17-0.79) and oral anticoagulation alone (univariable HR 0.33, 95% CI 0.13-0.74). The number of patients receiving both antiplatelet agents and oral anticoagulants was too small to allow a reliable comparison with patients receiving either drug.

The results of a secondary multivariable analysis carried out after stratification of the patients according to the type of first event are shown in Table 3 and confirmed the efficacy of cytoreductive treatment in patients with first arterial thrombosis (53% reduction in the risk), whereas in patients with first venous thrombosis the influence was more limited (34% reduction in the risk) and not statistically significant (Table 3). Subgroup analysis showed that in patients with a previous acute coronary syndrome, cytoreductive treatment was particularly effective (70% reduction in the risk) while the influence of antiplatelet agents was limited and not statistically significant. In contrast, in patients with previous cerebrovascular disease the benefit of pharmacological cytoreduction was not statistically significant whereas antiplatelet agents were found to be highly effective (67% reduction in the risk).

In the subgroup of patients with first venous thromboembolism antithrombotic treatment had significant efficacy in preventing re-thrombosis; this was true for both long-term oral anticoagulation (68% reduction in the risk) and antiplatelet agents (58% reduction in the risk). After exclusion of the patients with first venous thrombosis at unusual sites, multivariable analysis of outcomes in the remainig 114 patients showed that long-term oral anticoagulation was more effective than antiplatelet agents at preventing recurrence (HR 0.31, 95%CI 0.13-0.69, and HR 0.53, 95%CI 0.27-1.03, respectively).

In general, the rate of re-thrombosis in patients receiving antiplatelet agents soon after the index thrombosis was quite similar to that in the patients in whom the antiplatelet treatment was preceded by a period under oral anticoagulants or low molecular weight heparin (31.3% vs. 31.4%, respectively, p=1.00). However, in patients having had a first venous thromboembolism the rate of recurrences was higher in those who received antiplatelet agents soon after thrombosis (48.6%) compared to in those in whom the antiplatelet treatment was preceded by a period under oral anticoagulants or low molecular weight heparin (36.1%), although the difference was not statistically significant (p=0.34).

Out of 106 patients who had a re-thrombosis when on cytoreductive treatment, at the time of recurrence 46 (43.3%) showed an unsatisfactory level of cytoreduction, defined as hematocrit >0.50 and/or a leukocyte count >12.4×10^9/L and/or a platelet count >600×10^9/L; the recurrence involved arterial vessels in 35 of them. Twenty-eight of the patients with re-thrombosis on cytoreductive treatment did not take any antiplatelet agent: the level of cytoreduction was unsatisfactory in 12 of these (42.8%) at the time of recurrence, which involved arterial vessels in nine cases. Twenty-three patients had re-thrombosis when on oral anticoagulant treatment. The International Normalized Ratio value at the time of recurrence was available for only 12 of them; in six (50%) the value was below the therapeutic range (i.e. <2.0). Only one of these patients was on cytoreduction, which was unsatisfactory at the time of recurrence.

Twenty-seven major bleeds were recorded, with an incidence of 0.9% patient-years (Table 3 and *Online Supplementary Table S4*): the bleeding was gastrointestinal in 17 cases, hemorrhagic stroke in six, muscle hematoma in two, hematuria in one, and epistaxis in one. Bleeding occurred during administration of antiplatelet agents in 17 cases (62.9%), vitamin K antagonists in four (14.8%), antiplatelet agents combined with vitamin K antagonists in three (11.1%), and in the absence of antithrombotic treatment in three (11.1%). Hemorrhagic stroke (which occurred in the absence of antithrombotic treatment) was fatal in one male patient with essential thrombocythemia, aged 61. The incidence of major bleeding in per cent patient-years was 0.8 (antiplatelet agents), 0.9 (vitamin K antagonists), 2.8 (antiplatelet agents plus vitamin K antagonists), and 1.2 (no antithrombotic treatment).

Discussion

The thrombotic risk in polycythemia vera and essential thrombocythemia is more pronounced in patients over 60 old years and in those with a history of previous thrombosis, both groups being included in the high-risk category.[1-7] Nevertheless the risk of recurrence in this category is obviously not homogeneous, since some patients over 60 years old remain free of thrombotic events. Few studies have been specifically aimed at evaluating the efficacy of treatment in preventing re-thrombosis and they included small numbers of subjects.[13-15] The primary objective of our study was to estimate the rate of re-thrombosis and the efficacy of cytoreductive and/or antithrombotic treatment in preventing recurrence in patients with polycythemia vera or essential thrombocythemia. We examined a large multicenter retrospective cohort of more than 1,000 patients with polycythemia vera and about 2,000 patients with essential thrombocythemia and selected 494 high-risk patients who had suffered from at least one major thrombotic event. The rate of patients with thrombosis in the initial cohort was lower than that reported in other investigations.[1-7] There are several possible reasons for this difference. First, in most studies the number of patients was considerably smaller than in our cohort, so that a referral bias cannot be excluded.[1] Second, we investigated only patients diagnosed after 1985, whereas in the largest published cohorts the period of diagnosis had no limits

and included patients diagnosed in the 1950s to 1970s.[2-7] It could, therefore, be suggested that the higher rate of asymptomatic individuals found in our study is due to a more extensive use of routine blood analyses in the general population as well as to an improvement of antithrombotic primary prophylaxis in patients with polycythemia vera or essential thrombocythemia.

In our study the recruited patients with polycythemia vera and essential thrombocythemia showed substantial homogeneity as regards age, the presence of vascular risk factors, and the type of first clinical manifestation; moreover multivariable analysis did not show any difference in the risk of recurrence according to diagnosis of polycythemia vera or essential thrombocythemia. Our results are, therefore, applicable to both categories of patients as a whole. The overall incidence of recurrences was 5.9% patient-years among patients <60 years old and 8.9% patient-years among those >60 years old, with rates similar to those found in a large prospective cohort of polycythemia vera patients.[3] Multivariable analysis adjusted for a number of potential confounders showed that age >60 years is the main risk factor for recurrence too, being associated with a 1.7-fold increase in risk with respect to that in patients aged <60 years and that this increased risk in the elderly was even more pronounced in patients with cerebrovascular disease and venous thromboembolism. The overall probability of recurrence was not predicted by whether the first manifestation was in the arterial or venous district, but re-thrombosis occurred preferentially in the same vascular district previously affected, in line with the results found in a large prospective cohort of polycythemia vera patients.[9]

We were not able to confirm in the overall cohort the higher risk recently reported in patients with increased leukocyte count,[8,9] probably because more than one fourth of the study patients had been administered cytoreductive treatment soon after diagnosis and prior to the index thrombosis. Nevertheless, in the evaluable patients leukocytosis at the time of the index thrombosis was associated with a 3.5-fold increased probability of recurrence in younger patients, confirming that the role of leukocytes in predicting thrombosis can be disclosed more easily in such individuals.[8]

Thrombophilia was found to be associated with a higher risk of recurrence, in agreement with previous reports.[12] In the general population most of the inherited thrombophilic alterations are associated with only a mild risk of recurrence,[27] so that the increase in risk in patients with polycythemia vera and essential thrombocythemia could be produced by an interaction between inherited thrombophilia and the myeloproliferative disorder. However in our study only a minority of the patients were investigated for thrombophilia, so that a selection bias cannot be excluded.

After thrombosis 71% of the 494 patients received cytoreductive therapy which, in the large majority of cases, was hydroxyurea. The remaining patients were not prescribed cytoreduction because in those with polycythemia vera the hematocrit value was controlled by phlebotomies or because in those with essential thrombocythemia the platelet count was lower than $1{,}000\times10^9$/L. Multivariable analysis showed that cytoreduction was independently effective in preventing re-thrombosis, reducing the risk by 47%. This finding was fully confirmed after exclusion of the patients receiving cytoreduction not only for their history of thrombosis but also because of hematocrit values not controlled by phlebotomies or because of platelet counts exceeding $1{,}000\times10^9$/L. A possible bias due to the heterogeneity of the patients receiving cytoreduction is, therefore, unlikely. The use of antiplatelet agents was associated in the overall patient cohort with a 38% reduction in risk, which was of borderline statistical significance. The contemporary administration of a cytoreductive and an antiplatelet agent was associated with a significant improvement of efficacy in preventing re-thrombosis in comparison with the efficacy of either drug alone. Similarly, the combination of cytoreduction and oral anticoagulation was more effective than the single treatments given individually.

The efficacy of cytoreduction was more pronounced in patients who had acute coronary syndrome as the index thrombosis, producing a 70% reduction in the risk of re-thrombosis. In patients with previous cerebrovascular disease or venous thromboembolism, cytoreduction was associated with an approximately 30% decreased risk of recurrence, which was not statistically significant. The special efficacy of cytoreductive treatment in patients with acute coronary syndrome could mirror the knowledge that leukocytosis in the general population is a consistent risk factor for coronary heart disease[28] and in polycythemia vera is associated with a 2.8-fold increase in the risk of myocardial infarction, whereas the increase in the risk of cerebrovascular disease or venous thromboembolism is not statistically significant.[9]

In patients with first arterial thrombosis antiplatelet agents were associated with a 23% reduction in the risk of recurrence, which was of borderline statistical significance, showing a benefit similar to that observed in the general population of patients with previous myocardial infarction or previous cerebrovascular ischemia;[29] in this special setting of patients with polycythemia vera or essential thrombocythemia the highest efficacy was found in those with cerebrovascular disease, who had a 67% reduction in the risk of recurrence. Conversely, in patients with acute coronary syndrome antiplatelet agents produced only marginal benefit, confirming that in this group the most effective treatment for preventing recurrence is pharmacological cytoreduction. In the ECLAP trial, which tested the efficacy of prophylaxis with aspirin, the reduction in risk in polycythemia vera patients receiving aspirin with respect to those receiving placebo was 68% for patients with cerebrovascular disease and 46% for those with myocardial infarction.[22]

Although the number of thrombotic events was low , these findings seem to confirm that in such a setting antiplatelet therapy is more effective in patients with cerebrovascular disease than in patients with acute coronary syndrome.

Cytoreduction in patients with first venous thrombosis was associated with a 34% reduction in the risk of recurrence, this only bordering statistical significance. Long-term treatments with antiplatelet or anti-vitamin K agents were found to be independently effective in preventing recurrence, with reductions of re-thrombosis of 58% and 68%, respectively. Both strategies showed quite satisfactory safety profiles, the incidence of major bleeding not being higher than that observed in patients with no treatment. In contrast, the association of antiplatelet agents plus vitamin K antagonists resulted in a higher incidence of major bleeding (2.8% patient-years). The role of aspirin in the prevention of venous thromboembolism has been the object of many studies. Pulmonary embolism was reduced by 25-30% in patients given antiplatelet agents both in trials targeted at the prevention of arterial events[29] and in trials targeted at the prevention of post-operative events.[30] This it is not surprising, given that platelets play a role in the formation of thrombi and in particular in the propagation of the thrombus growth in the case of venous thrombosis.[31] Moreover, in patients with polycythemia vera and essential thrombocythemia the formation of a thrombus is obviously influenced by special factors not present in the general population; for instance, the interplay between platelets and activated leukocytes is enhanced, as reflected by higher levels of polymorphonuclear leukocyte-platelet aggregates; interestingly, aspirin mitigates such a phenomenon.[32] Our findings could imply that long-term treatment with anti-vitamin K agents is effective and safe in patients with polycythemia vera or essential thrombocythemia and venous thromboembolism, but that indefinite administration of antiplatelet agents could be acceptable as an alternative strategy after a conventional short-term period of oral anticoagulant treatment.

Finally, the observation that at least 40% of recurrences occurred in the presence of unsatisfactory levels of cytoreduction or anticoagulation strongly indicates the need for careful laboratory surveillance of patients to achieve the desired therapeutic goals and improve the benefits of secondary prophylaxis, as already pointed out for patients on oral anticoagulants.[15]

In conclusion, cytoreductive treatment halves the incidence of re-thrombosis in patients with polycythemia vera or essential thrombocythemia, notably in those with first arterial thrombosis. The protection offered by cytoreduction is particularly marked in patients with acute coronary syndrome. As could be expected, long-term treatment with oral anticoagulants is effective in patients with first venous thromboembolism. Antiplatelet treatment reduces the risk of recurrence in patients with cerebrovascular disease but also in those with venous thromboembolism. The substantial equivalence in efficacy and safety of secondary antithrombotic prophylaxis with either antiplatelet agents or oral anticoagulants in patients with venous thromboembolism calls for prospective randomized trials specifically designed to investigate the optimal treatment in this setting.

Recently, patients carrying the acquired *JAK2* V617F mutation have been demonstrated to obtain better cytoreduction and have a lower rate of arterial thrombosis when treated with hydroxyurea rather than anagrelide.[33] Our findings could, therefore, depend in part on an interaction between the *JAK2* status and different treatments, indicating that patients in future investigations, tailored according to the type of first clinical event, should be stratified accordingly.

Authorship and Disclosures

VDS designed the study, coordinated the research project, and was responsible for the statistical analysis, the final interpretation of the data, and the final draft of the manuscript; VDS, TZ, and ER were responsible for the final database collecting the laboratory and clinical data; VDS, TZ, ER, AMV, MR, EE, CM, AT, RRC, CS, GG, NV, PG, LP, FS, FR, EMP, GF, LG, GL, and TB were the physicians responsible for the clinical managment of the patients, the collection of the clinical and laboratory data, as well as the adjudication of the thrombotic or bleeding events; RM supervised the statistical analysis; AMV, MR, NV, FR, EMP, GF, LG, RM, GL as senior authors, critically revised the paper and gave important intellectual contributions; TB conceived the study, supervised the research project, critically revised the paper and gave an important intellectual contribution. All authors were involved in the final revision of the article, interpretation of the data and final approval of the version to be published. The authors reported no potential conflicts of interest.

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