Leukocytosis is a risk factor for recurrent arterial thrombosis in young patients with polycythemia vera and essential thrombocythemia

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There is evidence that leukocytosis is associated with an increased risk of first thrombosis in patients with polycythemia vera (PV) and essential thrombocythemia (ET). Whether it is a risk factor for recurrent thrombosis too is currently unknown. In the frame of a multicenter retrospective cohort study, we recruited 253 patients with PV (n = 133) or ET (n = 120), who were selected on the basis of a first arterial (70%) or venous major thrombosis (27.6%) or both (2.4%), and who were not receiving cytoreduction at the time of thrombosis. The probability of recurrent thrombosis associated with the leukocyte count recorded at the time of the first thrombosis was estimated by a receiver operating characteristic analysis and a multivariable Cox proportional hazards regression model. Thrombosis recurred in 78 patients (30.7%); multivariable analysis showed an independent risk of arterial recurrence (hazard ratio [HR] 2.16, 95% CI 1.12–4.18) in patients with a leukocyte count that was >12.4 × 10^9/L at the time of the first thrombotic episode. The prognostic role for leukocytosis was age-related, as it was only significant in patients that were aged <60 years (HR for arterial recurrence 3.35, 95% CI 1.22–9.19). Am. J. Hematol. 85:97–100, 2010. © 2009 Wiley-Liss, Inc.

Introduction

Polycythemia vera (PV) and essential thrombocythemia (ET) are typically complicated by arterial or venous thrombosis, which may be the inaugural presentation or complicate the course of disease [1]. Advanced age and a prior history of thrombosis are the main risk factors for vascular complications [1–6]. More recently, leukocytosis has been reported as an independent risk factor for thrombosis in both PV and ET [6–13]. In a previous article, we evaluated a cohort of patients with PV and ET who had suffered at least one thrombotic event; we showed that age >60 years is also an independent risk factor for recurrences, and cytoreduction significantly protects against novel events [14]. In this study, we investigated the prognostic role of leukocytosis that was registered at the time of the first event to predict future recurrent thrombotic events.

Patients and Methods

Study patients. A retrospective study was conducted using the medical records of 494 patients with PV and ET who were diagnosed at one of the hematological centers of GIMEMA (Gruppo Italiano Malattie Ematologiche dell'Adulto) from January 1985 to December 2005. The main criterion for inclusion in the study was that all 494 individuals had suffered at least one major thrombotic event related to their haematological disease. Details of the procedure used to recruit the cohort have been reported elsewhere [14]. In this analysis, we only investigated the patients who were not receiving any cytoreductive treatment and had a recorded white blood cell (WBC) count at the time of the initial thrombosis.

Definition of the events and of the risk factors. A thrombotic event related to the patient's hematologic disease was defined as an event that occurred following diagnosis and referral to the specialized hematological center, or an event that occurred no earlier than 2 years preceding the diagnosis. Thrombotic events that occurred more than 2 years before the hematologic disease diagnosis were considered to be remote thromboses.

The major thrombotic events of interest were ischemic stroke, transient ischemic attack (TIA), acute myocardial infarction, unstable angina pectoris, peripheral arterial thrombosis, retinal artery or vein occlusion, deep venous thrombosis (including thrombosis of cerebral and splanchnic veins), pulmonary embolism, and superficial venous thrombosis. Our definition of acute coronary syndrome encompasses acute myocardial infarction as well as unstable angina pectoris. We consider cerebrovascular disease to include ischemic stroke as well as TIA. Splanchnic venous thrombosis included occlusion of the hepatic, portal, mesenteric, and splenic veins. Diagnosis of the first or a subsequent major thrombotic event was only accepted, if objectively proven or in the case of medical documentation that unambiguously confirmed the occurrence of angina or a TIA, as previously described [14]. Microcirculatory events, including vascular headaches, dizziness, visual disturbances, sensations of burning pain in the palms of the hands and soles of the feet, distal paresthesia and acrocyanosis, were not considered as events of interest.

Vascular risk factors included smoking habit, hypercholesterolemia, hypertension, diabetes mellitus, and chronic atrial fibrillation. Leukocytosis was defined as the upper quartile of the WBC count for those patients who did not receive cytoreduction at the time of their first thrombosis.

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Statistical methods. Differences in the proportions were estimated using the Fisher's exact test (statistical significance threshold set at $P < 0.05$).

A preliminary univariable estimate of the association of the WBC count with the probability of future recurrent thrombosis was performed by a receiver operating characteristic (ROC) analysis, to select a cut-off value to be employed in the multivariable model. The probability of recurrence as a function of time was estimated according to the method of Kaplan and Meier, analyzing the interval between the initial thrombosis and a recurrent thrombotic event (uncensored observations), or the duration until death, or the time elapsed until the patient's final visit to the center (censored observations). The probability of recurrence was compared between groups using the log-rank test (statistical significance threshold at $P < 0.05$), and the relative risk of recurrence was estimated as a hazard ratio (HR) using a Cox proportional hazards regression model. The HR was adjusted using recurrence as the dependent variable, and the selected covariates were gender, diagnosis (PV or ET), age at the time of the initial thrombosis (>60 or <60 years), presence of one or more vascular risk factors, history of remote thromboses, type of first thrombosis (arterial or venous), hematological parameters at the time of the first thrombosis, and type of treatment following thrombosis. The haematological parameters used in the multivariable model as putative risk factors were hematocrit, WBC count, and a platelet count that was higher than the respective upper quartile. The treatment used within our sample included antithrombotic prophylaxis using antiplatelet agents, or long-term oral anticoagulants, any type of pharmacological cytoreductive treatment, and phlebotomy.

Statistical analyses were performed using the HLSTAT software (Addinsoft, Paris, France) for the ROC analysis, the GraphPad PRISM 3.0 software (GraphPad Software, San Diego, CA) for univariable methods, and the GB-STAT V6.5 software (Dynamic Mycrosystems, Silver Spring, MD,) for multivariable testing.

Results

Study patients

We investigated 253 patients who had suffered at least one major thrombotic event and who, at the time of the initial thrombotic event, were not receiving any cytoredutive treatment. In 194 of them, thrombosis occurred as heralding event of the myeloproliferative neoplasm. In the remaining 59 patients who were asymptomatic at the time of diagnosis and experienced thrombosis during the follow up, cytoreduction had not been prescribed after diagnosis by the care physicians, because in those with PV phlebotomy was considered effective in controlling the haematocrit value, or because in those with ET the platelet count was lower than $1{,}500 \times 10^9$/L.

The clinical characteristics of patients are shown in Table I. Two-thirds of the initial thromboses were arterial (Table II): The thrombosis was associated with cerebrovascular disease in 108 of the patients (42.6%), acute coronary syndrome in 48 of the patients (18.9%), and peripheral arterial occlusion in 24 of the patients (9.4%). The first event was a venous thrombosis in 76 of the patients (30%), including those with either a cerebral ($n = 2$) or an abdominal vessel ($n = 19$) involvement.

Following thrombosis, most patients received therapy with antiplatelet agents and/or cytoreduction (Table I). One hundred and ninety-four of the patients (76.6%) received antiplatelet agents, while 36 of the patients (14.2%) were prescribed long-term treatment (over one year) with vitamin k antagonists. Six of the patients (2.4%) received both antiplatelet and vitamin k antagonists. One hundred and fifty-eight of the patients (62.4%) were prescribed cytoreductive agents, namely hydroxyurea (83.6%), pipobroman (6.3%), busulphan (5.7%), interferon (2.5%), and anagrelide (1.9%). Overall, 150 of the patients (59.2%) received a combined treatment that included both a cytoreductive agent and an antithrombotic drug.

TABLE I. Characteristics of the Patient Cohort

Diagnosis (no.)	PV (133)	ET (120)	PV + ET (253)
Sex (M/F), no. (% of male sex)	78/55 (58.6)	46/74 (38.3)	124/129 (49.0)
Median age at diagnosis, years (range)	63 (18–84)	60 (21–87)	62 (18–87)
Presence of at least one vascular risk factor, no. (%)	83 (62.4)	70 (58.3)	153 (60.4)
Smoke, no. (%)	16 (12.0)	17 (14.1)	33 (13.0)
Hypertension, no. (%)	68 (51.1)	50 (41.6)	118 (46.6)
Hypercholesterolemia, no. (%)	14 (10.5)	16 (13.3)	30 (11.8)
Diabetes mellitus, no. (%)	10 (7.5)	10 (8.3)	20 (7.9)
Chronic atrial fibrillation, no. (%)	8 (6.0)	7 (5.8)	15 (5.9)
History of remote thromboses, no. (%)	16 (12.0)	7 (5.8)	23 (9.0)
Median hematocrit at the time of the first thrombosis, % (range)	47.4 (40.0–75.0)	42.3 (30.6–49.8)	44.0 (30.6–75.0)
Median white blood cell count at the time of the first thrombosis, $\times 10^9$/L (range)	10.5 (4.2–24.9)	9.4 (3.1–20.0)	10.2 (3.1–24.9)
Median platelet count at the time of the first thrombosis, $\times 10^9$/L (range)	556 (144–1372)	716 (361–1964)	630 (144–1964)
Patients receiving one treatment or more than one treatment following the initial thrombosis			
Antiplatelet agents, no. (%)	103 (77.4)	97 (80.8)	200 (79.0)
Long-term oral anticoagulation, no. (%)	22 (16.5)	20 (16.6)	42 (16.6)
Phlebotomy, no. (% of the PV patients)	101 (75.9)	–	101
Any pharmacological cytoreduction, no. (%)[a]	76 (57.1)	82 (68.3)	158 (62.4)

PV, polycythemia vera; ET, essential thrombocythemia.

[a] Cytoreductive treatment includes hydroxyurea, pipobroman, busulphan, interferon, or anagrelide.

TABLE II. Rate of Disease-Related Thrombotic Events in the Patient Cohort

Diagnosis (no.)	PV (133)	ET (120)	PV + ET (253)
First arterial thrombosis, no. (%)	93 (69.9)[a]	90 (75.0)[a]	183 (72.3)[a]
First venous thrombosis, no. (%)	45 (33.8)[a]	31 (25.8)[a]	76 (30.0)[a]
Median age at thrombosis, years (range)	63 (22–84)	61 (21–89)	63 (21–89)
First thrombosis at diagnosis of PV and ET or during the prior 2 years, no. (%)	97 (72.9)	97 (80.8)	194 (76.6)
First thrombosis during follow-up, no. (%)	36 (27.1)	23 (19.1)	59 (23.3)
Second thrombosis during follow-up, no. (%)	44 (33.0)	34 (28.3)	78 (30.8)
Third thrombosis during follow-up, no. (%)	9 (6.7)	9 (7.5)	18 (7.1)

PV, polycythemia vera; ET, essential thrombocythemia.

[a] In five of the patients with PV and one of the patients with ET, the first event involved both arterial and venous vessels.

Recurrent thrombosis

Seventy-eight of the patients (30.8%) had a first recurrent thrombotic event over a total observation time of 1,602 patient-years following the first thrombosis (median 5.5), with an incidence of events of 4.8% patient-years. The observation time exceeded 5 years for 141 patients (55.7%) and 10 years for 50 patients (19.7%).

The second thrombotic event (first recurrence) involved arterial vessels in 46 of the patients (59% of recurrences) and venous vessels in 32 of the patients (41%). Recurrence was in the form of an ischemic stroke in 12 of the patients, TIA in 19 patients, a myocardial infarction in 6 patients, unstable angina in 5 patients, a peripheral arterial thrombosis in 5 patients, a retinal artery occlusion in 1 patient, deep venous thrombosis of the legs in 18 patients, a pulmonary embolism in 4 patients, superficial venous thromboses in 6 patients, mesenteric vein thrombosis in 1 patient, and cerebral venous thrombosis in 1 patient.

TABLE III. Rate of Recurrent Events in the Patient Cohort According to Age and to White Blood Cell (WBC) Count Over the Upper Quartile at the Time of the First Thrombosis

		All patients	WBC $>12.4 \times 10^9$/L, no. (%)	WBC $<12.4 \times 10^9$/L, no. (%)	P WBC $>12.4 \times 10^9$/L versus WBC $<12.4 \times 10^9$/L
All patients	Recurrent thrombosis, no. (%)	78/253 (30.8)	23/63 (36.5)	55/190 (28.9)	0.27
	Recurrent arterial thrombosis, no. (%)	49/253 (19.3)	16/63 (25.3)	33/190 (17.3)	0.19
	Recurrent venous thrombosis, no. (%)	29/253 (11.5)	7/63 (11.2)	22/190 (11.6)	1.00
<60 years	Recurrent thrombosis, no. (%)	30/109 (27.5)	12/27 (44.4)	18/82 (21.9)	0.04
	Recurrent arterial thrombosis, no. (%)	20/109 (18.3)	9/27 (33.3)	11/82 (13.4)	0.04
	Recurrent venous thrombosis, no. (%)	10/109 (9.2)	3/27 (11.1)	7/82 (8.5)	0.78
>60 years	Recurrent thrombosis, no. (%)	48/144 (33.3)	11/36 (30.5)	37/108 (34.2)	0.83
	Recurrent arterial thrombosis, no. (%)	29/144 (20.1)	7/36 (19.4)	22/108 (20.3)	1.00
	Recurrent venous thrombosis, no. (%)	19/144 (13.2)	4/36 (11.1)	15/108 (13.9)	0.78

After the second thrombotic event, 18 of the patients (7.1%) experienced a third thrombosis (second recurrence), which consisted of an ischemic stroke in two of the patients, TIA in four patients, a myocardial infarction in two, unstable angina in three, peripheral arterial thrombosis in one, deep venous thrombosis of the legs in four, hepatic vein thrombosis in one, and cerebral venous thrombosis in one.

Risk factors for recurrent thrombosis

The WBC counts at the time of first thrombosis ranged between 3.1 and 24.9 $\times$ 10^9/L (Table I); the median value was 10.2 $\times$ 10^9/L, and the upper quartile was $>12.4 \times 10^9$/L. The upper quartiles of the hematocrit values and the platelet count were >51.0% and $>806 \times 10^9$/L, respectively.

We explored the univariable probability of recurrent thrombosis associated to different WBC count cut-off values, estimating by a ROC analysis the positive likelihood ratio (LR) between the true positives (sensitivity) and the false positives (1 − specificity). Using as cut-off the median value of the WBC counts, the positive LR was 1.06 in the overall cohort, 1.11 in the patients who were younger than 60 years, and 1.00 in the patients who were older than 60 years. Using as cut-off the upper quartile of the WBC counts, the positive LR was 1.22 in the overall cohort, 1.86 in the patients who were younger than 60 years, and 0.81 in the patients who were older than 60 years. Applying in the patients who were younger than 60 years as cut-off the upper quartile of the WBC counts, the positive LR was 1.29 for recurrent venous thrombosis (sensitivity 30%, specificity 76%) and 1.97 for recurrent arterial thrombosis (sensitivity 36%, specificity 78%).

Considering the whole population, the rate of events was not influenced by leukocytosis at the time of the first thrombosis (defined as WBC count over the upper quartile, i.e., $>12.4 \times 10^9$/L); in contrast, among the patients who were younger than 60 years, the rate of recurrence was higher in those who had leukocytosis compared with those who did not (44.4% vs. 21.9%, $P = 0.04$). The recurrent events that were more associated with leukocytosis were arterial (Table III).

In a multivariable analysis that was adjusted for gender, diagnosis, age at the time of the first thrombosis, presence of vascular risk factors, history of remote thromboses, type of first thrombotic event (arterial or venous), hematocrit, leukocytosis, platelet count, and antithrombotic or cytoreductive treatment following the initial thrombosis, we show that an age >60 years is an independent predictor of recurrences (HR, 2.00, 95% CI 1.23–3.37). Actually, the role played by leukocytosis for recurrence reached a level that bordered on statistical significance (HR 1.72, 95% CI 1.00–2.95) when the model included the entire population; the risk was significant for arterial recurrences (HR 2.16, 95% CI 1.12–4.18), but not for venous ones (HR 1.14, 95% CI 0.43–2.98). The risk for arterial recurrences remained significantly increased even after the exclusion of recurrent TIA as outcome of interest (HR 3.13, 95% CI 1.22–7.98). It is noteworthy that recurrence was significantly prevented either by antiplatelet treatment (HR 0.38, 95% CI 0.19–0.77) or by cytoreduction (HR 0.45, 95% CI 0.28–0.73). We were unable to establish a predictive role for vascular recurrences played by gender, diagnosis of PV or ET, presence of vascular risk factors, history of remote thromboses, hematocrit value or platelet count (data not shown).

After the second thrombotic event, the leukocyte number did not significantly influence the risk for a further episode (HR 2.60, 95% CI 0.80–8.42).

In the subgroup of younger patients (<60 years), a WBC of $>12.4 \times 10^9$/L was an independent predictor of arterial recurrences (HR 3.35, 95% CI 1.22–9.19), but not for venous events (HR 2.87, 95% CI 0.40–20.53); no significant risk associated with leukocytosis was noticed among the older patients (>60 years).

Discussion

Leukocytosis has been reported to be an independent risk factor for thrombosis in both PV and ET [6–13]. This is in keeping with the well-established relationship in the general population where the increased leukocyte count predicts a first or a recurrent coronary heart disease and ischemic stroke [15–18]. Cytoreductive therapy with hydroxyurea, which leads to a decrease in the WBC count, has a protective effect on the incidence of thrombosis both in PV and ET [7,14], which further supports a role for leukocytosis in the occurrence of a thrombotic event. In line with this concept are the results of the PT-1 randomized clinical trial where hydroxyurea was tested against anagrelide, which specifically inhibits platelet production and leaves the leukocyte number unaffected. The investigators showed a significant decrease in the number of arterial events in the patients with ET treated with hydroxyurea compared with the patients treated with anagrelide, namely in the group of the JAK2 V617F-positive patients, and it is possible that these results are due to the control of the leukocyte number by hydroxyurea [19,20]. The biological plausibility of the role of leukocytes in thrombogenesis, that is, associated with these disorders has been confirmed by many studies that all indicate increased neutrophil and platelet activation, increased platelet–leukocyte aggregates, endothelial damage, and eventually increased levels of blood hypercoagulability markers [21,22].

The presence of the somatic JAK2 V617F mutation influences both the number of leukocytes and their activation, particularly when the allele burden is more pronounced [23,24], and has been reported to be associated with an increased risk for thrombosis [23–26]. Thus, it is very likely that the JAK 2 mutation exerts its thrombogenic role via leukocyte number and activation. The aforementioned data refer to a correlation between baseline leukocytosis and

the first thrombotic event registered in the follow-up. In our article, we explored whether leukocytosis could also be an additional risk factor for recurrent thrombosis.

In a previous study cohort, patients with PV or ET showing a substantial homogeneity with respect to age, presence of vascular risk factors, and the nature of their first clinical manifestation, did not show any difference in the risk of recurrence [14]. Therefore, the analysis performed in this article grouped PV and ET patients into a single category. Multivariable analysis adjusted for a number of potential confounding factors confirmed that an age over 60 years at the time of the first thrombotic event is associated with a risk of recurrence that is double that of patients aged less than 60 years. Moreover, the presence of leukocytosis at the time of first thrombosis doubled the risk for an arterial but not a venous recurrence. Remarkably, cytoreduction was highly protective halving the incidence of recurrent events. The risk for arterial recurrence associated with leukocytosis was definitely more pronounced among the younger patients (3.3-fold), suggesting that older age is such a strong risk factor that it obscures the role of other predictors.

In conclusion, this study aimed to search the risk factors that predict recurrences in PV and ET, we found that leukocytosis has an impact on the occurrence of subsequent events. This information is of clinical importance, as it suggests that to avoid recurrences cytoreductive therapy after the first event should be aimed at normalizing leukocytes, and calls for prospective randomized trials specifically designed to investigate this issue.

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Appendix

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