To elucidate the role of thrombocytosis, alone or in combination with standard (age, previous cardiovascular events) and novel (leukocytosis, JAK2V617F mutational status) risk factors, in the cardiovascular events of essential thrombocythemia (ET), we analyzed a cohort of 1063 patients. We found that a platelet count at diagnosis greater than 1000 $10^9/L$ was associated with significantly lower rate of thrombosis in multivariable analysis and, if combined with leukocytosis less than 11 $10^9/L$, pointed to a “low-risk” category with a rate of thrombosis of 1.59% of patients/year. On the contrary, the highest risk category (thrombosis rate, 2.95% of patients/year) was constituted of patients with leukocytosis, lower platelet count, and a JAK2V617F mutated genotype in most cases (77% vs 26% in the low-risk group), independently from standard risk factors. These data challenge the theory that elevated platelet count increases thrombosis risk in ET and suggest prospective clinical trials to support this hypothesis. (Blood. 2008;112:3135-3137)

Introduction

Thrombosis is a major complication in essential thrombocythemia (ET). Therapy is driven by patient cardiovascular risk, conventionally defined by age and history of previous thrombosis. Recently, leukocytosis and JAK2V617F mutation have been proposed as disease related prognostic factors. In contrast, the link between thrombocytosis and vascular complications remains uncertain. Nevertheless, expert-produced guidelines recommend that therapy in ET should be aimed at correcting thrombocytosis.

In order to explore the respective role of conventional and new risk factors predicting the incidence of vascular complications, we examined a large cohort of 1063 ET patients and report here the prognostic interaction between patient related and disease related risk factors including platelet and leukocyte counts, and JAK2V617F mutational status.

Methods

The study cohort consisted of 1063 patients with ET diagnosed according to the Polycythemia Vera Study Group and, since 2000, World Health Organization criteria and followed in 3 Italian academic institutions (Ospedali Riuniti di Bergamo, Azienda Ospedaliera-Universitaria Careggi, Florence, and Ospedale San Bortolo, Vicenza). Permission was obtained from the institutional review boards to review the medical records. This study was conducted in accordance with the Declaration of Helsinki.

There were 709 females and 354 males (ratio 2:1) and median age at diagnosis was 55 years (range 8 to 95 years). Median platelet and leukocyte counts were 806 $10^9/L$ (376-3000 $10^9/L$) and 8.8 $10^9/L$ (3.3-35 $10^9/L$), respectively. JAK2V617F mutation was found in 465 of 860 patients (54%) and 5% of them had greater than 50% JAK2V617F allele. A total of 193 of the patients (18%) had a prior history of thrombosis, which was made up of an arterial event in 133 (69%) and venous thrombosis in 60 (31%). Major hemorrhagic events were reported in 38 patients of 1063 (3.6%).

Patients were classified as being at low- or high-risk for thrombosis according to standard risk factors (age ≥ 60 years and/or a previous major thrombotic event). Low risk patients (n = 517, 49%) were followed with no cytoreductive therapy whereas high-risk patients (n = 546, 51%) were given cytotoxic drugs, which were hydroxyurea (HU) in the great majority (90%) of cases and busulfan in a small group of aged patients (10%). The target of therapy was to keep platelet numbers below 600 $10^9/L$. Low-dose aspirin (100 mg daily) was prescribed in 703 patients (66%) according to the indication of the physician in charge.

Only major vascular thrombotic events were examined, including ischemic stroke, cerebral transient ischemic attacks (TIA), acute myocardial infarction (AMI), peripheral arterial thrombosis (PAT), and venous thromboembolism (VTE). Diagnostic procedures for thrombosis and JAK2V617F and MPLW515K/L mutations were performed as previously described.

Cox multivariable models were evaluated unadjusted and subsequently adjusted for center, sex, standard risk factors (age ≥ 60 years and/or previous thrombotic event), hemoglobin at diagnosis, use of antiplatelet drugs, chemotherapy, and JAK2V617F status. All probability values were 2-tailed; $P$ less than or equal to .05 was considered significant.

Results and discussion

During up to 38 years of follow-up (median 4.8 years), 118 major thrombosis (2.3% of patients/year) were objectively diagnosed and included 48 ischemic strokes or TIA, 25 AMI, 11 PAT, and 34 VTE. Severe bleeding episodes (gastrointestinal in 80%) were 39 (0.76% patients/year).

Multivariable analysis confirmed that age and previous thrombosis were independent factors for occlusive events (hazard ratio...
The other major finding of this study is the interaction of thrombocytosis with leukocyte number and JAK2V617F mutation in predicting the thrombotic risk. The lowest risk has been observed in ET patients with low white blood cell count, high platelet count, and low prevalence of JAK2V617F. On the other hand, the typical phenotype of JAK2-mutated ET patients, that is, higher leukocyte and lower platelet count, is associated with the highest thrombotic risk. Even though results of this study deserve to be confirmed by prospective observations, they strengthen the view that overall myeloproliferation rather than platelet count only should be the target of therapy in ET. This is in keeping with Primary Thrombocytopenia 1 (PT1) trial results showing superiority of hydroxyurea

Table 1. Multivariable analysis of the relative risk of total thrombosis among 1063 ET patients

<table>
<thead>
<tr>
<th>Models</th>
<th>Platelet count* (×10^9/L)</th>
<th>White blood cell count* (×10^9/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>650 to 1000</td>
<td>More than 1000</td>
</tr>
<tr>
<td>1. Unadjusted</td>
<td>0.7 (0.4-1.1) .2</td>
<td>0.5 (0.3-0.9) .03</td>
</tr>
<tr>
<td>2. Variables adjusted</td>
<td>0.6 (0.4-1.0) .1</td>
<td>0.5 (0.3-0.8) .01</td>
</tr>
<tr>
<td>3. JAK2V617F adjusted</td>
<td>0.6 (0.4-1.1) .1</td>
<td>0.6 (0.3-1.1) .10</td>
</tr>
</tbody>
</table>

Data are HR (95% CI) P value.
*Reference categories: platelet count less than 650 ×10^9/L; white blood cell count less than 8 ×10^9/L.
†Percentage calculated on 860 patients evaluated for JAK2V617F status (yes/no).
‡Age younger than 60 years and no previous thrombosis; untreated (90%).
§Low risk = age younger than 60 years and no previous thrombosis; untreated (90%).

Results of this study extend and confirm a number of previous observations concerning the uneven relationship between thrombocytosis and thrombosis in ET. The novel finding is the inverse relationship between platelet count and thrombosis. Similar results were obtained in an analysis of 1638 polycythemia vera (PV) patients enrolled in the European Collaboration on Low-Dose Aspirin in Polycythemia Vera study. In this study, time-dependent multivariable analysis showed that patients with PV and thrombocytosis above 500 ×10^9/L had a 30% lower risk of total thrombosis than patients with less than 300 ×10^9/L (relative risk [RR] = 0.64, P = .02). In the present ET patient population, results of multivariable analysis argue against a more aggressive use of cytoreductive therapy as the reason for the lower rate of total thrombosis in patients with extreme thrombocytosis. The most likely explanation of this countintuitive finding is the acquired von Willebrand disease occurring in ET with very elevated platelet count. The decrease of von Willebrand factor (VWF) protein, due to an increased adsorption of large VWF multimers to platelet surfaces consistent with variant type 2A von Willebrand disease, is significantly correlated with the level of thrombocytosis in ET and other myeloproliferative neoplasms. Reduction of the platelet count below 1000 ×10^9/L usually restores the multimeric integrity of the VWF protein with the reversal of the bleeding tendency. Thus, high platelet number is a possible risk factor for major bleeding, but at the same time, it is protective against thrombosis, as observed in patients with congenital von Willebrand disease. In addition to acquired von Willebrand disease, bleeding may also be due to biochemical and functional abnormalities of platelets.

Table 2. Interaction of leukocyte and platelet counts at diagnosis (multivariable model)

<table>
<thead>
<tr>
<th>Interaction</th>
<th>Rate of vascular event (% patients/year)</th>
<th>Low risk† (% patients)</th>
<th>JAK2V617F‡ (% patients)</th>
<th>RR (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. WBC less than 11, Plt more than 1000</td>
<td>1.59</td>
<td>95 (56)</td>
<td>35 (26)</td>
<td>1</td>
</tr>
<tr>
<td>2. WBC less than 11, Plt less than 1000</td>
<td>2.26</td>
<td>317 (49)</td>
<td>304 (56)</td>
<td>1.92 (.034)</td>
</tr>
<tr>
<td>3. WBC more than 11, Plt more than 1000</td>
<td>2.88</td>
<td>41 (41)</td>
<td>40 (61)</td>
<td>2.38 (.026)</td>
</tr>
<tr>
<td>4. WBC more than 11, Plt less than 1000</td>
<td>2.95</td>
<td>52 (40)</td>
<td>75 (77)</td>
<td>2.43 (.017)</td>
</tr>
</tbody>
</table>

WBC and Plt counts are ×10^9/L.
*Twenty patients were excluded because of WBC or platelet count data missing at diagnosis.
†Age younger than 60 years and no previous thrombosis; untreated (90%).
‡Percentage calculated on 860 patients evaluated for JAK2V617F.
(panmyelosuppressive drug) over treatment with anagrelide (specific thrombopoietic inhibitor) in reducing arterial events in JAK2-mutated ET patients.

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Authorship

Contribution: A.C. performed research, analyzed and interpreted data, and wrote the manuscript; G.F. performed research and wrote the manuscript; E.A. performed laboratory experiments and collected data; P.G. performed laboratory experiments; A.M.V. wrote the manuscript and supervised laboratory experiments; F.D., M.R., and F.R. collected clinical data; V.G. performed laboratory experiments; A.R. performed research; and T.B. designed and supervised the research project, wrote the manuscript, and raised funds.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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References

Thrombocytosis and leukocytosis interaction in vascular complications of essential thrombocythemia

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