Thrombocytosis and Leukocytosis Interaction in Vascular Complications of Essential Thrombocythemia

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SHORT TITLE: Platelets and thrombosis in ET patients

SCIENTIFIC SECTION HEADING: Clinical observations, interventions, and therapeutic trials

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Abstract

To elucidate the role of thrombocytosis, alone or in combination with standard (age, previous cardiovascular events) and novel (leukocytosis, JAK2617V>F mutational status) risk factors, in the cardiovascular events of Essential Thrombocythemia (ET) we analyzed a cohort of 1,063 patients. We found that a platelet count at diagnosis greater than 1,000x10^9/L was associated with significantly lower rate of thrombosis in multivariable analysis, and if combined with leukocytes less than 11x10^9/L pointed to a “low-risk” category with a rate of thrombosis of 1.59%patients/year. On the contrary, the highest risk category (thrombosis rate, 2.95%patients/year) was constituted of patients with leukocytosis, lower platelet count and a JAK2617V>F mutated genotype in most cases (77% versus 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.
**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\(^1\). Recently, leukocytosis and *JAK2*\(^{617V>F}\) mutation have been proposed as disease related prognostic factors\(^2\). In contrast, the link between thrombocytosis and vascular complications remains uncertain\(^3\). Nevertheless, expert-produced guidelines recommend that therapy in ET should be aimed at correcting thrombocytosis\(^4,5\).

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 1,063 ET patients and report here the prognostic interaction between patient related and disease related risk factors including platelet and leukocyte counts, and *JAK2*\(^{617V>F}\) mutational status.

**Patients and Methods**

The study cohort consisted of 1,063 patients with ET diagnosed according to PVSG and, since 2000, WHO criteria and followed in three Italian academic institutions (Ospedali Riuniti di Bergamo, Azienda Ospedaliera-Universitaria Careggi, Florence, and Ospedale San Bortolo, Vicenza). Permission was obtained from the IRBs 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 count were 806 (376-3,000) and 8.8 (3.3-35) x10\(^9\)/L, respectively. *JAK2*\(^{617V>F}\) mutation was found in 465 of 860 patients (54%) and 5% of them had greater than 50% *JAK2*\(^{617V>F}\) 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 1,063 (3.6%).

Patients were classified as being at low- or high-risk for thrombosis according to standard risk factors (age \(\geq 60\) 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, that 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 number below 600 x10\(^9\)/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\textsuperscript{6} and \textit{JAK2617V>F} and \textit{MPL W515K/L} mutations were performed as previously described\textsuperscript{7,8}.

Cox multivariable models were evaluated unadjusted, and subsequently adjusted for center, gender, standard risk factors (age $\geq$ 60 years and/or previous thrombotic event), hemoglobin at diagnosis, use of antiplatelet drugs, chemotherapy, and \textit{JAK2617V>F} status. All probability values were two-tailed; $p \leq 0.05$ was considered significant.

\textbf{Results and Discussion}

During up to 38 years of follow up (median 4.8 years), 118 major thrombosis (2.3\%/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 (HR=1.7, 95\% CI= 1.1 – 2.6, $p=0.01$; data not shown). None of the above variables influenced the risk for major bleeding.

Table 1 reports the risk estimates given as Hazard Ratio of two platelet counts and two White Blood Cell (WBC) levels respectively. Compared to a reference platelet count below 650 x10\textsuperscript{9}/L, risk estimates were searched in patients presenting with platelet counts at presentation ranging from 650 to 1,000 x10\textsuperscript{9}/L or above 1,000 x10\textsuperscript{9}/L. In this latter group, a significant reduction of the incidence of arterial and venous thrombosis was seen in univariate analysis (unadjusted model) and in the progressively adjusted models including center, gender, standard risk factors, hemoglobin level, leukocyte values, antiplatelet drugs and chemotherapy. Only when the model was adjusted also for \textit{JAK2617V>F} status, this association was reduced and no longer significant ($p=0.10$). When compared to patients with WBC $< 8$ x10\textsuperscript{9}/L, those with WBC above 11 x10\textsuperscript{9}/L had a significant higher risk of total thrombosis both in unadjusted analysis and in the progressively adjusted predictive models including \textit{JAK2617V>F} status, that \textit{per se} was found an independent predictor for thrombosis in this latter model.

To see whether the prognostic risk of thrombosis was not only driven by individual WBC and platelet counts, but also by their interaction, we analyzed four groups of patients according to the baseline leukocyte (lower or greater than 11 x10\textsuperscript{9}/L) and platelet count (lower or greater than 1,000 x10\textsuperscript{9}/L) count (Table 2). In patients presenting with WBC $< 11\times10^9$/L and extreme thrombocytosis (group 1) the annual rate of events was 1.59 \% patients/year and the prevalence of \textit{JAK2617V>F} mutation was 26\%. In the other three groups the incidence and the relative risk of thrombosis progressively increased, as well as the proportion of \textit{JAK2} mutated patients (up to 77\%).
No difference across groups was demonstrated in terms of conventional risk factor distribution, percentage of JAK2617V>F allele burden and MPL W515K/L mutation frequency (found in 3% of examined patients) (data not shown).

Results of this study extend and confirm a number of previous observations concerning the uneven relationship between thrombocytosis and thrombosis in ET\(^2\). The novel finding is the inverse relationship between platelet count and thrombosis. Similar results were obtained in an analysis of 1,638 Polycythemia Vera (PV) patients enrolled in the ECLAP study\(^9\). In this study, time-dependent multivariable analysis showed that patients with PV and thrombocytosis above 500 x10\(^9\)/L had a 30% lower risk of total thrombosis than patients with less than 300 x10\(^9\)/L (RR=0.64, p=0.02). 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 counterintuitive finding is the acquired von Willebrand disease occurring in ET with very elevated platelet count\(^10\). 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 to <1,000 x10\(^9\)/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\(^10\), but at the same time, it is protective against thrombosis, as observed in patients with congenital von Willebrand disease\(^11\). In addition to acquired von Willebrand disease, bleeding may also be due to biochemical and functional abnormalities of platelets\(^10\).

The other major finding of this study is the interaction of thrombocytosis with leukocyte number and JAK2617V>F 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 JAK2617V>F. 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 PT1 trial results\(^12\) showing superiority of Hydroxyurea (panmyelosuppressive drug) over treatment with Anagrelide (specific thrombopoietic inhibitor) in reducing arterial events in JAK2 mutated ET patients.
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Authorship and Conflict of Interest Statements

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

All authors have no conflict of interest to declare
References

Tables

Table 1. Multivariable analysis on the relative risk of total thrombosis among 1,063 ET patients

<table>
<thead>
<tr>
<th>Hazard Ratio (95% CI) p-value</th>
<th>Platelet count* (x10^9/L)</th>
<th>White Blood Cell count* (x10^9/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>650 to 1,000</td>
<td>More than 1,000</td>
</tr>
<tr>
<td></td>
<td>8.0 to 11.0</td>
<td>More than 11.0</td>
</tr>
<tr>
<td>Total thrombosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1) Unadjusted</td>
<td>0.7 (0.4-1.1)</td>
<td>0.2 (0.3-0.9) 0.03</td>
</tr>
<tr>
<td></td>
<td>1.3 (0.8-1.9)</td>
<td>0.7 (1.0-2.7) 0.04</td>
</tr>
<tr>
<td>2) Variables adjusted</td>
<td>0.6 (0.4-1.0)</td>
<td>0.1 (0.3-0.8) 0.01</td>
</tr>
<tr>
<td></td>
<td>1.4 (0.9-2.1)</td>
<td>1.8 (1.1-2.9) 0.02</td>
</tr>
<tr>
<td>3) +JAK2V617F adjusted</td>
<td>0.6 (0.4-1.1)</td>
<td>0.1 (0.3-1.1) 0.10</td>
</tr>
<tr>
<td></td>
<td>1.5 (0.9-2.5)</td>
<td>2.0 (1.1-3.6) 0.02</td>
</tr>
</tbody>
</table>

*Reference categories: platelet count < 650x10^9/L; white blood cell count < 8x10^9/L
Model 1: platelet count (3 categories) or white blood cell count (3 categories)
Model 2: model 1 plus center (3 categories), sex (2 categories), standard risk factors: age ≥ 60 years and/or previous thrombotic event (yes/no), hemoglobin at diagnosis (2 categories, median value used as cut-off), white blood cell count or platelet at diagnosis respectively (2 categories, median value used as cut-off), antiplatelets use (yes/no), chemotherapy use (yes/no)
Model 3: model 2 plus JAK2V617F status (yes/no)

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

<table>
<thead>
<tr>
<th>Interaction</th>
<th>N (%)*</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 &lt;11 + PLT &gt; 1000</td>
<td>170 (16)</td>
<td>1.59</td>
<td>95 (56)</td>
<td>35 (26)</td>
<td>1</td>
</tr>
<tr>
<td>2) WBC &lt;11 + PLT &lt; 1000</td>
<td>644 (62)</td>
<td>2.26</td>
<td>317 (49)</td>
<td>304 (56)</td>
<td>1.92 (0.034)</td>
</tr>
<tr>
<td>3) WBC &gt;11 + PLT &gt; 1000</td>
<td>99 (9)</td>
<td>2.88</td>
<td>41 (41)</td>
<td>40 (61)</td>
<td>2.38 (0.026)</td>
</tr>
<tr>
<td>4) WBC &gt;11 + PLT &lt; 1000</td>
<td>130 (12)</td>
<td>2.95</td>
<td>52 (40)</td>
<td>75 (77)</td>
<td>2.43 (0.017)</td>
</tr>
</tbody>
</table>

* 20 patients were excluded because of WBC or platelet count data missing at diagnosis
** Age < 60 years and no previous thrombosis; untreated (90%)
*** Percentage calculated on 860 patients evaluated for JAK2V617F
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