Influence of the JAK2 V617F Mutation and Inherited Thrombophilia on the Thrombotic Risk among Patients with Myeloproliferative Disorders

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ABSTRACT

Background: A number of studies showed that the JAK2 V617F mutation increases the thrombotic risk in patients with myeloproliferative disorders (MPN) while others did not reveal this correlation, and it is unknown whether inherited thrombophilia is an additive risk factor in mutated subjects. Our aim was to clarify the contribution of JAK2 V617F to a hypercoagulable state, as well as its interaction with other thrombophilic factors in patients with thrombosis and myeloproliferative disorders.

Method: We studied 192 patients with myeloproliferative disorders, 90 with Essential thrombocytopenia (ET), 42 with Polycythemia vera (PV) and 60 with Primary or idiopathic myelofibrosis (PMI). From these patients a subgroup of only 62 patients underwent laboratory screening for thrombophilia.

Results: The JAK2 V617F mutation was present in 62.8% patients with myeloproliferative disorders, 97.6% with PV, 54.5% with ET and 53.44% patients with PMI. The mutated patients had a relative risk (RR) for thrombosis at any time of 2.94 in comparison with “wild-type” patients which was 0.93; in those patients having both the mutation and thrombophilia the RR was 3.56 (95% CI 2.41-7.34) compared to patients with neither the mutation nor thrombophilia, suggesting an additive interaction between the two risk factors.

Conclusion: In patients with myeloproliferative neoplasias, the thrombotic risk is higher in the JAK2 V617F-mutated subgroup and it is further increased by the presence of inherited thrombophilia (especially by the presence of mutated F V Leiden and lupus anticoagulant).

Keywords: myeloproliferative disorders, JAK2 V617F mutation, thrombosis, inherited thrombophilia
INTRODUCTION

The clinical course of the predominant Myeloproliferative diseases (MPDs) e.g. Essential Thrombocytemia (ET), Polycythemia vera (PV), Primary Myelofibrosis (PMI) excluding Chronic myeloid leukemia (CML) is characterized by thrombotic and hemorrhagic events that significantly impact upon prognosis and quality of life. Thrombosis predominates in ET and PV and is more rare in PMI in proliferative stages. Considering large selected studies, prevalence rates for major thrombosis, at time of diagnosis, range from approximately 34 to 39 % for PV, 10 to 29 % for ET and 4 to7% in PMI; the corresponding figures for thrombosis at follow-up are approximately 8 to 19 % for PV, 8 to 31 % for ET and 2 to 4 % in MPI. In all instances, arterial events (acute coronary disease, stroke/transient ischemic attack, acute limb ischemia) are more common than venous thrombotic events (splanchnic thrombosis, lower or upper limb deep venous thrombosis). The splanchnic thromboses (suprahepatic, portal, and splenic) are quite specific for PV and ET and represent sometimes catastrophic, even fatal events. Minor thrombotic and microcirculatory events, such as superficial venous thrombosis, untreatable headache, Raynaud phenomenon, or erythromelalgia are also common among these patients. It is well known that advanced age and a prior history of thrombosis are the two most important risk factors for vascular complications.

The term thrombophilia includes any inherited and acquired disorders associated with an increased tendency to venous thromboembolism (VTE). Factor V Leiden and prothrombin G20210A mutations are the most frequent causes of inherited thrombophilia in Caucasians. Due to their relatively high frequency in the general population, a contribution of these mutations to the thrombotic risk of MPN patients seems an attractive hypothesis. Hyperhomocysteinemia may influence also the risk for both arterial and venous thrombosis. Two common mutations of the methylenetetrahydrofolate reductase (MTHFR) gene, namely 677 C>T and 1298 A>C are associated with decreased enzymatic activity of the MTHFR and may lead to hyperhomocysteinemia.

A number of studies have been trying to determine whether an inherited thrombophilia is an additive risk factor for thrombosis, but their results remained elusive. Such discrepancies could be explained, in part, by different mutational loads in the patients investigated, related to whether the individuals harbored the mutation in the homozygous status (i.e. >50% mutant allele burden), who are more prone to overall thrombosis. The effect of the combined carriership of the JAK2 V617F mutation and the inherited thrombophilia on the thrombotic risk is unknown.

In the present study, we investigated in a cohort of 192 patients with myeloproliferative neoplasias the risk of thrombosis according to the JAK2 V617F mutational load and correlation between thrombotik risk and the presence of thrombophilia.

DESIGN AND METHODS

We conducted a retrospective cohort study among 192 patients with MPN diagnosed according to either previous or updated WHO criteria between 2000-2008, 90 patients with ET, 42 patients with PV and 60 patient with PMI. From these patients we made a subgroup of 62 patients who underwent laboratory screening for thrombophilia.

The patients were recruited from the Hematology Department of Coltea Clinical Hospital Bucharest, the laboratory tests were performed at The National Institute of Virology „Stefan Nicolau“ Bucharest, and the tests for thrombophilia were performed at Colentina Clinical Hospital Bucharest.

Genomic DNA was extracted from peripheral blood granulocytes by standard procedures. The JAK2 V617 mutation was detected by allele-specific polymerase chain reaction, restriction-PCR, TaqMan PCR quantity and sequencing at the Institute of Virology „Stefan S. Nicolau“. Heterozygous or homozygous status was defined as a mutant allele burden under 50% or up to 50%, respectively.

Screening for thrombophilia included measuring of antithrombin, protein C functional activities, free protein S activity, homocysteine; FV Leiden, PT C20210A, and antiphospholipid antibodies (lupus anticoagulant).

The events of interest were major thromboses, arterial or venous (ischemic stroke or transient ischemic attack (TIA), acute coronary syndrome (acute myocardial infarction or unstable angina pectoris), peripheral arterial thrombosis,
retinal artery or vein occlusion, thrombosis of deep veins (including cerebral and splanchic veins), and pulmonary embolism, splanchic venous thrombosis includes occlusion of hepatic, portal, mesenteric, and splenic veins. Moreover, superficial vein thromboses diagnosed by ultrasound objective methods were also computed. We noted separately microthrombotic complications including regular presence of symptoms of microvascular damage (headache, acral paresthesia, erythromelalgia, transient neurological and visual impairment). They were reported both at diagnosis and newly diagnosed cases as they occurred after the diagnosis. The thrombotic events were diagnosed based on clinical and imaging (computed tomography, arterial or venous Doppler ultrasound, or coronarography) criteria.

For statistical analysis we used chi-square test for nominal variables, t-test and ANOVA for continuous variables comparing averages. Differences between groups were estimated by the Fisher’s exact test, the χ² test, and the Mann-Whitney test employed when appropriate (statistical significance p<0.05). The relative risk (RR) for thrombosis with the 95% confidence interval (95%CI) was estimated by a 2 x 2 contingency table.

Based on this statistical analysis, correlations between the presence or absence of mutations and clinical and biological presentation were extracted and compared with those reported in the literature.

RESULTS

After we performed the mutational screening, we reported the presence of JAK2 V617F in 62.8% of patients with MPN (97.6% of patients with PV, 54.5% of patients with ET and 53.4% patients with PMI).

Thrombotic complications were more common in ET and PV subgroups, where we reported 17% thrombosis in ET group, and 18.6% respectively in the group of patients with PV, while in the PMI group there were only 2% such cases.

Regarding the microthrombotic complications we found a rate of 19% of the total group, with 8.4% in the group of PV, 6.4% in the group of ET and 4.2% in the group of PMI.

Among the frequent circumstantial factors known to increase the risk of thrombosis, arterial hypertension was noted in 58 patients (30.21%), prior thrombosis was noted in 24 patients (12.5%), old age (more than 65 years old) in 49 patients (25.52%), obesity in 24 patients (12.5%), while diabetes mellitus was seen in 19 patients (7.81%). Fifty-three patients (27.60%) were cigarette smokers.

We report a rate of thrombotic complications in the entire group of MPN of 42.8%, of which 27.4% had JAK2 V617F mutation and 15.4% were “wild-type”. Among these patients, those with the JAK2 V617F mutation had a relative risk of thrombosis of 2.94 (95% Confidence Interval 0.96-3.96), while in “wild-type” patients, this was 0.93 (95% Confidence Interval 0.13-1.12).

Regarding microthrombotic complications they were found at a rate of 32.4%. 16.4% of these patients had JAK2 V617F mutation and 17% were “wild type”.

There is statistical correlation between this mutation and the risk of thrombotic events in the ET group (p = 0.002; confidence interval (CI) of = 95%) and in the PV group (p = 0.001; CI = 95%), but not in the PMI group.
The prevalence of inherited thrombophilia did not differ among patient groups (JAK2 V617F absent versus present). In the whole group, the relative risk for thrombosis associated with thrombophilia was 3.20 (95% CI 1.75-4.70). Among the patients with the JAK2 V617F mutation, those with thrombophilia had a risk of thrombosis of 3.56 compared to those without thrombophilia where it was 2.94. Among patients without thrombophilia those with the JAK2 V617F mutation had a relative risk of thrombosis of 3.04 (95% CI 0.96-3.96) compared to those without the mutation who had a risk of 2.01.

The carriers of both the JAK2 V617F mutation and inherited thrombophilia had a risk of 3.56 (95% CI 2.41-7.34) compared to patients with neither the mutation nor thrombophilia. During follow-up, the carriers of both the JAK2 V617F mutation and inherited thrombophilia had a relative risk of 5.32 (95% CI 0.58-7.40) compared to patients with neither the mutation nor thrombophilia, without achieving statistical significance likely due to the small number of events. None of them had a recurrent thrombosis.

**DISCUSSION**

The JAK2 V617F mutation was present in 62.8% patients with myeloproliferative disorders, 97.6% with PV, 54.5% with ET and 53.44% patients with PMI.

In 2007, Ruggeri M, et al. published in American Journal of Hematology the article Factor V Leiden mutation carrierhip and venous thromboembolism in polycythemia vera and essential thrombocythemia in which they reported the results of a study on a cohort of 304 patients with PV and ET. 16% of those with venous thromboembolism had a relative risk of 3.56 (95% CI 2.41-7.34) compared to patients with neither the mutation nor thrombophilia.

During follow-up, the carriers of both the JAK2 V617F mutation and inherited thrombophilia had a relative risk of 5.32 (95% CI 0.58-7.40) compared to patients with neither the mutation nor thrombophilia.

Most of the patients were treated with hydroxyurea (49%), a percentage of 11.2 were treated with anagrelide, 11.7% did not attend any treatment and only 5.6% were treated with interferone (IFN). A percentage of 22.4 of the patients were given combination therapy. The chart below illustrates the correlations between the treatment used and the presence of JAK2 V617F mutation on the entire lot of MPN.

A percentage of 22.4 of the patients were given combination therapy. The chart below illustrates the correlations between the treatment used and the presence of JAK2 V617F mutation on the entire lot of MPN.

### Table 1. The distribution of complications according to JAK2 V617F mutation.

<table>
<thead>
<tr>
<th>Complications</th>
<th>Micro-thrombotic</th>
<th>Thrombotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>JAK2 V617F Absent N=69</td>
<td>number</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>17%</td>
</tr>
<tr>
<td>JAK2 V617F Present N=123</td>
<td>number</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>16.4%</td>
</tr>
<tr>
<td>Total N=192</td>
<td>number</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>32.4%</td>
</tr>
</tbody>
</table>

### Table 2. The results of the screening for thrombophilia and its correlation with thrombosis.

<table>
<thead>
<tr>
<th>Patients (No,%), Control (No,%), Correlation with thrombosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor V: 5 (80.66), 57 (11.94)</td>
</tr>
<tr>
<td>Lupus anti-coagulant: 9 (14.51), 53 (85.49)</td>
</tr>
<tr>
<td>Protein C deficiency: 0 (0), 0 (0)</td>
</tr>
<tr>
<td>Protein S deficiency: 0 (0), 0 (0)</td>
</tr>
<tr>
<td>Anti thrombin III deficiency: 0 (0), 0 (0)</td>
</tr>
<tr>
<td>Factor III mutation: 12 (19.35), 50 (80.65)</td>
</tr>
<tr>
<td>Hyper-homo-cysteinemia: 34 (45.16), 28 (54.84)</td>
</tr>
<tr>
<td>JAK2 V617F mutation: 34 (45.16), 28 (54.84)</td>
</tr>
<tr>
<td>Correlation with thrombosis: p = 0.031, p = 0.023, NA, NA, 0.554, 0.003, 0.002</td>
</tr>
</tbody>
</table>
thrombosis in the general population, without interaction with the thrombotic risk due to myeloproliferative neoplasms.

Another study was conducted on a cohort of 214 patients with PV and ET in 2007. Gisslinger H, et al. published the results in the article Mutation of the prothrombin gene and thrombotic events in patients with polycythemia vera or essential thrombocythemia: a cohort study. In this study, the risk for venous thromboembolism associated with the prothrombin (PT) G20210A was claimed to be higher than that expected.

In our study the mutated patients had a relative risk (RR) for thrombosis at any time of 2.94 in comparison with „wild-type“ patients which was 0.93; in those patients having both the mutation and thrombophilia the RR was 3.56 (95% CI 2.41-7.34) compared to patients with neither the mutation nor thrombophilia, suggesting an additive interaction between the two risk factors.

A meta-analysis of 2,436 patients with ET, conducted by Dahabreh IJ, Zoi K, Giannouli S, Zoi C, Loukopoulos D, Voulgarelis M. was published in Leuk Res in 2009;33:67-73 in the article named Is JAK2 V617F mutation more than a estimated diagnostic index? A meta-analysis of clinical outcomes in essential thrombocythemia. They concluded that the JAK2 V617F mutation was associated with a 1.8-fold increased risk for thrombosis.

Valerio De Stefano from the Institute of Hematology, Catholic University, Rome studied 132 patients with essential thrombocythemia and concluded that in younger patients with ET the thrombotic risk is higher in the JAK2 V617F mutated group and is further increased by the presence of inherited thrombophilia.

In contrast, in another study, Carobbio A, Antonioli E, Cuglielmi M, Vannucchi AM, Delfini F, Guerini V, et al. published in J Clin Oncol 2008;26:2732-6 the article Leukocytoysis and risk stratification assessment in essential thrombocythemia in which they reported the results on a cohort of 657 patients with ET. Their conclusion pointed that the JAK2 V617F mutation did not influence the risk for thrombosis.

We had 12 patients with abnormal levels of homocysteine that were correlated with a high incidence of acute coronary syndrome (acute myocardial infarction or unstable angina pectoris), 5 patients were heterozygous for FV Leiden (8.06%), and 3 heterozygous for PT G20210A (2.3%). This is quite comparable to the prevalence of those polymorphisms among caucasian individuals.

The carriers of both the JAK2 V617F mutation and inherited thrombophilia had a risk of 3.56 (95% CI 2.41-7.34) compared to patients with neither the mutation nor thrombophilia, suggesting an additive interaction between the two risk factors.

**CONCLUSION**

The JAK2 V617F mutation was associated with an increased risk for thrombosis in patients with myeloproliferative neoplasias. Inherited thrombophilia produced a limited impact on the overall risk for thrombosis at any time, but this risk was increased among carriers versus noncarriers. However, in the presence of both the JAK2 V617F mutation and inherited thrombophilia, the risk was 5-fold increased in compared to non-carriers of either alteration during follow-up, suggesting an additive interaction.

We acknowledge that the results of our study are based on a small number of individuals carrying both inherited thrombophilia and the JAK2 V617F mutation and we did not measure all the thrombotic parameters.

The characteristics of our cohort reflect the current knowledge concerning MPN regarding the rate of thrombosis, the rate of the JAK2 V617F mutation and the phenotype associated. The prevalence of inherited thrombophilia...
was similar to that found in the general population.

Accordingly, it can be suggested that the awareness of the JAK2 V617F mutation (especially in the homozygous state) and of thrombophilia could allow a further risk stratification among the low-risk patients <60 years without history of thrombosis. The magnitude of the thrombotic risk in such patients and the final opportunity of employing such criteria for risk stratification and for individually tailored therapy during follow-up must be confirmed by prospective trials.

Based on these findings, we propose that patients with MPN, should be screened for thrombophilic mutations. It is unclear whether MPN patients without major thrombosis, positive for thrombophilic mutations should require a new standard of care, such as prophylactic anticoagulation. Of course, these questions can only be answered by the results of large randomized clinical trials.

Conflict of interests: none declared.

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REFERENCES


