Vascular events in Korean patients with myeloproliferative neoplasms and their relationship to JAK2 mutation

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Summary

Evaluation of the Janus kinase 2 (JAK2) V617F mutation has been widely used for the diagnosis of myeloproliferative neoplasms (MPN). However, its prognostic relevance to clinical outcome is not completely understood. We investigated the association of JAK2 V617F with vascular events in Korean patients with myeloproliferative neoplasms (MPN). We studied 283 patients from 15 centers, who were diagnosed with MPN. The JAK2 V617F status was evaluated by allele-specific polymerase chain reaction (PCR) and sequencing. The patients’ diagnoses were essential thrombocythemia (ET n=146), polycythemia vera (PV n=120), primary myelofibrosis (n=12), and unclassifiable MPN (MPNu n=5). JAK2 V617F was detected in 89 (61%) patients with ET, 103 (86%) with PV, four (33%) with myelofibrosis, and four (80%) with MPNu. A higher number of leukocytes, haemoglobin levels and BM cellularity as well as an older age, lower platelet counts, and diagnosis of PV were significantly correlated with JAK2 V617F. Eighty-three and 43 episodes of thrombosis and bleeding occurred in 100 patients each before and after the diagnosis. Vascular events more frequently occurred in 37% of patients with JAK2 V617F than in 29% of those without the mutation (p=0.045). Among 175 patients whose samples were available for sequencing, 28 patients with homozygous JAK2 V617F had vascular events more frequently (57%) than those who were heterozygotes (39%) or had the wild type (27%) (p=0.03). The multivariate analysis showed that a JAK2 homozygous mutation, hypercholesterolemia and older age were independent risk factors for a vascular event. The results of this study showed that Korean patients with MPN had a similar JAK2 mutation rate and frequency of vascular events when compared to Western patients. The presence of V617F was significantly related to vascular events. Therefore, initial evaluation for the JAK2 mutation and careful monitoring for vascular events should be performed in MPN patients.

Keywords

Myeloproliferative disorders, Janus kinase 2, thrombosis, bleeding

Introduction

The identification of the Janus kinase 2 (JAK2) V617F mutation (1–5) has been followed by significant changes in the diagnostic criteria and classification of chronic myeloproliferative disorders. The 2008 World Health Organization (WHO) criteria for the classification and diagnosis of myeloproliferative disorders were recently released (6). The most important change in the 2008 WHO report was the replacement of ‘chronic myeloproliferative disorders’ with ‘myeloproliferative neoplasms (MPN)’. It
is now well established that clonal neoplasms share the JAK2 mutation, including V617F and other sites, and the phenotypic diversity of these disorders is partly attributed to JAK2 mutation.

The three common diseases included in the MPNs are polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF). The JAK2 V617F mutation is found in up to 97% of PV patients and in about half of the ET and PMF patients. The proportion of homozygous mutations has been reported to be 21~70% in PV, 9~22% in PMF and 0~6% of ET (7). The differential diagnosis of PV and ET, in patients with similar clinical features may be aided by JAK2 mutation studies; for example, if the JAK2 mutation is homozygous, this favors the diagnosis of PV. Recent studies have reported that a higher JAK2 V617F allele burden could be associated with a disease phenotype such as older age, greater frequency of pruritus, higher white blood cell (WBC) counts, lower platelet (PLT) counts, and higher rates of splenomegaly (8–10). In addition, disease outcomes such as thrombosis and leukemic transformation have been reported to be related to the JAK2 mutation status in patients with MPN (9–10). However, there is limited information on vascular events in Asian patients with MPN. One report suggested that the thrombotic risk, in Chinese patients with ET, was comparable to Western patients, but that the bleeding risk was much lower (11–13). Therefore, we investigated the incidence and the characteristics of vascular complications in Korean patients with MPN and studied the association of JAK2 mutation with vascular events.

Materials and methods

After approval from the institutional review boards, clinical data and peripheral blood samples of 330 MPN patients from 15 centers were collected by the Korean MPN working party from March 2006 to September 2007. These patients were diagnosed as MPN according to the criteria of Polycythemia Vera Study Group or WHO, and adequately treated according to the thrombotic risk such as age, prior vascular event and cardiovascular risk factors (14). Among them, 40 patients were excluded due to insufficient data on their vascular complications, four because of the absence of adequate samples and three with the diagnosis of the hypereosinophilic syndrome. Finally, 283 patients were enrolled in this study. Cytoreductive agents such as hydroxyurea, anagrelide, interferon, and chlorambucil were used in 212 (74.9%), 68 (24.0%), one (0.4%) and one (0.4%) patients, respectively. One hundred nine patients (38.5%) received aspirin except one with ticlopidine for the prevention of thrombosis. Phlebotomy for the control of polycythemia performed in 91 (32.2%) patients. Only 11 (3.9%) patients were untreated with at a regular follow-up. For the symptom palliation of 12 PMF patients, transfusion was main treatment in eight patients and also steroid, oxymetholone, danazol and thalidomide were tried in two, one, one, and one patients, respectively. DNA samples were delivered to the central laboratory for JAK2 V617F testing using allele-specific PCR in all 283 patients and followed by sequencing in 175 patients (2).

Vascular complications were reviewed both prior to or at the diagnosis of MPN as well as during the follow-up. Thrombosis included arterial or venous thrombosis and ischaemic events such as angina pectoris or transient ischaemic attacks (TIA), Erythromelalgia, a digital microvascular insufficiency, was also included in the assessment. A Grade 2 or more bleeding was graded according to the WHO bleeding scale (grade 0, no bleeding; grade 1, petechiae; grade 2, mild blood loss; grade 3, gross blood loss; grade 4, debilitating blood loss).

Statistical analysis

We compared the clinical characteristics at diagnosis according to the mutational status of JAK2. The chi² test was used to test for significant differences with regard to gender, diagnosis, splenomegaly, M:E ratio, bone marrow cellularity or fibrosis, low serum erythropoietin, and vascular events at diagnosis and during the follow-up. In addition, a t-test was used to detect significant differences in age and blood counts including haemoglobin, WBCs and PLTs. The correlation of the JAK2 mutation and vascular events was analyzed using the chisq test. Multivariate analysis for significant factors associated with vascular events was performed using binary logistic regression. Two-way interactions between the JAK2 mutation and other covariates were also tested. All calculations were performed with the SPSS system, version 11.0.

Results

JAK2 mutational status and its clinical relevance

The JAK2 mutation was detected in 89 (61%) of 146 patients with ET, 103 (86%) of 120 patients with PV, 4 (33%) of 12 patients with PMF, and four (80%) of five patients with unclassifiable MPN. The initial clinical and laboratory findings according to the JAK2 V617F status are compared in Table 1. The presence of the JAK2 mutation correlated with an older age (p=0.008), higher haemoglobin (p=0.000) and leukocyte counts (p=0.000), and BM hypercellularity (p=0.022). High platelet counts were more common with the JAK2 wild type (p=0.017). Vascular events, bleeding or thrombosis, frequently occurred in patients with JAK2 mutation (p=0.045).

The incidence and profiles of vascular events

During a mean follow-up of 53 months (range; 0~230 months), 14 patients died. The causes of death were mainly infection in nine patients. In addition, there was one case each of multiorgan failure, transformation to acute myeloid leukemia and myelofibrosis, and hepatic failure after the activation of hepatitis B virus and C virus. No thrombosis or bleeding related mortality was observed. The vascular events (83 episodes in 80 patients) prior to or at the time of diagnosis occurred more frequently than during the follow-up period (43 episodes in 39 patients) in the 283 patients. Thrombosis (87 episodes in 74 patients) was more prevalent than bleeding (39 episodes in 37 patients). Arterial thrombosis or transient ischaemia accounted for most of the episodes of thrombosis (84/87, 97%). The most frequent site of arterial thrombosis was a cerebral artery (48/69, 70%). Bleeding episodes also occurred more frequently before the treatment of the MPN than after the treatment. During the follow-up, two patients stopped taking medication on their own, and then had an intracranial haemorrhage and haemoptysis, (respectively). The most prevalent site of bleeding was the mucosa and skin, followed by the central nervous system, muscle and viscera. These
findings are summarized in Table 2. Aspirin use did not affect significantly to the occurrence of thrombosis, bleeding, and transient ischaemia, and all vascular events, respectively.

**Mutation level of JAK2 V617F and association with vascular events**

Sequencing was performed in 175 patients; their diagnoses were PV in 78, ET in 84, PMF in 10, and unclassifiable MPN in three patients. The proportion of homozygous mutations was 31% for PV, 1% for ET, 20% for PMF, and 33% for unclassifiable MPN. Vascular events at anytime occurred in 57% of patients with homozygous mutations, 39% of patients with heterozygous mutations and 27% of patients with the wild type (p=0.003). The multivariate analysis showed that the patients with homozygous mutations had more frequent vascular events than did the patients with heterozygous mutations or the wild type (p=0.012). Also among the known risk factors for thrombosis, hypercholesterolemia (total cholesterol ≥240mg/dl) and an older age (age ≥60) were significant independent risk factors (p=0.028 and p=0.008).

**Discussion**

This is the first detailed report on the vascular events associated with *JAK2* mutation in Korean patients with myeloproliferative neoplasms. The *JAK2* V617F mutation rate and genetic load for PV, ET and PMF were similar to those reported for Western MPN patients (7). The association of the mutation with the disease phenotype characterized by advanced age, high haemoglobin and white blood cell counts and increased BM cellularity was also concordant with previously published data (8–10). There have been many contradictory results reported for the correlation of the JAK2 mutation with vascular events; prior reports have mostly focused on thrombosis, a more common and serious complication in patients with MPN than bleeding. However, our data showed the association of *JAK2* mutation with thrombotic and haemorrhagic complications. In addition, the genetic load of the *JAK2* mutation significantly increased the chance of vascular events by the multivariate analysis. The separate analysis for thrombotic and haemorrhagic events showed no correlation to the *JAK2* mutation itself or allele burden (data not shown).

The mechanism underlying bleeding complications in patients with MPN has been studied with a focus on platelets. Over-activated platelets lose their normal function at the second wave of aggregation in response to adrenaline. In addition, the activation of platelets causes the consumption of the large von Willebrand factor multimer, which results in bleeding (15). Thrombosis is thought to be related to leukocytosis (15,000/mm³) rather than thrombocytosis. Granulocytes form complexes with platelets and endothelial cells mediated by tissue factors secreted from the monocytes and neutrophils (13).

The results of our study, and a Chinese report on ET (12), showed that the rate of thrombotic complications was similar to the findings of a review of Western studies (13). A major thrombotic event was reported to be a complication in 11–38.6% of Western patients with MPN at the time of diagnosis and in 8–30.7% during treatment. The results of our study showed that a major thrombosis occurred in 19% at diagnosis and in 6% dur-

<table>
<thead>
<tr>
<th>Vascular events</th>
<th>Prior to or at the diagnosis</th>
<th>After the diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Thrombosis</strong></td>
<td>61</td>
<td>26</td>
</tr>
<tr>
<td><strong>Arterial</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebral artery</td>
<td>38</td>
<td>10</td>
</tr>
<tr>
<td>Coronary artery</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Peripheral artery</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Erythromelagia</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Visceral</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td><strong>Transient ischaemia</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angina</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>TIA</td>
<td>3</td>
<td>3</td>
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<tr>
<td><strong>Venous</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visceral vein</td>
<td>1</td>
<td></td>
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<tr>
<td>Deep vein</td>
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<td></td>
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<tr>
<td><strong>Bleeding</strong></td>
<td>22</td>
<td>17</td>
</tr>
<tr>
<td>Mucocutaneous</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Muscular or visceral</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Intracranial</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>83</td>
<td>43</td>
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TIA: transient ischaemic attack.
ing treatment. Thrombosis was reported to be present in 13% of patients at diagnosis and in 10% during treatment in a Chinese study (12). The shorter median time of follow-up in our study may have affected the slightly lower incidence of thrombosis during treatment. The incidence of bleeding complications was reported to be 3~18% at diagnosis and 2.9 ~11.5% at follow-up in Western studies (13); our results showed an incidence of bleeding of 7% at diagnosis and 4% at follow-up. Therefore, Korean patients with MPN appear to have vascular events with a similar frequency when compared to Western patients. In general, the risk of arterial and venous thrombosis in the general population of native Asians is low (16, 17). The incidence of ischaemic heart disease and idiopathic venous thrombembolism has been shown to be lower in Native Asians than in Western countries including many different ethnic groups (white and black). However, the presence of stroke has been reported to be more prevalent in Asians than in European persons (white). In addition, thrombosis associated with solid tumor cancers has been reported to be less frequent in Asian patients when compared to other races (18).

The patterns of the vascular events identified in this study had three salient features. The first was the frequent occurrence of stroke, including TIAs and intracranial haemorrhage, which accounted for 48.4% of the 126 vascular episodes. This confirms the prior findings of frequent stroke in Chinese patients with MPN (12). Cerebrovascular events accounted for 50.7% of the 75 vascular events. In a large English study, cerebrovascular accidents accounted for 20.3% of the vascular events (19). The second important feature was that serious bleeding from intracranial, muscular or visceral organs occurred mainly at the time of diagnosis. The two patients who had an intracranial haemorrhage and haemoptysis each had had poor compliance with treatment before the serious bleeding occurred. The last point was about the very low incidence of venous thrombosis in our patients. Only one patient with Budd-Chiari syndrome (BCS) and two patients with deep-vein thrombosis were noted. Overt and latent MPN with JAK2 mutation accounted for up to 50% in Western BCS patients (20). Patients with multiple prothrombotic disorders took a proportion of around 28% among BCS (21, 22).

The most frequent type of congenital thrombophilia in Western patients is factor V Leiden mutation, but no Korean patient with this mutation has been documented until now. This may account partly the low incidence of BCS and deep-vein thrombosis in Korean MPN patients. Also prospective study performing vigorous search for silent venous thrombosis in a highly suspicious patient (23) is necessary.

In summary, the results of this study showed that Korean patients with MPN had a similar incidence of the JAK2 mutation and vascular events compared to Western patients with MPN. In addition, we found that the JAK2 mutation, especially the homozygous mutation, was associated with a significantly increased risk for vascular events. Therefore, patients with MPN and JAK2 V617F homozygous status should be monitored carefully for their vascular events. Also, all patients with MPN should be monitored carefully for their thrombogenic risks and all potential correctable factors, which might include the modification or control of the following cardiovascular risks: smoking, hypertension, hyperlipidemia, diabetes mellitus, obesity, physical inactivity, and diet. Further study is needed to determine whether the modification of cardiovascular risk factors reduces the frequency of vascular events and minimizes the influence of JAK2 mutations.

What is known about this topic?

- Janus kinase 2 (JAK2) V617F mutation was found in 50~97% of the myeloproliferative neoplasms (MPN), and its relation to vascular events is not completely understood.

What does this paper add?

- Korean MPN patients had a similar JAK2 mutation rate and frequency of vascular events to Western patients.
- The presence of JAK2 V617F was significantly related to vascular events with other risk factors such as hypercholesterolemia and old age.

References