JAK2 Mutations Across a Spectrum of Venous Thrombosis Cases

Shrimati Shetty, PhD, Bipin Kulkarni, PhD, Navin Pai, MSc, Preeti Mukundan, MSc, Priyanka Kasatkar, MSc, and Kanjaksha Ghosh, MD, MRCP, MRCPI, FRCPath

Key Words: JAK2 mutations; Venous thrombosis; Splanchnic venous thrombosis

The JAK2V617F mutation is recurrent in polycythemia vera and essential thrombocytopenia, which are myeloproliferative neoplasms frequently associated with arterial and venous thromboembolism. It has also been reported as a marker for occult myeloproliferative disorder (MPD) in patients with splanchnic venous thrombosis. Limited data are available regarding the prevalence of the JAK2V617F mutation in patients with thrombosis outside the splanchnic region. The present study was undertaken to address these issues in a large series of patients with thrombosis in the splanchnic and nonsplanchnic regions.

Abstract

The JAK2V617F mutation is recurrent in polycythemia vera and essential thrombocytopenia, which are myeloproliferative neoplasms frequently associated with arterial and venous thromboembolism. It has also been reported as a marker for occult myeloproliferative disorder (MPD) in patients with splanchnic venous thrombosis. Limited data are available regarding the prevalence of the JAK2V617F mutation in patients with thrombosis outside the splanchnic region. For the study, 321 cases of venous thrombosis in the splanchnic and nonsplanchnic regions (cerebral venous thrombosis [CVT], 70; deep venous thrombosis [DVT], 36; Budd-Chiari syndrome [BCS], 137; portal venous thrombosis [PVT], 78) were studied for the presence of JAK2 mutations. The prevalence values for the JAK2 mutation were 3% (1/36), 8.8% (12/137), 5% (4/78), and 3% (2/70) in DVT, BCS, PVT, and CVT, respectively; 19 (5.9%) of 321 cases were positive for the JAK2 mutation. Of 111 healthy subjects screened for this mutation, none were found to be carriers. Determination of the JAK2V617F mutation may be useful to identify patients who should be carefully observed for the development of overt MPDs. The significance of screening for this mutation in nonsplanchnic thrombosis cases needs to be analyzed in a larger series.

Materials and Methods

Patients and Control Subjects

This is a retrospective analysis of the data for patients who were referred to our center for thrombophilia screening with
a history of venous thromboembolism. The data for patients with overt cancer, chronic myeloproliferative disorders, or liver cirrhosis were not included in the present analysis.

Blood Sample Collection
Peripheral blood was collected, after obtaining informed consent, into citrate Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ). Blood sampling was done 15 days to 8 months after the thrombotic event (median, 4 months). Collection and analysis of samples for different applications was approved by the National Institute of Immunohaematology Institute Ethics Committee, KEM Hospital, Mumbai, India.

Diagnosis of deep venous thrombosis (DVT), cerebral venous thrombosis (CVT), hepatic vein, and extrahepatic portal vein obstruction was confirmed by using objective methods such as Doppler ultrasonography, magnetic resonance imaging, computed tomography, and venography.

JAK2 Mutation Screening
Analysis of the V617F (1848G>T) mutation was done by using the amplification refractory mutation system (ARMS) restriction endonuclease analysis using BsaXI (New England Biolabs UK, Hitchin, England).6,12

In the allele-specific polymerase chain reaction (PCR), the presence of the V617F (1848G>T) mutation was identified by using 4 primers: forward outer, 5’-TCC TCA GAA CGT TGA TGG CAG-3’; reverse outer, 5’-ATT GCT TTC CTT TTT CAC AAG AT-3’; forward wild-type specific, 5’-CAT TTG GTT TTA AAT TAT GGA GTA TAT G-3’; and reverse mutant specific, 5’-GTT TTA TTT ACT CTC GTC TCC ACA AAA-3’. The forward and reverse outer primers flank the JAK2 exon 12 and amplify a 453-base-pair (bp) product control in all cases. The forward wild-type and reverse outer primers amplify a 229-bp wild-type-specific band (1848G), while the forward outer and reverse mutant primers amplify a 279-bp mutant-specific band (1848T). PCR was performed using Taq polymerase (Bioron, Ludwigshafen, Germany), 1.5 mmol/L magnesium chloride, 150 ng of blood donor DNA, and 1 and 0.5 μmol/L of the outer primers and the mutant/wild-type-specific inner primers, respectively. The amplification conditions consisted of a denaturation step of 1 cycle at 94°C for 5 minutes followed by 35 cycles of denaturation (95°C, 30 seconds), annealing (60°C, 30 seconds), and extension (72°C for 60 seconds). These cycles were followed by a final extension step of 1 cycle at 72°C for 5 minutes. The restriction endonuclease analysis included PCR amplification using the forward 5’-AGC AAG CTT TCT CAC AAG CA-3’ and reverse 5’-CTG ACA CCT AGC TGT GAT CCT G-3’ followed by overnight digestion at 37°C with BsaXI (New England Biolabs UK). Products were resolved on 10% acrylamide gel and examined after staining with ethidium bromide.

All samples were initially screened by a PCR–restriction fragment length polymorphism (RFLP) technique, and the positive samples were reanalyzed by ARMS-PCR.

Results
In this cohort of 321 patients (median age, 43 years; range, 2-68 years; 219 males and 102 females), 70 (21.8%) had CVT, 36 (11.2%) had DVT, 137 (42.7%) had BCS, and 78 (24.3%) had portal venous thrombosis (PVT). The median age of patients with the mutation was 46 years (range, 18-65 years). Among the 19 patients who were positive for JAK2 mutations, 6 (32%) were younger than 25 years.

The prevalence of the JAK2 mutations in different groups of thrombosis are given in Table I. Only 1 case of homozygous JAK2 mutation was detected in a woman with BCS. Of patients with BCS positive for the JAK2 mutation, 2 were also heterozygous carriers of the factor V Leiden (FVL) mutation, as were 1 of 4 patients with PVT. None of the patients with DVT or CVT were found to be carriers of the FVL mutation. All samples positive by the ARMS-PCR technique were also positive by the RFLP technique.

Discussion
There are contradictory reports about the association between JAK2 mutation and thrombosis in patients with myeloproliferative disorders (MPDs). A study by Kralovics et al13 involving 244 patients with MPDs, of whom 117 (48.0%) were JAK2<sup>V617F</sup> carriers, showed that the rate of thrombotic complications was significantly higher in this group than the wild-type group (26% vs 15%). Wolanskyj et al,14 on the other hand, observed 150 patients with essential thrombocythemia: 73 were V617F<sup>+</sup>, and the incidence of thrombotic complications was not different from their V617F<sup>−</sup> counterparts. In catastrophic intra-abdominal thrombosis in cases of polycythemia vera and essential thrombocythemia, the median posttransplantation survival of V617F<sup>+</sup> patients was

<table>
<thead>
<tr>
<th>Table I</th>
<th>Prevalence of JAK2 Mutations in Different Groups of Patients With Thrombosis and Healthy Control Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombotic Group</td>
<td>Total No. Screened</td>
</tr>
<tr>
<td>Deep venous thrombosis</td>
<td>36</td>
</tr>
<tr>
<td>Budd-Chiari syndrome</td>
<td>137</td>
</tr>
<tr>
<td>Portal vein thrombosis</td>
<td>78</td>
</tr>
<tr>
<td>Cerebral venous thrombosis</td>
<td>70</td>
</tr>
<tr>
<td>Control subjects</td>
<td>111</td>
</tr>
</tbody>
</table>

© American Society for Clinical Pathology
found to be significantly lower than that of V617F– patients.15 Thus, there is conflicting evidence for the importance of JAK2V617F mutation status in predicting the risk of thrombosis in patients with MPD; nevertheless, screening for JAK2 mutations is justified in splanchnic venous thrombosis because the management of these cases differs significantly from cases without thrombosis.

We used ARMS-PCR as a screening technique, and results were further confirmed by using a restriction-based technique. The advantage of ARMS is its apparent high sensitivity to small amounts of mutant DNA in a wild-type background with a detection sensitivity of mixed clonality ranging from 0.01% to 3%.16,17 Presently, the clinical significance of detecting small amounts of mutant clones is not clear. Despite the relative lack of sensitivity, RFLP analysis is simple to perform and was used to confirm the positive results of ARMS-PCR. There was 100% concordance between the 2 techniques.

The finding that the JAK2 mutation may confer increased activation of leukocytes and platelets in MPDs has led several investigators to study whether the JAK2 mutation can be a causative factor for thrombosis at sites other than the splanchnic area and in patients without MPD. Remacha et al18 reported that of 295 patients with venous thrombosis, only 1 was found to be positive for the JAK2V617F mutation, although the study later revealed that the patient had occult MPD. A few other studies on large series of patients with venous thrombosis referred for thrombophilia testing have found that 2% to 3% have the mutation.19,20 The prevalence of the mutation in patients with CVT has ranged between 0% and 14% in the different studies reported so far, although most of the reports have shown the absence of this mutation.21,22 Taken together, all of these studies further add to the dilemma of whether to include this screening test in routine thrombophilia screening. The prevalence of the JAK2 mutation in idiopathic venous thrombosis in all studies reported is too low and does not seem to be significant enough to be part of routine thrombophilia screening. A detailed follow-up study of patients with idiopathic venous thrombosis who are carriers of the JAK2 mutation may provide some insight about the clinical implications of this mutation.

In the present study, 8.8% of the BCS cases and 5% of the PVT cases were shown to be carriers of the JAK2 mutation. It is possible that if BCS is an initial sign of MPD, detection of the JAK2 mutation may help to understand the underlying etiology of thrombosis. The most common thrombophilia marker in BCS cases from our center has been reported to be the FVL mutation, which has been as high as 27%, compared with 3% in other groups of DVT cases in the nonsplanchnic region.23-25 However, no clinically discriminant synergistic effect of the FVL with JAK2 mutation could be observed in this series. The presence of other common thrombophilic risk factors, like protein C and protein S, and antithrombin levels could not be determined in the splanchnic venous thrombosis cases, whereas 4 cases of protein C and protein S deficiency detected in CVT and DVT cases were negative for the JAK2 mutation. Thus, the combined effect of other thrombophilia markers could not be studied in these cases.

Regarding the prevalence in the general population, contrasting reports are available in literature. One study did not find the mutation in 700 healthy persons, whereas a more recent study demonstrated that nearly 1% of blood samples collected in China from a hospital population revealed the JAK2 mutation.26,27 We did not find any carriers in the normal population (in 111 healthy subjects), but it is possible that on screening a larger number of healthy control subjects we might encounter occasional carriers.

The present report reveals that JAK2 mutations are present universally across different thrombotic groups. Clear follow-up data should enable clinicians to segregate this small subset from the remaining patients for optimal clinical management.

From the National Institute of Immunohaematology, KEM Hospital, Mumbai, India.

Supported by the Council of Scientific and Industrial Research, New Delhi, India.

Address reprint requests to Dr Ghosh: National Institute of Immunohaematology (ICMR), KEM Hospital, Parel, Mumbai 400 012, India.

References


