Correlation of JAK2 V617F Mutant Allele Quantitation with Clinical Presentation and Type of Chronic Myeloproliferative Neoplasm

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Abstract. Activating JAK2 V617F mutation is present in many patients with chronic myeloproliferative neoplasms. We evaluated, retrospectively, clinical and laboratory data from 70 patients with BCR-ABL1 negative, JAK2 positive, chronic myeloproliferative disease. Quantity of the JAK2 mutant allele was tested for correlation with the clinical presentation, type of chronic myeloproliferative disease, hemoglobin level, white blood cell and platelet counts, spleen size, and/or cardiovascular complications. RealTime–PCR was used for amplification of DNA from marrow or peripheral blood. Polycythemia vera was more frequently diagnosed among patients with ≥50% mutational load than among those with <50% mutational load (71% vs 25%; p = 0.003). Patients with ≥50% mutational load had higher mean white blood cell count (18.6 billion/L vs 11.3 billion/L; p = 0.043). Essential thrombocythemia patients were more likely to have <50% mutational load (48% vs 7%; p = 0.005). Splenomegaly was more frequent in patients with ≥50% mutational load independent of the diagnosis (89% vs 48%; p = 0.03). Cardiovascular complications were reported in 50% of patients with ≥50% mutational load vs 21% of patients with <50% mutational load. JAK2 quantitation was highest in polycythemia vera followed by chronic myeloproliferative disease, unclassifiable, and essential thrombocythemia patients. JAK2 quantitation appears important in clinical evaluation. Mutational load appears to be helpful in stratification of patients into subgroups with different frequency of complications. Quantitation of JAK2 mutational load may be useful in evaluating response to therapy.

Keywords: janus kinase 2 (JAK2) mutation V617F, myeloproliferative neoplasms, primary myelofibrosis

Introduction

The hypothesis that genetic factors play a role in the pathogenesis of Philadelphia chromosome negative, BCR-ABL1 negative, chronic myeloproliferative neoplasms has inspired scientists to search for new genetic aberrations. A recent change in terminology warrants a brief mention of the synonymous terms chronic myeloproliferative disease (CMPD) and myeloproliferative neoplasm (MPN), which will be used interchangeably [1,2].

In addition, the currently preferred term primary myelofibrosis (PMF) used in the 2008 edition of WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues [2] replaces the previously used terms referenced in this manuscript, such as idiopathic myelofibrosis (IMF), chronic idiopathic myelofibrosis (CIMF), and myelofibrosis with myeloid metaplasia (MMM)] [4-8].

The importance of protein-tyrosine kinases in growth regulation of eukaryotic cells was first outlined by Wilks in 1989 [3]. Janus kinase 2 (JAK2) V617F gene mutation has been postulated as a pathogenetic basis of the disease process in many patients with MPNs. This postulate is based on the observation that in many of these patients microsatellite mapping and DNA sequencing of the short arm of chromosome 9 (9pLOH region),

Available online at www.annclinlabsci.org


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0091-7370/09/0400-0345. $2.10. © 2009 by the Association of Clinical Scientists, Inc.
which includes the JAK2 gene, reveal G-T transversion causing production of an altered protein in which phenylalanine is substituted for valine at position 617 of JAK2 (V617F). In 2005, groups of investigators from Europe and the United States demonstrated that a significant proportion of patients with BCR-ABL1 negative chronic myeloproliferative diseases are positive for the somatic mutation JAK2 V617F in hematopoietic progenitors [4–7]. Clinical significance of the V617F mutation has been increasingly investigated and has led to appreciation of its importance in the diagnosis, classification, and treatment of myeloproliferative neoplasms. During the last 4 years, studies have revealed that activating JAK2 mutation V617F is present in >95% of patients with polycythemia vera (PV) and in approximately half of patients with essential thrombocytopenia (ET) and idiopathic myelofibrosis (IMF). Patients with these myeloproliferative neoplasms often present with overlapping clinical signs, symptoms, and morphologic findings, causing difficulty in differential diagnosis and risk stratification. The percentage of the mutant allele is related to the extent of dominance of the JAK2 positive clone and of the clone genotype. Quantitation of JAK2 mutational load is important in monitoring the malignant clone and assessing response to therapy in patients previously found positive by the qualitative JAK2 mutation test. This study attempted to correlate the quantitative results of JAK2 mutational status with the type of MPN, the laboratory and clinical findings, and the presence or absence of clinical complications.

Materials and Methods

We reviewed the clinical courses and laboratory data of 70 patients with Philadelphia chromosome negative, BCR-ABL1 negative, JAK2 positive myeloproliferative neoplasms. Retrospectively those patients were selected who had the diagnosis of myeloproliferative disorder and JAK2 quantitation performed by Molecular Pathology Laboratory of William Beaumont Hospital. All patients were Philadelphia chromosome negative; 9 patients had additional karyotypic abnormalities including +9, +13, -Y, del 5q, del 13q12, and del 20q11. They were included in our study; however, it should be noted that complex abnormalities may have interfering effects on the disease course and symptoms and may warrant separate study to delineate their effects. The Institutional Review Board of William Beaumont Hospital approved this study.

DNA samples extracted from fresh bone marrow and peripheral blood specimens were amplified using Real-Time PCR with fluorescent labeled, allele specific, PCR primers (MultiCode-RTx system, EraGen Biosciences, Madison, WI). The amount of JAK2 V617F mutant allele in each patient’s sample was quantitated using a standard calibration curve. The detection limit of the method was 1% mutant allele. Patients were divided into two study groups based on mutational load: those with ≥50% mutational load and those with <50% mutational load. These groups have been designated “homozygotes” and “heterozygotes,” respectively, by some researchers [13,19].

Patient age was reported as min-max range, mean ± SD, followed by the (median). Age distribution was examined using the Wilcoxon rank test, a non-parametric approximation of the t-test. The other variables were examined by the Chi-square test where appropriate (expected frequency ≥5) or by Fisher’s exact test. These data were expressed as counts and % frequency. The SAS program (version 9.1.3) was used for statistical analyses; p <0.05 was considered significant.

Results

The 70 patients analyzed in this study were 29 males and 41 females, age 36 to 91 yr. Twenty-four patients were diagnosed with PV, 28 with ET, 2 with IMF, and 16 with chronic myeloproliferative disease, unclassifiable (CMPD.U). We reviewed their clinical manifestations and laboratory data at the time of quantitative JAK2 analysis and we compared patients with <50% mutant allele vs those with ≥50% mutant allele. We observed the
frequency and distribution of specific diagnoses within each group. We also studied the occurrence and frequency of the most common clinical signs, symptoms, and significant complications in each group. The clinical manifestations included assessment of blood hemoglobin level, white blood cell count, platelet count, and spleen size. The common medical complications included cardiovascular complications such as thromboembolism, bleeding, and/or myocardial infarction. The average duration of follow-up was 2-3 yr. The analysis included 56 patients (80%) with <50% mutant allele for JAK2 V617F mutation and 14 patients (20%) with ≥50% mutant allele (Fig. 1).

Patients with ≥50% JAK2 V617F mutant allele were slightly older (52 to 89 yr, mean 76, median 80) than those with <50% mutant allele (36 to 91 yr, mean 67, median 70). Female patients outnumbered males with M:F ratio: ~0.7:1 (similar proportions in both groups).

Correlation between clinical diagnosis and JAK2 V617F mutational load was strongest in patients with polycythemia vera. Patients with ≥50% mutant allele presented more frequently with clinical signs and symptoms of polycythemia vera than any other chronic myeloproliferative neoplasm (p = 0.003). Ten of 14 patients with ≥50% mutational load had the diagnosis of polycythemia vera as opposed to 14 of 56 patients with <50% mutant allele (p = 0.003). WBC count was elevated in 71% of patients with ≥50% mutant allele and 45% of those with <50% mutant allele. WBC count was elevated above 15 billion/L in 50% of patients with the higher mutational load and only in 15% of those with <50% mutant allele. No major differences were noted in hemoglobin levels or platelet counts. Patients with <50% mutant allele were more likely to present with clinical signs and symptoms of essential thrombocytopenia than any other chronic myeloproliferative neoplasm (p = 0.005). The data for patients in each diagnosis group are summarized in Table 1.

Among the polycythemia vera (PV) patients, 10 had ≥50% mutational load and 14 had <50% mutational load. Their age range was 52 to 84 yr. On average, patients with mutational load ≥50% were older with mean age of 74 yr (median 80) vs mean age of 68 yr (median 66) for those with <50% mutational load. M:F ratio was 1:3 in the group with high mutational load and 1:1 in those with <50% mutational load. In PV patients in the ≥50% mutational load group, 80% had elevated WBC count vs only 57% in the other group. Mean WBC count was markedly higher among those with ≥50% mutational load (18.6 billion/L and 11.3 billion/L respectively; p = 0.043). Mean hemoglobin level was slightly higher in patients with <50% mutational load (16.8 g/dL vs 15.7 g/dL) and mean platelet count was slightly higher in patients with ≥50% mutational load (509 billion/L vs 422 billion/L). Splenomegaly was slightly more frequent in those with ≥50% mutational load (50% vs 43%).

<table>
<thead>
<tr>
<th>Mutational load</th>
<th>PV</th>
<th>ET</th>
<th>IMF</th>
<th>CMPD,U</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, no. (%)</td>
<td>14 (58%)</td>
<td>10 (42%)</td>
<td>27 (96%)</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Male:Female</td>
<td>7:7</td>
<td>3:7</td>
<td>11:16</td>
<td>1:0</td>
</tr>
<tr>
<td>Age, yr</td>
<td>68 ± 9</td>
<td>74 ± 11</td>
<td>64 ± 18</td>
<td>70</td>
</tr>
<tr>
<td>WBC, billion/L*</td>
<td>11.3 ± 4.3</td>
<td>18.6 ± 10.2</td>
<td>10.9 ± 3.7</td>
<td>10.1</td>
</tr>
<tr>
<td>Platelets, billion/L*</td>
<td>422 ± 155</td>
<td>509 ± 390</td>
<td>859 ± 317</td>
<td>798</td>
</tr>
<tr>
<td>Hb, g/dl*</td>
<td>16.8 ± 3.4</td>
<td>15.7 ± 3.0</td>
<td>13.6 ± 2.5</td>
<td>16.1</td>
</tr>
<tr>
<td>Splenomegaly, no. (%)</td>
<td>6 (43%)</td>
<td>5 (50%)</td>
<td>6 (22%)</td>
<td>1 (100%)</td>
</tr>
</tbody>
</table>

* Mean ± SD;  NA, data not available
Thromboembolic complications were similar in both groups. Bleeding complications were too rare for meaningful statistical analysis.

Diagnosis of essential thrombocythemia (ET) was made in 28 patients. Of these 27 (96%) had <50% mutational load. They were 39 to 90 yr old with a mean age of 64 (median 70). M:F ratio was 3:4. Only one patient in the ET group had ≥50% JAK2 V617F mutational load. WBC count was elevated in 12 patients (43%) and normal in 16 patients (57%). All ET patients had platelet counts above the normal reference range. Hemoglobin was slightly increased in one patient with ≥50% mutant allele and four patients with <50% mutant allele. Spleen size was assessed in 15 of 28 patients and it was enlarged in approximately half of the ET patients. Cardiovascular complications were noted in 10 ET patients. Seven of them suffered from thromboembolic complications, two suffered from bleeding, and one had myocardial infarction.

Clinical diagnosis of idiopathic myelofibrosis (IMF) was made in 2 patients. Their ages were 65 and 89 yr. Both were females with <50% JAK2 V617F mutational load. Both had hemoglobin levels below the normal reference range, one had slightly elevated WBC count (12.3 bil/L) and both had platelet counts within the normal reference range. Splenomegaly was recorded in one patient and neither had cardiovascular complications.

Sixteen patients had a diagnosis of chronic myeloproliferative disease, unclassifiable (CMPD U). Three of them (19%) had ≥50% mutational load and 13 (81%) had <50% mutational load. These patients were 40 to 91 yr old. Patients with higher mutational load were older with a mean age of 84 yr (median 84) vs a mean age of 71 yr (median 77) in those with <50% mutational load. Two of 3 patients with ≥50% mutational load had elevated WBC counts, while only 4 of 13 in the other group had elevated WBC counts. Mean WBC count was higher among those with ≥50% mutational load than in the other group (21.7 billion/L vs 14.8 billion/L, respectively). Platelet counts were elevated in two-thirds of patients in both groups and on average, were only slightly higher in those with ≥50% mutational load (558 billion/L vs 515 billion/L). Mean hemoglobin was higher in patients with <50% mutational load (13.1 g/dl vs 10.6 g/dl).

Splenomegaly was more frequent in patients with ≥50% mutational load (66% vs 23%). Thromboembolic complications were also more frequent in patients with ≥50% mutational load (66% vs 23%). One patient with <50% mutational load (6%) had bleeding complications vs none in the other group.

Discussion

Detection of JAK2 V617F is an objective method of establishing the presence of clonality and a valuable tool in clinical evaluation of patients with suspected myeloid disorder [9,10]. The JAK2 V617F mutation has been recognized recently as one of the major diagnostic criteria for diagnosis of polycythemia vera, primary myelofibrosis, and essential thrombocytemia, and as one among several important mutations in those with myeloproliferative neoplasm, unclassifiable [2,11,12].

It has been shown that the mutational load is closely related to the clinical manifestations of myeloproliferative neoplasms. Mutational load also appears important in assessment of risk factors for the serious complications that may occur in the course of the disease [11,13-16]. Our purpose was to examine the relationship between quantitatively determined mutational load and the clinical presentation as well as the type and frequency of medical complications. The current literature indicates that evaluation of JAK2 mutational burden is not only helpful in establishing the diagnosis of MPN and differentiating it from benign myeloid disorders, but may be helpful in evaluating the prognosis. In this context it is important to note that the JAK2 mutation has been detected in occasional patients with other clonal hematologic diseases, including myelodysplastic syndrome, atypical myeloproliferative disorders, and acute myeloid leukemia [9,17,18].

Our study adds to previous evidence that determination of the quantity of JAK2 V617F mutation and demonstration of ≥50% mutational load is of primary clinical importance and should be included in the evaluation of cases of myeloproliferative neoplasms [13,19]. We focused our attention in particular on comparative analysis of patients with <50% vs those with ≥50% mutational load for JAK2 V617F mutation and its clinical
implications. Our analysis underlines the relevance and role of JAK2 quantitation in diagnosis and follow-up of MPN patients. In our study, ≥50% mutational load was demonstrated in 3.6% of ET patients vs 42% of PV patients (other studies reported ~2% for ET and 25 to 32% for PV) [4,6,8,13]. Variability in the percentage of patients with ≥50% mutational load vs those with <50% in different studies is likely due to number of patients analyzed and method used. The largest analysis included 962 patients with mutated and wild type JAK2 gene in a multicenter study performed by Vannucchi et al [13], reporting homozygosity in 2.2% of ET patients and 32% of PV patients.

The data suggest that ≥50% mutational load for JAK2 V617F is a late event, increasingly more common with advanced age. It also appears to be indicative of patients who are more likely to become symptomatic and more likely to suffer from serious, life threatening complications. These patients not only present with higher than average hemoglobin level and platelet count, but also have mild to moderate leukocytosis. Landolfi et al [20] examined the association of hematologic variables and cardiovascular risk factors in 1638 PV patients and found that a risk factor of primary importance was WBC count >15 billion/L, compared to <10 billion/L. Thus, correlation of mutational load with increased WBC count, particularly if the leukocyte count is greater than 15 billion/L, may prove to be a useful, quick, and simple way to select those patients in whom close clinical follow up and aggressive therapeutic and preventive measures may be indicated.

Presence of splenomegaly, which appears to be only slightly more frequent in patients with higher mutational load, may be a phenomenon related to increased turnover of cells rather than directly related to the pathophysiology of the underlying process. Splenomegaly, if marked, may pose a source of morbidity and mortality, but in its mild form may not have significant bearing on the clinical outcome.

In conclusion, in our study, patients with ≥50% JAK2 V617F mutational load had higher white blood cell and platelet counts and more frequently had splenomegaly vs those with <50% load. They were also much more likely to develop the clinical picture of polycythemia vera than any other MPN. There was no significant association between mutational load and hemoglobin level. Serious cardiovascular complications, eg, thromboembolic events or bleeding, were more common in patients with higher load of the mutation, although due to the small number of cases the statistical significance could not be determined. This finding is similar to other reports of correlation between the risk of thrombosis and JAK2 V617F mutational load [13,14] and between leukocytosis above 15 billion/L and thrombotic risk [20]. Mutational load appears to increase with increasing patient age.

This study indicates that quantitation of JAK2 mutational load is important not only in diagnostic considerations but also in monitoring of the malignant clone. In patients with high load of JAK2 V617F mutation, a diagnosis of polycythemia vera should be strongly considered. It can be postulated that high mutational load favors diagnosis of PV and those with high mutational load who do not fulfil the diagnostic criteria for PV should be periodically re-evaluated to incorporate the dynamic changes of disease into the diagnostic scheme. JAK2 V617F quantitation may also aid in risk stratification and development of follow-up and therapeutic strategies for patients with myeloproliferative neoplasms. Thus the correlation of JAK2 mutational load may be useful in assessing the response to therapy in patients diagnosed with myeloproliferative neoplasms and in those previously found to be JAK2 V617F positive by qualitative JAK2 testing.

Acknowledgements

The authors thank Xiuling Meng for assistance in gathering the quantitative JAK2 results and Judith A. Boura for assistance in the statistical analyses.

References


