Molecular genetic tests for JAK2V617F, Exon12_JAK2 and MPLW515K/L are highly informative in the evaluation of patients suspected to have BCR-ABL1-negative myeloproliferative neoplasms

Marcos Tadeu dos Santos,1 Miguel Mitne-Neto,1 Kozue Miyashiro,1 Maria de Lourdes L Ferrari Chauffaille,1,2 Edgar Gil Rizzatti1

INTRODUCTION
Polycythemia vera (PV), essential thrombocythemia (ET) and idiopathic myelofibrosis (MF), are the most common myeloproliferative neoplasms (MPN) in patients without the BCR-ABL1 gene rearrangement. They are caused by clonal expansion of haematopoietic stem cells and share, as a diagnostic criterion, the identification of JAK2V617F mutation. Classically, when other clinical criteria are present, a JAK2V617F negative case requires the analysis of Exon12_JAK2 for the diagnosis of PV, and of MPLW515K/L mutations for the diagnosis of ET and MF. Here, we evaluated 78 samples from Brazilian patients suspected to have MPN, without stratification for PV, ET or MF. We found that 28 (35.9%) are JAK2V617F carriers; from the 50 remaining samples, one (2%) showed an Exon12_JAK2 mutation, and another (2%) was positive for MPLW515L mutation. In summary, the investigation of JAK2V617F, Exon12_JAK2 and MPLW515K/L was relevant for the diagnosis of 38.4% of patients suspected to have BCR-ABL1-negative MPN, suggesting that molecular genetic tests are useful for a quick and unequivocal diagnosis of MPN.
JAK2V617F, Exon12_JAK2 and MPLW515K/L mutations in Brazilian patients clinically suspected to have MPN.

MATERIAL AND METHODS

We analysed 78 samples from patients suspected to have MPN, all of which were sent to our clinical laboratory to be tested for the JAK2V617F mutation over a 2-month period. DNA was extracted automatically through a QIACUBE system (QIAGEN) and evaluated using TaqMan-based real-time PCR method. Only the wild type JAK2 samples (JAK2V617F negative) were analysed for Exon12_JAK2 mutations (Sanger sequencing) and for MPLW515K/L mutations (TaqMan-based real-time PCR assay). Primers used are shown in table 1.

Real-time PCR reactions were run in an ABI 7900HT (Life Technologies) for JAK2V617F and in a Rotor-Gene 6000 (QIAGEN) for MPLW515K/L. Exon12_JAK2 was sequenced in a 3130 Genetic Analyzer (Applied Biosystems). Reactions parameters, cycling conditions and reagents volumes were used as universal conditions defined by the manufactures.

The study protocol was approved by the Internal Review Board, and all samples were anonymised from patient identifiers for the purposes of this study. To compare our data against previous studies, we assumed that, in the studies in which the stratification of MPNs were presented, the sum of PV, MF and ET patients represent the total of MPN samples, and this number was used as a denominator to identify the frequency of JAK2V617F-positive cases in the cohort.

RESULTS

From the 78 analysed samples, 50 (64%) were negative and 28 (35.9%) positive for JAK2V617F mutation. JAK2V617F mutation frequencies from the present study and those selected from the literature are presented in table 2.

Figure 1 Mutation identified on Exon12_JAK2 flanking region. The mutation is six nucleotides apart from the hotspot mutation area.

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**Table 1** Primers and probes used for screening

<table>
<thead>
<tr>
<th>JAK2 (V617F)</th>
<th>Exon12_JAK2</th>
<th>MPL515</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward primer</td>
<td>CATACITTCAAGTTTGAAGTG</td>
<td>TGGTGACCGGCACTCTCTT</td>
</tr>
<tr>
<td>Reverse primer</td>
<td>ATGCACATGAATGAACTCA</td>
<td>TCAGCGCCAGGTCTGCT</td>
</tr>
<tr>
<td>Probe WT_FAM_MGB</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Probe V617F_VIC_MGB</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

**Table 2** Detailed frequency of JAK2V617F mutation in different cohorts, stratified by PV, MF and ET, or not (*)

<table>
<thead>
<tr>
<th>Authors</th>
<th>PV</th>
<th>MF</th>
<th>ET</th>
<th>Total</th>
<th>JAK2V617F Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monte-Mór et al</td>
<td>47/49</td>
<td>14/25</td>
<td>8/29</td>
<td>69/103</td>
<td>66.9</td>
</tr>
<tr>
<td>Baxter et al</td>
<td>71/73</td>
<td>8/16</td>
<td>29/51</td>
<td>108/140</td>
<td>77.1</td>
</tr>
<tr>
<td>James et al</td>
<td>40/45</td>
<td>3/7</td>
<td>9/21</td>
<td>52/73</td>
<td>71.2</td>
</tr>
<tr>
<td>Kralovics et al</td>
<td>83/128</td>
<td>13/23</td>
<td>21/93</td>
<td>117/244</td>
<td>47.9</td>
</tr>
<tr>
<td>Levine et al</td>
<td>121/164</td>
<td>16/46</td>
<td>37/115</td>
<td>174/325</td>
<td>53.5</td>
</tr>
<tr>
<td>dos Santos et al</td>
<td>18/20</td>
<td>9/21</td>
<td>8/17</td>
<td>35/58</td>
<td>60.3</td>
</tr>
<tr>
<td>Kiladjian et al</td>
<td>No stratification</td>
<td>94/241</td>
<td>39.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>This study*</td>
<td>No stratification</td>
<td>28/78</td>
<td>35.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*ET, Essential thrombocythemia; MF, myelofibrosis; PV, polycythaemia vera.
*There was no stratification for PV, MF or ET on both studies.
Analysis of/MPL515K/L mutations also identified a single carrying patient where the tryptophan is exchanged by a leucine (W515L). The mutation/MPL515K was not identified in our series.

Taken together, JAK2V617F, Exon12_JAK2 and MPLW515L mutations were present in 38.4% of the cases suspected to have MPN from our cohort. Although JAK2V617F is the most frequent mutation, it is important to note that the other mutations correspond to 4% of JAK2V617F-negative samples (table 3).

DISCUSSION
JAK2V617F frequencies in previous studies are higher than in our series (table 2), even when Brazilian patients were evaluated. A possible reason for this could be the stratification of PV, MF or ET patients as performed by others. Prestratification for PV, ET or MF generally increases the pretest probability of a positive result, given that in this context molecular genetic tests are frequently ordered on a confirmatory basis. In non-stratified cases, on the other hand, these tests are ordered in a broader clinical context, with a lower pretest probability of a MPN diagnosis.

This situation is quite common in the clinical laboratory setting, where molecular genetic tests are frequently ordered for patients with sustained high blood counts in a ‘rule out MPN’ approach. The probability of a positive test in this setting is, therefore, much lower than that for prestratified cases, as we have observed in our series. Kiladjian et al13 used a similar approach in their study, without prestratification for PV, ET or MF, and observed similar frequencies. However, no MPLW515K/L or Exon12_JAK2 mutations were identified in their study, despite the higher number of patients evaluated. In our series, those mutations corresponded to 2% of JAK2V617F-negative cases, or 1.28% of all cases suspected to have MPN clinically.

The analysis of Exon12_JAK2 identified a single mutation carrier. Although no clinical data was related to this mutation in the COSMIC, HGMD, LOVD, HGVS, OMIM and ClinVar databases, it is present in a splice site region, probably leading to constitutive activation of the JAK2 protein. In the 1000 genome database, it is present in 0.1% of the overall population, being identified solely in Americans, but not in African, Asian and European individuals (0.002). Although further clinical data would be invaluable to better characterise this finding, the retrospective and anonymised design of our study has prevented this approach. In summary, our study suggests that molecular genetic tests for JAK2V617F, Exon12_JAK2 and MPL515K/L mutations are relevant for the investigation of patients suspected to have BCR-ABL1-negative MPN.

Table 3 Mutation frequency on MPN samples

<table>
<thead>
<tr>
<th>Mutations</th>
<th>Positive samples</th>
<th>% On tested samples</th>
<th>% On JAK2V617F negative samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>JAK2V617F</td>
<td>28/78</td>
<td>35.9</td>
<td>–</td>
</tr>
<tr>
<td>Exon12_JAK2</td>
<td>1/78</td>
<td>1.28</td>
<td>2 (1/50)</td>
</tr>
<tr>
<td>MPLW515K/L</td>
<td>1/78</td>
<td>1.28</td>
<td>2 (1/50)</td>
</tr>
<tr>
<td>Total</td>
<td>30/78</td>
<td>38.4</td>
<td>4 (2/50)</td>
</tr>
</tbody>
</table>

MPN, myeloproliferative neoplasms.

Take-home messages

▸ Molecular tests emerge as a convenient tool to quickly confirm the diagnosis in patients suspected to have BCR-ABL1-negative myeloproliferative neoplasms (MPN).
▸ JAK2V617F, Exon12_JAK2 and MPLW515K/L molecular analysis are useful for the investigation of cases suspected to have BCR-ABL1-negative MPN even without polycythaemia vera, essential thrombocythaemia or myelofibrosis prestratification.
▸ In our series, a test panel evaluating JAK2V617F, Exon12_JAK2 and MPLW515K/L was relevant to confirm MPN diagnosis in 38.4% of BCR-ABL1 negative MPN cases.

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Contributors MtDs, MM-N, KM designed the study, performed the experiments and drafted the manuscript. MollfC and EgR contributed as clinical haematologists. EgR revised the manuscript. All authors read and approved the final version.

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REFERENCES

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