Molecular genetic tests for *JAK2*V617F, Exon12_*JAK2* and *MPL*W515K/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 Rizzatti[1]

[1]Research and Development Department, Fleury Group, Sao Paulo, Brazil
[2]Department of Haematology and Hemotherapy Discipline, Federal University of São Paulo (EPM), Sao Paulo, Brazil

Correspondence to
Dr Miguel Mitne-Neto, Research and Development Department, Fleury Group, Sao Paulo 04344-070, Brazil; miguel.mitne@grupofleury.com.br

MTdS and MM-N contributed equally

ABSTRACT
Polycythaemia 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 *JAK2*V617F mutation. Classically, when other clinical criteria are present, a *JAK2*V617F negative case requires the analysis of Exon12_*JAK2* for the diagnosis of PV, and of *MPL*515K/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 *JAK2*V617F carriers; from the 50 remaining samples, one (2%) showed an Exon12_*JAK2* mutation, and another (2%) was positive for *MPL*W515L mutation. In summary, the investigation of *JAK2*V617F, Exon12_*JAK2* and *MPL*W515K/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.

INTRODUCTION
Polycythaemia vera (PV), essential thrombocythemia (ET), idiopatic myelofibrosis (MF) and BCR-ABL1 chronic myeloid leukaemia (CML) are distinctive haematopoietic diseases from the group of the myeloproliferative neoplasms (MPN). Clinical and biological features set CML apart from PV, ET and MF, and these three entities have some important characteristics in common. They are all caused by the clonal expansion of a multipotent haematopoietic progenitor cell, maintain a relatively preserved haematopoietic differentiation, develop some degree of bone marrow fibrosis, and can evolve into each other or transform into more aggressive haematological cancers.[1] In PV, the oncogenic process targets a multipotent precursor committed to the erythroid lineage, causing an expansion of the red cell mass in the peripheral blood and a loss of response to the physiologic regulation of erythropoiesis. In MF, the target is a pluripotent haematopoietic precursor whose transformation leads to an intense stromal proliferation in the bone marrow, and a variety of peripheral blood manifestations.[2] In ET, the oncogenic process results in megakaryocytic hyperplasia in the bone marrow and increased platelet counts in the peripheral blood.[1] Despite such distinctive phenotypic features, PV, MF and ET can share some important findings at the molecular level, as the identification of a shared clonal marker. Molecular studies have identified a point mutation in the *JAK2* gene on PV, MF and ET. The *JAK2*V617F mutation leads to the constitutive activation of the JAK2 kinase that can be found in 95% of patients with PV, and in 50–60% of patients with MF and ET.[3] Due to such high frequencies, the *JAK2*V617F mutation has become one of WHO's criteria for the diagnosis of PV, MF and ET. Although the *JAK2*V617F mutation has been also described with low frequencies in different groups of haematological malignancies,[4] its identification in cells from the peripheral blood in the appropriate clinical context is virtually diagnostic of a BCR-ABL1-negative MPN. On the other hand, a negative test does not definitely exclude PV, MF and ET in the presence of other clinical and haematologic features of MPN. In this scenario, further molecular investigations are suggested, as the search for mutations in the Exon 12 of the *JAK2* gene (Exon12_*JAK2*), or in the position 515 of the *MPL* gene (*MPL*W515K/L). Exon12_*JAK2* mutations have been found in approximately 2–3% of PV patients; those mutations are clustered between codons 533 and 547, where they disrupt the pseudo-kinase domain, promoting its constitutive activation.[4] *MPL* gene is predicted to be a haematopoietic cytokine receptor acting through JAK2 intracellular transduction,[5] and the *MPL*W515L mutation results in a constitutive activation of JAK-STAT signalling. Another mutation in this position—*MPL*W515K—was later identified, but its precise effects on signalling are not yet explained. The 515 tryptophan (W515) is the key amino acid located in a unique amphipathic domain that prevents spontaneous activation of MPL,[6] and either *MPL*W515K/L mutations meet the WHO diagnostic criterion for ET or MF.

Although major diagnostic criteria for MPN are based on clinical features, blood cell counts, hormone levels and bone marrow morphology, these data are not always available or sufficient to conclude a diagnosis. In this regard, novel tools that are fast, convenient and widely available could be helpful in this clinical setting. Thus, the present work was designed to evaluate the frequency of

Table 1 Primers and probes used for screening

JAK2 (V617F)	Exon12_JAK2	MPL515
Forward primer GCAGCAAGTATGATGAGCAAGCT	*Forward primer* CATACTTTCAGTGTATTTTGAAGTG	*Forward primer* TGGTGACCGCTCTGCATCTA
Reverse primer GGCATTAGAAAGCCTGTAGTTTTACTTAC	*Reverse primer* ATGTCACATGAATGTAAATCAAG	*Reverse primer* TCCACCGCCAGTCTCCTG
Probe WT_FAM_MGB TGGAGTATGTGTCTGTGGA	–	*Probe WT_VIC_MGB* TGAGGTGGCAGTTTC
Probe V617F_VIC_MGB TGGAGTATGTTTCTGTGGAG	–	*Probe W515L_FAM_MGB* CTGCTGAGGTTGCAGTT
–	–	*Probe W515K_FAM_MGB* CTGCTGAGGAAGCAGT

Table 2 Detailed frequency of *JAK2*V617F mutation in different cohorts, stratified by PV, MF and ET, or not (*)

Authors	JAK2V617F+/tested				JAK2V617F Frequency (%)
	PV	MF	ET	Total	
Monte-Mór et al[7]	47/49	14/25	8/29	69/103	66.9
Baxter et al[8]	71/73	8/16	29/51	108/140	77.1
James et al[9]	40/45	3/7	9/21	52/73	71.2
Kralovics et al[10]	83/128	13/23	21/93	117/244	47.9
Levine et al[11]	121/164	16/46	37/115	174/325	53.5
dos Santos et al[12]	18/20	9/21	8/17	35/58	60.3
Kiladjian et al*[13]	*No stratification*			94/241	39.0
This study*	*No stratification*			28/78	35.9

ET, Essential thrombocythemia; MF, myelofibrosis; PV, polycythaemia vera.
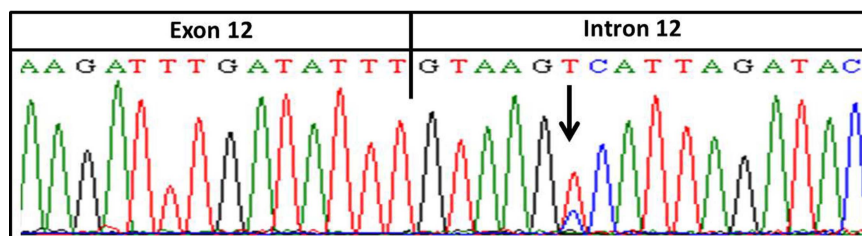*There was no stratification for PV, MF or ET on both studies.

*JAK2*V617F, Exon12_*JAK2* and *MPL*W515K/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 *JAK2*V617F 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 (*JAK2*V617F negative) were analysed for Exon12_*JAK2* mutations (Sanger sequencing) and for *MPL*W515K/L mutations (TaqMan-based real-time PCR assay). Primers used are shown in table 1.

Real-time PCR reactions were run in a ABI 7900HT (Life Technologies) for *JAK2*V617F and in a Rotor-Gene 6000 (QIAGEN) for *MPL*W515K/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 *JAK2*V617F-positive cases in the cohort.

RESULTS

From the 78 analysed samples, 50 (64%) were negative and 28 (35.9%) positive for *JAK2*V617F mutation. *JAK2*V617F mutation frequencies from the present study and those selected from the literature are presented in table 2.

The 50 *JAK2*V617F negative samples were evaluated for both Exon12_*JAK2* and *MPL*W515K/L mutations. A single patient was identified with an Exon12_*JAK2* mutation (c.1194+6T>C) (figure 1). This alteration, deposited as rs182123615, is only six nucleotides apart from the Exon12_*JAK2* hotspot area, and is located in a splicing site region.

Figure 1 Mutation identified on Exon12_*JAK2* flanking region. The mutation is six nucleotides apart from the hotspot mutation area.

Table 3 Mutation frequency on MPN samples

Mutations	Positive samples	% On tested samples	% On JAK2V617F negative samples
*JAK2*V617F	28/78	35.9	–
Exon12_*JAK2*	1/78	1.28	2 (1/50)
*MPL*W515K/L	1/78	1.28	2 (1/50)
Total	30/78	38.4	4 (2/50)

MPN, myeloproliferative neoplasms.

Analysis of *MPL*515K/L mutations also identified a single carrying patient where the tryptophan is exchanged by a leucine (W515L). The mutation *MPL*W515K was not identified in our series.

Taken together, *JAK2*V617F, Exon12_*JAK2* and *MPL*W515L mutations were present in 38.4% of the cases suspected to have MPN from our cohort. Although *JAK2*V617F is the most frequent mutation, it is important to note that the other mutations correspond to 4% of *JAK2*V617F-negative samples (table 3).

DISCUSSION

*JAK2*V617F frequencies in previous studies are higher than in our series (table 2), even when Brazilian patients were evaluated.[7–12] 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 al*[13] used a similar approach in their study, without prestratification for PV, ET or MF, and observed similar frequencies. However, no *MPL*W515K/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 *JAK2*V617F-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 *JAK2*V617F, Exon12_*JAK2* and *MPL*515K/L mutations are relevant for the investigation of patients suspected to have BCR-ABL1-negative MPN.

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 thrombocythemia 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.

REFERENCES

1 Spivak JL, Silver RT. The revised World Health Organization diagnostic criteria for polycythemia vera, essential thrombocytosis, and primary myelofibrosis: an alternative proposal. *Blood* 2008;112:231–9.
2 Chauffaille MLLF. Neoplasias mieloproliferativas: revisão dos critérios diagnósticos e dos aspectos clínicos. *Rev Bras Hematol Hemot* 2010;32:1–7.
3 Bench AJ, White HE, Foroni L, et al. Molecular diagnosis of the myeloproliferative neoplasms: UK guidelines for the detection of JAK2 V617F and other relevant mutations. *Br J Haematol* 2013;160:25–34.
4 Kiladjian JJ. The spectrum of JAK2-positive myeloproliferative neoplasms. *Hem Am Soc Hematol Educ Program* 2012;2012:561–6.
5 Scott LM. The JAK2 exon 12 mutations: a comprehensive review. *Am J Hematol* 2011;86:668–76.
6 Chaligné R, James C, Tonetti C, et al. Evidence for MPL W515L/K mutations in hematopoietic stem cells in primitive myelofibrosis. *Blood* 2007;110:3735–43.
7 Monte-Mór BRC, Cunha AFC, Pagnano KBB, et al. JAK2 V617F prevalence in Brazilian patients with polycythemia vera, idiopathic myelofibrosis and essential thrombocytemia. *Genet and Mol Biol* 2007;30:336–8.
8 Baxter EJ, Scott LM, Campbell PJ, et al. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. *Lancet* 2005;365:1054–61.
9 James C, Ugo V, Le Couedic JP, et al. A unique clonal JAK2 mutation leading to constitutive signalling causes Polycythaemia vera. *Nature* 2005;434:1144–8.
10 Kralovics R, Passamonti F, Buser AS, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. *N Engl J Med* 2005;352(17):1779–90.
11 Levine RL, Wadleigh M, Cools J, et al. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. *Cancer Cell* 2005;7:387–97.
12 Dos Santos LC, Ribeiro JCC, Silva NP, et al. Cytogenetics, JAK2 and MPL mutations in polycythemia vera, primary myelofibrosis and essential thrombocythemia. *Rev Bras Hematol Hemoter* 2011;33:417–24.
13 Kiladjian JJ, Cervantes F, Leebeek FW, et al. The impact of JAK2 and MPL mutations on diagnosis and prognosis of splanchnic vein thrombosis: a report on 241 cases. *Blood* 2008;111:4922–9.