Concomitant presence of JAK2V617F mutation and BCR-ABL translocation in two patients: A new entity or a variant of myeloproliferative neoplasms (Case report)

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Abstract. Myeloproliferative neoplasms (MPNs) are classically divided into BCR RhoGEF and GTPase activating protein (BCR)-ABL proto-oncogene 1 non-receptor tyrosine kinase (ABL) positive chronic myeloid leukemia (CML) and BCR-ABL negative MPNs, including essential thrombocytopenia (ET). One of the major diagnostic criteria for ET is the absence of the Philadelphia chromosome, thus when present it is almost indicative of CML. ET and CML are considered to be mutually exclusive; however, there are rare situations in which patients with ET present positive BCR-ABL without the features of CML. Although from the literature review, the frequency of JAK2V617F mutation and BCR-ABL translocation coexistence in MPNs is low, it may be higher than expected. The current study reported cases of two patients with an initial diagnosis of ET in the presence of JAK2V617F mutation and BCR-ABL translocation by fluorescent in situ hybridization. Both patients presented with a heterozygous BCR-ABL translocation, and absence of p190 and p210 transcripts, seemingly a der(9) in the background of an ET JAK2V617F mutation.

Introduction

According to World Health Organization (WHO) 2008 Classification of tumours of haematopoietic and lymphoid tissues and 2016 revision, myeloproliferative neoplasms (MPNs) can be classified into two major groups, chronic myeloid leukemia (CML) and Philadelphia-negative MPNs (PN-MPNs), such as polycythemia vera (PV), essential thrombocytopenia (ET) and primary myelofibrosis (PMF) (1). These disorders are more frequently found in elderly patients, mostly in men (1).

One of the major genetic insights into the pathogenesis of the PN-MPNs included the identification of the somatic point gain-of-function mutations in Janus kinase 2 gene (JAK2), leading to the activation of the JAK/STAT signaling pathway (signal transducer and activator of transcription), culminating in exacerbated cellular proliferation, resistance to apoptosis and evolution to MPNs (2-4). On the other hand, the identification of Philadelphia chromosome (Ph), a translocation involving chromosomes 9 and 22 that results in the formation of the BCR-ABL fusion gene, constitutes the defining leukemic event in CML (5,6). ET is characterized by a high platelet count, often associated with thrombotic and hemorrhagic events, and the presence of JAK2 mutation in about 50-60% of cases (7-9).

As far as we know from literature revision, the frequency of concurrent presence of JAK2V617F mutation and BCR-ABL translocation in a single individual with a MPN is a rare event, independently of what phenotype expresses earlier, PN-MPN or CML (10-13).

Although ET and CML are considered to be mutually exclusive, rare cases of concomitant presence of BCR-ABL translocation positive CML and JAK2V617F mutation positive ET have been reported in the literature (10,13).

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We report here the case of two patients initially included in a data base population of 58 patients with the diagnosis of ET in the presence of JAK2V617F mutation, with the suspicion of coexistence with BCR-ABL translocation. Patient anonymity was guaranteed and consent was provided, in agreement with the Declaration of Helsinki. The Institutional Ethics Board of the Hospital of São Francisco Xavier, West Lisbon Hospital Centre (Lisbon, Portugal) approved the present study.

Case report

Case report 1. A 75-year-old man with a medical history of dyslipidemia, hypertension, acute myocardial infarction, and ischemic stroke in August 2013. In December 2013 this patient was hospitalized with his second ischemic stroke. Although he had confirmed poor adherence to the prescribed therapy for cardiovascular risk patients, in January 2014 he was referred to the hematology consultation for maintained thrombocytosis and leukocytosis, since at least August 2013 (as there was no previous laboratory data available).

Evaluation revealed platelet count of 1,405x10^9/l, leukocytosis (15x10^9/l), with normal formula, and without immature precursor cells as well as normal hemoglobin (Table I).

Abdominal ultrasound confirmed absent splenomegaly and Bone marrow (BM) aspirate showed megakaryocytic hyperplasia and enlarged megakaryocytes, with no abnormalities of the myeloid and erythroid series (Fig. 1). BM biopsy showed a hypercellular marrow (80%), megakaryocytic and granulocytic (slight) hyperplasia (Fig. 2).

Molecular biology (Fig. 3) and cytogenetic tests were performed in peripheral blood and the results revealed positivity for the JAK2V617F mutation and a karyotype of 45,X,-Y[5]/46,XY[15]. The fluorescence in situ hybridization (FISH) was positive for the BCR-ABL translocation in 16% with an atypical pattern. The BCR-ABL transcript was not detected by the conventional reverse transcriptase-polymerase chain reaction (RT-PCR) method (specific for p190 and p210 transcripts). This high risk patient received a daily hydroxyurea (HU), and low dose aspirin regimen as secondary thrombotic prevention. A good response to treatment was achieved, with normalization of leukocytes and platelet reduction of greater than 50% after one month and normalization of platelets after five months of therapy (Table I). This patient had very poor compliance to the therapy and hospital check-ups, so tyrosine kinase inhibitor (TKI) that was planned to be introduced, was discussed, and taking into account the presence of these mutations, the diagnosis of ET JAK2V617F and BCR-ABL positive was admitted. The patient started on HU 500 mg (alternate day progressing to 1 g/alternate day) and TKI (Imatinib, 400 mg/day). A few months later, TKI was suspended and the patient remained under treatment with HU, actually with well controlled disease.

Regarding the methodology used for genetic study, extraction of whole DNA from peripheral blood was accomplished by cell lysis followed by ethanol precipitation and recovery of the DNA by elution in a buffer solution (QIAamp DNA Mini kit; Qiagen GmbH, Hilden, Germany). The presence/absence of JAK2V617F mutation was determined by amplification refractory mutation system (ARMS)-PCR (in-house), based on amplification of a genomic fragment which includes the region corresponding to amino acid 617 of the JAK2 protein, and on the differential detection on agarose gel of the normal or mutated alleles through the use of allele-specific primers. The test result is qualitative and the test sensitivity is 1%. Quantification of JAK2 was obtained by high resolution melting PCR (HRM-PCR) (LightCycler® 480 Instrument; Roche Molecular Diagnostics, Pleasanton, CA, USA), with a sensitivity of about 10% of mutated cells. Conventional RT-PCR was performed for the identification of BCR-ABL transcripts (specific for p190 and p210), after RNA extraction, according to the methodology described by van Dongen et al (14). Results are analyzed on agarose gel electrophoresis. FISH analysis was done on 100 nuclei after hybridization with specific probes for t(9;22) BCR-ABL (Vysis LSI BCR-ABL Dual Color, Dual Fusion Translocation Probe).

Discussion

Several authors have investigated the relationship between JAK2V617F and BCR-ABL anomalies and many theories have been postulated in the last years, especially after the identification of JAK2V617F mutation in 2005.
The Janus kinase 2 gene (JAK2; cytogenetic location: 9p24.1) provides instructions for making a protein that promotes the growth and division (proliferation) of cells. This protein is part of a signaling pathway called the JAK/STAT pathway, which transmits chemical signals from outside the cell to the cell's nucleus. The JAK2 protein is especially important for controlling the production of blood cells from hematopoietic stem cells. These stem cells are located within the bone marrow and have the potential to develop into red blood cells, white blood cells, and platelets.

The Philadelphia chromosome (chromosome 22) results from the reciprocal translocation of genetic material between chromosome 9 and chromosome 22, and contains the fusion gene BCR-ABL, which codes for a tyrosine kinase signaling protein that causes the cells to divide uncontrollably (particularly CML cells).

From 2007 to 2015, at least 42 patients with this double mutated phenotype were reported in the literature (10,12,15,16). Moreover, the italian group of Pieri et al (17) studied 314 patients with CML and identified 8 cases (2.55%) with concomitant JAK2V617F mutation. Pagnano et al detected only one case with JAK2V617F mutation among 55 cases of CML analyzed (13).

Among these different studies reported, several patterns were described: i) Initially diagnosed with CML and treated
V617F mutations are present in hematopoietic positive ET without features of positive ET is featured. The translocation is confined to a small compartment of myeloid progenitor cells, only in granulocytic colonies (10). In contrast, other reports showed the simultaneous presence of both BCR-ABL transcript and JAK2V617F mutation in the majority of granulocytic and erythroid colonies at the time CML diagnosis was established, corroborating the hypothesis that only one cellular clone is bearing concomitantly the two anomalies (10,11).

Therefore, the phenotypic heterogeneity can be the result of the expression of a pre-existing mutated clone previously ‘silent’ or of the accumulation of several genetic events conferring genetic instability and leading to a ‘new’ anomaly (12).

As far as we know from literature revision, there are no other reports of positivity for JAK2 mutations, other than V617F, with the concomitant presence of BCR-ABL translocation.

One of the diagnostic criteria for ET, is the absence of the Ph chromosome. BCR-ABL positive ET without features of CML in blood and bone marrow is a rare entity and constitute less than 5% of ET diagnosis. Some authors have proposed to consider those cases as CML associated with a rather poor prognosis because of the high tendency to progress to myelofibrosis and blastic transformation after a few to several years (16,20,21).

An important difference between BCR-ABL positive ET and BCR-ABL positive CML at time of presentation is the absence of splenomegaly in the first situation (16).

The bone marrow in BCR-ABL positive ET is featured by predominant smaller than normal and hypo/mononucleated megakaryocytes caused by BCR-ABL gene and protein induced maturation defect of the hematopoietic stem cells. This contrasts with clustered enlarged megakaryocytes in BCR-ABL negative ET due to growth advantage and proliferation of constitutively activated JAK2 or MPL somatic mutated megakaryocytes (16).

The first patient reported had diagnostic features that matched CML and ET. However, his overall clinical presentation including bone marrow features was more commonly suggestive of ET. Since the t(9;22) was positive in FISH, according to the results, there should have been found a positivity for JAK2V617F, BCR/ABL and GTPase activating protein-ABL proto-oncogene 1 non-receptor tyrosine kinase; FISH, fluorescent in situ hybridization; HU, hydroxyurea; TKI, tyrosine kinase inhibitor.

Table II. Results over time and therapy prescribed for case study 2.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>2005</th>
<th>2009</th>
<th>2013</th>
<th>2016</th>
<th>2017</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelets (x10⁰/l)</td>
<td>1022</td>
<td>478</td>
<td>684</td>
<td>909</td>
<td>413</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>14.6</td>
<td>14.7</td>
<td>12.2</td>
<td>13.1</td>
<td>14.1</td>
</tr>
<tr>
<td>Leucocytes (x10⁰/l)</td>
<td>9.4</td>
<td>6.9</td>
<td>22.4</td>
<td>30.1</td>
<td>38.4</td>
</tr>
<tr>
<td>JAK2V617F mutation</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Positive 17%</td>
</tr>
<tr>
<td>BCR/ABL t(9;22) (FISH)</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
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</table>

The patient was started on HU for thrombocytosis, with subsequent increase in dose and the addition of TKI. A few months later, TKI was suspended and HU treatment was maintained. BCR/ABL, BCR RhoGEF and GTPase activating protein-ABL proto-oncogene 1 non-receptor tyrosine kinase; FISH, fluorescent in situ hybridization; HU, hydroxyurea; TKI, tyrosine kinase inhibitor.

Figure 4. Case study 2: FISH test. It revealed positivity for the the BCR-ABL translocation with an atypical pattern, in which a unique fusion signal is detected. BCR/ABL, BCR RhoGEF and GTPase activating protein-ABL proto-oncogene 1 non-receptor tyrosine kinase; FISH, fluorescent in situ hybridization; HU, hydroxyurea; TKI, tyrosine kinase inhibitor.

with imatinib that proceeded to a JAK2V617F myeloproliferative phenotype; ii) initially diagnosed with CML coexisting with JAK2V617F mutation positive PV, ET or PMF; or iii) initially diagnosed with JAK2V617F mutation positive PN-MPN, ET more rarely, evolving years later to CML (10). Commonly, men above 50 years old were the most frequently affected (10).

A question still has to be clarified: Which is the first anomaly to occur? Several working groups reported that in some cases of PN-MPNs that evolved to CML, JAK2V617F mutation was the first leukemogenic event and BCR-ABL the second positive clone (10). Moreover, it was also speculated that JAK2V617F mutations are present in hematopoietic stem cells, with an additional BCR-ABL translocation being subsequently acquired in a sub-clone (10,18). However, other groups didn't confirm these results, and postulate that the two anomalies are present since the beginning of the process (10). Indeed, about the amount of cellular clones involved, there are reports that state that two different clones are involved, with the phenotypic expression depending on which one of the aberrations is ‘dominating’, as a result of therapy targeted to the other anomaly (10,11,19,20). On the other hand, there are some authors evidencing that all the myeloid cells bear JAK2V617F mutation, including granulocytic and erythroid colonies, while BCR-ABL translocation is confined to a small compartment of cellular clones.
chromosome was detected by karyotype. The second patient reported was more suggestive of ET and did not have typical clinical, nor morphologic findings for CML. The t(9;22) was also positive only by FISH, with a negative result in molecular biology tests. In this case it was not possible to evaluate karyotype due to metaphases absence.

In both patients, search for JAK2V617F mutation and BCR-ABL was concomitant, making very difficult to know if both mutations were present from the beginning or the order of appearance of each one of them. The fact that the study has been performed before therapy institution, excludes the possible inhibitory effect of it over one of the altered clones, making the other more expressive.

Given the above, several questions have to be raised: Are these genomic alterations found in these two patients and their atypical pattern really true and clinically significant or are they false positive results? May those be new/distinct clinical entities? Should we consider Ph positive ET as distinct entity, separate from Ph negative ET and Ph positive CML?

As mentioned above, studies describing cases initially diagnosed with JAK2V617F mutation positive PhN-MPN, evolving later to CML, were rarely ET patients (10), in contrast to our report.

Although, the concomitant presence of these two anomalies in these patients didn't seemed to exclude the diagnosis of ET, at diagnosis or in some point of their clinical course, both patients evidenced a distinct clinical (thrombocytosis with associated leukocytosis) or morpho-histological (megakaryocytic) phenotype from what was expected for ET with isolated JAK2V617F positive or Ph positive CML, but apparently not influencing the course of the disease.

Both patients showed an atypical pattern for BCR-ABL translocation search by FISH, said to be atypical because only one fusion signal was observed, instead of the two signals expected, with a percentage of BCR-ABL translocation of approximately 20%. RT-PCR was performed using only a single primer pair, failing the identification of p190 and p210 transcripts of BCR-ABL fusion gene, and making the presence of BCR-ABL tyrosine kinase activity questionable. Real time PCR was a distinct possible technique to be used for the identification and quantification of BCR-ABL p210 (mainly b3a2 and b2a2 types) transcripts, however it was not performed.

Since no BCR-ABL transcripts were detected by RT-PCR, one hypothesis is that the unique fusion signal detected by FISH could correspond only to der(9), and not to Ph chromosome with associated tyrosine kinase activity (on chromosome 22). Confirmation could be achieved doing FISH in metaphases, which was only possible in the first case, since the second patient had no metaphases to allow it. This way, we were not able to be sure of the localization of break points and consequent fusion.

On the other hand, a missense on the primer site or the probe pairing region could also explain such RT-PCR result, but there is a vast experience with the used primers, internationally designed and certified.

Regarding clarification of the possible mechanism of association of JAK2V617F mutation and a ‘true’ BCR-ABL translocation involved in our patients, it would be useful to analyze JAK2V617F mutation and BCR-ABL gene in each colony of BFU-E or CFU-C.

Given the above, probably these cases correspond to two patients with a variant ET, in which we possibly can hypothesize that the presence of der(9) chromosome might be involved in those phenotypic differences. As far as we are aware, no other studies describing these two ‘truly’ genomic alterations have found a BCR-ABL aberrant pattern similar to our cases. However, Larsen et al (22). Described the case of four patients JAK2V617F positive with associated distinct karyotypic aberrations [including der(9;18)], presenting with a distinct clinical and prognostic profile. Likewise, another study also reported the association of der(9) chromosome and acute lymphoblastic leukemia (23), with prognostic impact.

Moreover, WHO does not currently address the classification of MPNs that have more than one genetic abnormality, but it is well established that the presence of additional co-operating mutations in myeloid genes (along with other important risk factors) has a straight relationship with phenotype and clinical outcome definition (24,25). Cytogenetic analysis allows to identify subgroups of patients with a distinct phenotype and prognostic profile, and should be performed in conjunction with JAK2 mutation analysis PhN-MPNs patients (22).

Furthermore, the concomitant presence of two molecular markers is well defined for certain diseases, and raises several issues, including the best therapeutic strategy to adopt. But, therapeutic decisions should not be based only on molecular biology test results (18).

CML can express on the background of a JAK2V617F positive PhN-MPN, and treatment with TKI might reveal/make more expressive the Ph-phenotype. It IS of great importance to recognize and investigate the association of both anomalies, especially in CML patients who have an unusual clinical/laboratorial course, with hemoglobin and/or platelets count increase, or when they do not respond to therapy, making the diagnosis of other MPNs to have practical therapeutic consequences.

It seems that for these complex patients the most efficient therapeutic choice is to associate a TKI with a JAK2 inhibitor (10,11).

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors’ contributions

FM and APA contributed to the conception and design of the work, acquisition, analysis and interpretation of data; drafted and wrote the manuscript, revising it critically for important intellectual content; were accountable for all aspects of the work in ensuring that questions related to the accuracy and integrity of any part of the work were appropriately investigated.
and resolved. TM, PSS, RC, SM, SS, JFV and FL analyzed the data and revised the paper. SR provided the histological images and revised the paper. All authors approved the final manuscript.

Ethics approval and consent to participate

Patient anonymity and consent was guaranteed, in agreement with the Declaration of Helsinki, the Institutional Ethic Board of Hospital of São Francisco Xavier, West Lisbon Hospital Centre (ref. no. 120/CE_2009) approved this study.

Consent for publication

Patient anonymity and consent was guaranteed, in agreement with the Declaration of Helsinki.

Competing interests

The authors declare that they have no competing interests.

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