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Letter to the Editor

Concomitant detection of BCR–ABL translocation and JAK2 V617F mutation in five patients with myeloproliferative neoplasm at diagnosis

Sir,

Myeloproliferative neoplasms (MPN) are clonal stem cell disorders characterized by proliferation of one or more of the myeloid lineages, associated with genetic abnormalities that include translocations or point mutations of genes that encode cytoplasmic or membrane tyrosine kinase (TK) receptor proteins. This produces a constitutively abnormal activation of signal transduction pathways, leading to an unregulated proliferation. According to the WHO criteria, MPN are classified into BCR–ABL $^{+}$/Philadelphia Ph^{+}: chronic myeloid leukemia (CML) and MPN BCR–ABL^{-}/Ph^{-}. A single acquired point mutation, JAK2 ^{V617F}, has been described in 95% of polycythemia vera (PV), in 50% of essential thrombocythemia (ET), and idiopathic myelofibrosis (IMF), and it is generally absent in MPNs Ph+ [1]. In the last years, the co-occurrence of both BCR–ABL gene fusion and JAK2 ^{V617F} mutation in few cases of patients with CML has been described [2–6]. We report here the rare and concomitant occurrence of JAK2 ^{V617F} mutation with BCR–ABL translocation at presentation in five atypical MPN patients.

Five patients with clinical suspicion of MPN diagnosis, with a slightly increased white blood cell (WBC) level (7.2 –25.9 $\times$ 10^{3}/mm^{3}), thrombocytosis (700–1036 $\times$ 10^{3}/mm^{3} platelets), and splenomegaly grade I – two of them with anemia (5.2 and 7.1 g/dl, respectively) and the others with normal hemoglobin level – were referred to our laboratory in order to perform cytogenetic studies and molecular analysis of BCR–ABL fusion gene expression by conventional RT-PCR. All cases showed BCR–ABL (b3a2 isoform) expression. JAK2 ^{V617F} mutation was also studied in two of them (patients 2 and 3, Table 1) by ASO-PCR at the same time, both with positive results (Table 1). Regarding cytogenetic studies, three cases showed the lack of Ph chromosome, one patient showed 15% Ph+ metaphases, and in the remaining patient, no data were available.

Patients 1, 4, and 5, after MPN diagnosis, received imatinib therapy and were monitored by quantitative BCR –ABL real-time PCR. Quantitative PCR for BCR–ABL expression performed in these patients during follow-up (8–12 months) showed BCR–ABL/ABL ratio <0.005% (scored according to the International Scale). Due to persistent thrombocytosis, slightly increased WBC level, and splenomegaly grade I, JAK2 status was analyzed in these blood specimens. All samples were positives for JAK2^{V617F} mutation. Retrospective assessment of stored samples showed that JAK mutation was already present at the time of the diagnosis of MPN. There are no available data from patients 2 and 3 during follow-up because one of them moved to a foreign country and the other died due to colon tumor.

These five patients were studied from a total of 1320 patients with a suspected diagnosis of myeloproliferative neoplasm and were referred to our laboratory from different health institutions between 1994 and 2010 to establish karyotype and BCR–ABL status. Four hundred forty-two of them were Ph/BCR–ABL (+) confirming CML diagnosis. Since 2006, 58 of the BCR–ABL (+) patients were also studied for JAK2^{V617F} mutation at the same time of the BCR–ABL detection or later retrospectively. Only six patients harbored both genetic alterations at the time of the initial MPN diagnosis, and five of them were described in this report. We had not enough clinical information of the other one.

The coexistence of both genetic defects, BCR–ABL fusion gene and JAK2^{V617F} mutation, in patients with MPN is a rare and uncommon feature. We described five patients with MPN harboring both genetic features in diagnosed blood samples. In three cases, JAK2^{V617F} mutation was detected in patients with MPN who were BCR–ABL positive after the remission induction with imatinib and retrospectively in sample at the onset of the disease. The rapid remission of BCR–ABL transcript, after a short period of imatinib treatment and lack of Ph chromosome, led us to think that BCR–ABL fusion gene expression was present in a low burden at diagnosis.

Table 1. Cytogenetic and molecular biology assays performed at the diagnosis of the disease and postimatinib treatment

	At diagnosis					Follow-up				
		BCR–ABL					BCR–ABL			
Case	Cytogenetics	Fish	RT-PCR	Q-PCR	$JAK2^{V617F}$	Cytogenetics	Fish	RT-PCR	Q-PCR	$JAK2^{V617F}$
1	Ph−	Neg	b_3a_2*	NA	Pos†	NA	Neg	Neg	<0.005	Pos
2	Ph−	NA	b_3a_2*	<0.3	Pos	NA	NA	NA	NA	NA
3	Ph−	NA	b_3a_2*	NA	Pos	NA	NA	NA	NA	NA
4	Ph+ (15%)	NA	b_3a_2*	NA	Pos†	Ph−	NA	Neg	<0.005	Pos
5	NA	NA	b_3a_2*	NA	Pos†	Ph−	Neg	Pos*	<0.005	Pos

Q-PCR, quantitative PCR (diagnostic sensitivity: 1 neoplasm cell/100 000 normal cells); RT-PCR, reverse-transcription polymerase chain reaction; Ph, Philadelphia chromosome; Pos, positive result; Neg: negative result; NA, no data were available.
*b_3a_2 amplification in second round of nested PCR (diagnostic sensitivity: 1 neoplasm cell/1 000 000 normal cells).
†Retrospective assay performed in sample at diagnosis.

In concordance with previous reports, the complete reduction in BCR–ABL rearrangement, after the imatinib therapy, and the persistence of JAK2 mutation suggest two possible mechanisms for this double genetic alteration: (i) a hematopoietic cell subclone with a pre-existing $JAK2^{V617F}$ acquires the BCR–ABL fusion gene, which confers a selective advantage to double-mutant progenitor; (ii) two clones, one of them having BCR–ABL rearrangement and the other one the $JAK2^{V617F}$ mutation (biclonal origin). These cases intend to contribute to the discussion about the onset of the molecular alterations, and their correlation with the different phenotypes and clinical management.

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