

THE SIMULTANEOUS OCCURRENCE OF THE BCR-ABL TRANSLOCATION AND THE *Jak2V617F* MUTATION. DETECTION AND DYNAMICS EVOLUTION IN A CASE OF CHRONIC MYELOPROLIFERATIVE NEOPLASM

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Abstract: *Although the discovery of the BCR-ABL transcript and of the *Jak2 V617F* mutation lead to important clarifications, the pathogenesis of chronic myeloproliferative neoplasms remains extremely complex and still presents several unknowns. Our study reports the case of a 52 years old man with chronic myeloid leukemia (BCR-ABL positive) who, after 4 years of targeted therapy with tyrosine kinase inhibitors, also developed PV (*Jak2 V617F* positive). As such, our findings negate the theory according to which the two genetic transformations and the two different pathologies defined by them, respectively, were mutually exclusive.*

Key words: *Chronic Myeloid Leukemia (CML), Polycythemia Vera, (PV) BCR-ABL Transcript, *Jak2 V617F* Mutation, PCR*

1. Introduction

Chronic myeloproliferative neoplasms (MPN) are proliferations of hematopoietic stem cells characterized by phenotypic and genotypic heterogeneity.

The consequence of clonal proliferation consists in the accumulation of adult myeloid elements resulting in several hematologic neoplasms with different clinical manifestations depending on the proliferating cellular element. Classic

examples of chronic myeloproliferative neoplasms are: CML, PV, ET and MMM.

In the last decade, molecular testing has become essential in the diagnosis of these disorders. According to the latest WHO classification (2008), two molecular markers define the clinical entities of which MPN is comprised.

BCR-ABL translocation (resulting from t(9;22)(q34;q11) translocation, also known as the Philadelphia chromosome or Philadelphia translocation), is usually

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associated with chronic myeloid leukemia (CML). This marker is treated with targeted drugs called tyrosine kinase inhibitors (TKIs)

The second marker is the Jak2 V617F point mutation, an important criterion in the diagnosis of BCR-ABL negative chronic myeloproliferative diseases including Polycythemia Vera (PV), Essential Thrombocythemia (ET) and Myelofibrosis with Myeloid Metaplasia (MMM) [7].

It was initially thought that the two markers were mutually exclusive, but recent literature has uncovered several cases in which these two markers coexisted.

Biological molecular testing performed in our laboratory for the diagnosis and monitoring of two chronic myeloproliferative neoplasm patients revealed both biological markers in the course of the disease. The first case, of a polycythemia vera patient that within 7 years also developed BCR-ABL positive CML with JAK2 617F translocation was described by I. Ursuleac, MD, PhD in 2013 [8].

2. Case Report

This article reports the case of a 52 years old man initially diagnosed with CML, who, after 4 years, also developed PV. The diagnosis and molecular monitoring were performed in our laboratory.

Informed consent was signed by the patient under the protocol approved by the Ethics Committee of the Carol Davila University of Medicine and Pharmacy, Bucharest.

The patient first consulted a specialist in August 2009, presenting symptoms such as malaise, pallor, fatigue, and severe weight loss (22 lb in a month). There was no mention of a significant family or personal medical history.

Clinical (significant hepato-splenomegaly) and biological evaluation revealed the occurrence of chronic myeloproliferation, and the histopathological and cytogenetic exams confirmed the CML diagnosis (in accordance with WHO 2008 classification) [7].

We were not able to perform a molecular analysis at that time.

The most relevant transformations occurred at different key stages of diagnosis: LGC (in 2009) and PV (2013) are presented in the table 1.

Laboratory findings

Table 1

	2009 (CML state)	2013 (PVstate)	Normal ranges
Laboratory findings	Values	Values	Values
Haemoglobin :	9.8 g/dl	18g/dl	12-16 g/dl
Haematocrit :	29.5%	54%	36-48%
WBC:	139×10 ⁹ / mmc	4.8×10 ⁹ / mmc	4-10 x10 ⁹ / mmc
Blasts:	3%	0	0
Promyelocytes:	4%	0	0
Myelocytes:	3%	0	0
Metamyelocytes:	14%	0	0
Bands:	10%	0	0
Neutrophils:	48%	72%	35-55%
Eosinophils:	3%	2%	0-5%
Basophils:	9 %	1%	0-1%
Lymphocytes:	4%	20%	25-25%
Monocytes:	2%	5%	3-7%

Laboratory findings

Table 1

Laboratory findings	2009 (CML state)	2013 (PVstate)	Normal ranges
	Values	Values	Values
Platelets	418 × 10 ⁹ /mmc	665x10 ⁹ /mmc	150-450×10 ⁹ / mmc
Erythroblasts	5/100 WBC	-	none
Neutrophil Alkaline Phosphatase score	0	170	10-100
Bone marrow aspiration	Granulocytic hyperplasia (85%) mild reduction of erythroblasts(14%)	Pan hyperplasia	Normal
Bone marrow biopsy	Features of CML	Features of PV	Normal
Cytogenetic exam	t(9;22)(q34;q11) in 85% metaphases	-	Normal
Molecular exam	Not possible in 2009 Ratio bcr-abl major/abl=0.02% (oct:2010)	Homozygous Jak2V617Fmutation. Undetectable bcr-abl major (since febr.2011)	Without mutations

It should be noted that, at the time of the diagnosis, the hemoglobin and hematocrit levels were low.

The patient began cytoreductive therapy (with Hydroxyuree), and after 3 months, targeted therapy with tyrosine kinase inhibitors was initiated (Imatinib 400 mg/day).

Molecular testing was performed in October 2010.

Detection and quantification of BCR-ABL transcript was performed by Standard Real-time PCR protocol [1] with primers specific for major BCR-ABL (p2 fusion protein correspondent), as well as ABL (Abelson gene) as a control gene on the Applied Biosystems® StepOnePlus™ Real-Time PCR System with TaqMan probes.

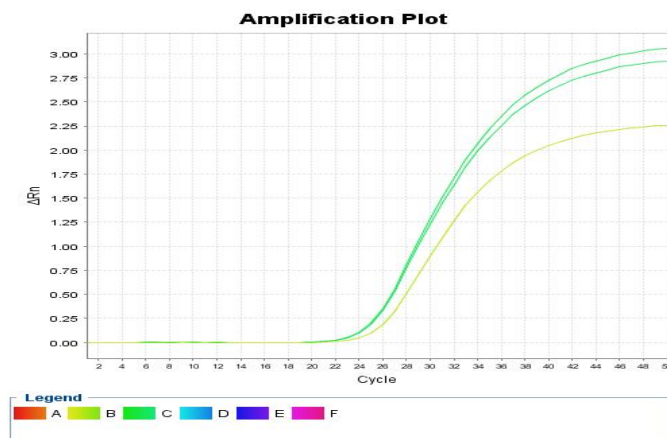


Fig. 1. Real-time PCR amplification curves

Subsequently, the treatment with tyrosine kinase inhibitors was monitored as recommended by European Leukemia Net,

by quantifying the major BCR-ABL transcript (Fig. 1).

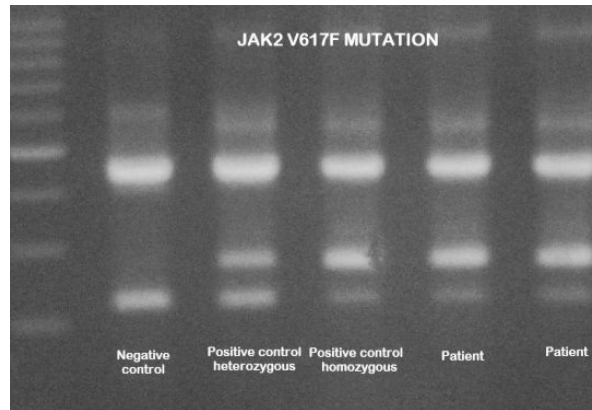


Fig. 2. *Agarose gel electrophoresis*
Duplicate samples from patient with *Jak2 V617F* homozygous mutation

Within 12 months, the patient achieved MMR (with a value of 0.02% major BCL-ABL/ABL) in October 2010, and within 21 months, in February 2011, the transcript became undetectable. Subsequent evaluations show the same results.

Four years after the diagnosis, in August 2013, clinical changes (pruritus, facial erythema) and important hematologic changes occurred (Hb:18g/dl, Ht:54%, L- $4.8 \times 10^9/\text{mmc}$, Plt- $665 \times 10^9/\text{mmc}$), which raised the suspicion of polycythemia.

The diagnosis algorithm was used to detect the presence of the *Jak2 V617F* mutation. The result was positive for *Jak2 V617F* mutant allele, homozygous genotype.

The nucleic acids (genomic DNA and total RNA) were obtained by standard procedures of purification of integral peripheral blood IVD kits (Qiagen, UK). *V617F* mutation detection was performed by the ARMS (Amplification refractory mutation system), technique described by Jones et al [3]. The technique uses two pairs of primers (tetra-primers) which, in a single step reaction, amplify a large fragment of the control gene, as well as fragments corresponding to the *V617F*

mutation and to the wild type *Jak2*. The resulting amplicons are: control fragments (463 bp), and fragments corresponding to the *V617F* mutation (279bp) and wild type *Jak2* (229 bp). The amplicons were visualized in a 3% agarose gel electrophoresis, ethidium bromide staining under a UV illuminator. Control samples were employed in every experiment, as follows: positive control samples (with the mutant allele) and negative control sample (with the wild type allele) (Fig. 2).

Subsequent molecular analyses show that the BCR-ABL transcript remains undetectable and that the proportion of the *Jak2 V617F* mutant allele remains constant.

The patient is still under treatment with Imatinib. Additionally, the patient undergoes phlebotomy and is being given Aspenther and he is in a good clinical and hematologic condition.

3. Discussion

The fact that the two mutations that were deemed to be mutually exclusive can coexist in the same patient raises some questions about the clonal origin of these proliferations: did the two clones occur

simultaneously or is it the case that a preexisting clone induced a genomic instability which, in turn, resulted in the occurrence of a phenotypically distinct subclone? [2], [5], [9]

Many studies regarding the Leukemic transformation of MPN have proven that unrelated leukemic and non-leukemic clones can coexist, also detecting additional mutations (TET2, CBL, and TP53 mutations) in blast cells, rather than in chronic proliferating cells. In some cases, the TET2 mutation preceded the occurrence of Jak2 V617F, suggesting a pre-Jak cell [4].

Older studies have also shown that leukemic blast cells derived from a positive transformation of a post-MPN Jak2 V617F positive are Jak2 V617F negative.

By studying endogenous colonies in a semisolid state, important data were obtained for the reconstruction of clonal events.

In a recent study, following the analysis of colonies derived from single cells, at different stages in the clinical evolution of a similar case (Jak2 myeloid metaplasia that within 3 years also developed CML), Yamada Y. made the following observations [10]:

- There are Jak2 V617F positive colonies that also presented BCR-ABL translocation, but none of the Jak2 wild type alleles present BCR-ABL clones.

- It is possible that the Jak2 somatic mutation is preexistent, determining a genetic instability which, in turn, results in the occurrence of a major BCR-ABL translocation, giving it a growth advantage on the Jak2 V617F and, consequently, leads to an excessive proliferation of granulocytes with CML phenotypic expression.

The phenotypic expression of the Jak2 V617 mutation reported in this article also occurred only after BCR-ABL levels

became low or undetectable, as a consequence of the tyrosine kinase inhibitors therapy.

41 such cases were reported on the PubMed biomedical studies platform between 2007 and 2013. See: [6].

There were described three different "scenarios":

- 1) Initial CML that within several years of tyrosine kinase inhibitors therapy also develops Jak2 V617F positive MPN

- 2) Initial (phenotypic) CML that also presents the Jak2 V617F mutation (genotypic coexistence of both markers)

- 3) Jak2 V617F positive MPN that, after years of clinical evolution, develops BCR-ABL positive CML

PV and MMM have a high frequency, while ET has a lower frequency.

4. Conclusions

The concomitant occurrence of two markers defining two different MPNs is a rare, but not impossible event.

Performing both molecular tests is highly recommended at the time of diagnosis or during the disease progression if any atypical sign occurred.

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