

# Detection of Jak2 V617f Mutation, Secondary to the Presence of Bcr-Abl1 Translocation in a Patient with Chronic Myeloid Leukemia: Report of a Case and Review of the Literature

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## Abstract

The myeloproliferative neoplasms are classified in four major diseases: Chronic Myeloid Leukemia, Polycythemia Vera, Primary Myelofibrosis and Essential Thrombocythemia. The JAK2 V617F mutation is found in 95% of Polycythemia Vera, and 50% of Essential Thrombocythemia and Primary Myelofibrosis patients. It was thought that the JAK2 V617F mutation and BCR-ABL1 translocation were mutually exclusive; but now a few cases have been reported with both alterations. We report a rare case with the presence of JAK2 V617F mutation, secondary to a diagnosis of BCR-ABL positive chronic myeloid leukemia. The patient was initially diagnosed as chronic myeloid leukemia and was BCR-ABL1 positive, so he started to receive Imatinib. He responded well to the therapy for three years, but after this time the patient had a hematological relapse still with no detectable copies of BCR-ABL1. For this reason, we thought of the possible development of another genetic alteration. Because the patient had a very high platelet count, we decided to look for the JAK2 V617F mutation, which result was positive. This case is just one of the few that have been reported worldwide that have a coexistence of these two genetic alterations: the BCR-ABL1 transcript and JAK2 V617F mutation in chronic myeloproliferative syndromes. This is the first case in the Central American population, found in our series of a total of 168 patients with Philadelphia positive chronic myeloid leukemia.

**Keywords:** BCR-ABL1; JAK2; Coexistence; Myeloproliferative neoplasm

## Introduction

The Myeloproliferative Neoplasms (MPN) are classified as four major diseases: Chronic Myeloid Leukemia (CML), Polycythemia Vera (PV), Primary Myelofibrosis (PMF) and Essential Thrombocythemia (ET) [1]. The translocation t(9;22)(q34;q11) produces the Philadelphia chromosome and causes the *BCR-ABL1* transcript; this alteration is commonly found in CML [1]. In 2008 the World Health Organization (WHO) reported that MPN patients with the *BCR-ABL1* transcript (Philadelphia positive) should be classified as chronic myeloid leukemia, and those who are Philadelphia negative (*BCR-ABL1* negative) should be classified as Polycythemia Vera (PV), Essential Thrombocythemia (ET) or myelofibrosis (PMF) [1]. *JAK2* is a tyrosine kinase gene that has a role in the signaling pathways of hematopoietic factors. The *JAK2* V617F mutation is found in 95% of PV patients, and 50% of ET and PMF patients [2,3]. Previously, it was thought that the *JAK2* V617F mutation and *BCR-ABL1* translocation were mutually exclusive. However a few rare cases have been reported that are positive for both alterations [4-17]. We report the first case in Central American with the presence of *JAK2* V617F mutation, in a patient with the diagnosis of Philadelphia positive chronic myeloid leukemia.

## Material and Methods

### Patients' samples

We collected blood samples from patients with a diagnosis of myeloproliferative neoplasm. The samples were collected from patients referred from different national hospitals. Every patient signed the informed consent form approved by the local ethics committee. The technique used for extracting leukocytes from blood was via ammonium lysis. Cells obtained by this technique were stored at -20°C.

### Case report information

All the information analyzed in this case report was given by the

patient and the participant hospitals. The diagnosis of the *BCR-ABL1* transcript was made by FISH by other national laboratory.

**RNA AND DNA EXTRACTION:** The RNA was extracted by the trizol (Life technologies, NY, USA) method according to the manufacturer's procedures. The DNA was extracted using the Purelink Genomic DNA Minikit (Invitrogen, NY, USA). The RNA and DNA quantitation was made by fluorometry, using the quantifluor equipment and method (Promega, WI, USA).

### JAK2 V617F Allele specific-PCR

The reaction was standardized according to the thermal cycler used. The primers used were described by Jones et al. and Chen, et al. [18,19]. The PCR conditions included 10 minutes at 94°C, 30 cycles each of 30 seconds at 94°C, 45 seconds at 54°C, 1 minute at 72°C, and a final extension of 5 minutes at 72°C.

### BCR-ABL1 real time PCR quantitation

The real time PCR was made according to the Molecular MD One-Step qRT-PCR BCR-ABL kit procedures. (Molecular MD, Portland OR, USA).

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## Results

### Case presentation

In November, 2010 a Guatemalan man of 68 years presented with a problem of thrombosis in one finger. Initial laboratory tests showed that the white cell count was  $22.7 \times 10^3/\text{ml}$  with 86.8% neutrophils, 6.9% lymphocytes, 2.2% monocytes 3.7% eosinophils and 0.4% basophils. The red blood count was  $7.12 \times 10^6/\text{ml}$ , the hemoglobin was 18 mg/ml, the HCT was 55.50% and the platelets count was  $6.97 \times 10^5/\text{mL}$ . Bone marrow analysis was compatible with a chronic myeloproliferative process. FISH analysis revealed the chromosome translocation t(9;22)(q34;q11). From this, the patient was diagnosed as chronic myeloid leukemia *BCR-ABL1*-positive. The patient was treated with 400 mg.

Imatinib, and then the dose was increased to 800 mg since there was not a complete hematologic response. The patient reached hematologic remission, and remained there, until January 2013, at which time the patient had a hematological relapse.

Molecular monitoring of *BCR-ABL1* copy numbers, began in November 2011 (one year after diagnosis) and was at 0.06% IS (international system), which means a major molecular response (MMR) to therapy; unfortunately there were no monitoring of *BCR-ABL1* prior to this date. Four additional *BCR-ABL1* monitoring were conducted periodically, and all showed a complete molecular response. The last monitoring was performed in April 2013 (Figure 1). Note that the patient has no detectable copies *BCR-ABL1*, yet still has clinically

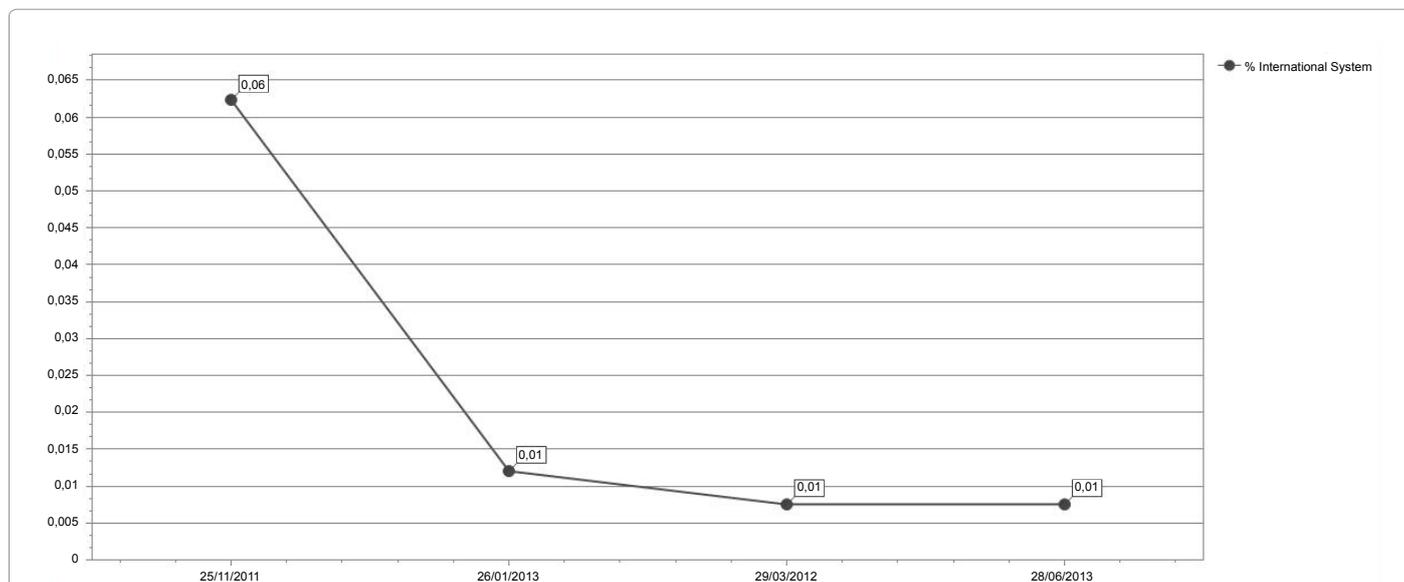


Figure 1: *BCR-ABL1* MOLECULAR MONITORING, showing the initial major molecular response and then the complete molecular response in each monitoring.

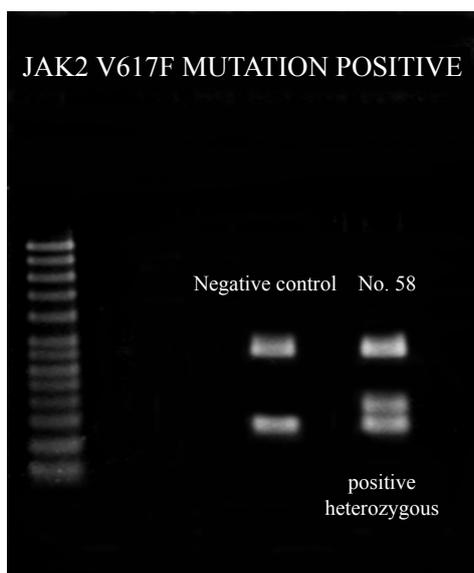


Figure 2: JAK2 V617F, This figure shows the presence of JAK2 V617F heterozygous mutation.

INITIAL DIAGNOSIS	NUMBER OF CASES	PRESENCE OF BCR-ABL1 AND JAK2 MUTATION	AGE	LEUKOCYTES COUNT ( $\times 10^3/\text{ml}$ )	PLATELETS ( $\times 10^5/\text{ml}$ )
CML	3	Coexistence of both genetic alterations at diagnosis	21-67 years	38.0-143.0	3.45-4.40
CML	4	Presence of BCR-ABL1 at diagnosis, and subsequent development of the JAK2 mutation	42-53 years	36.7-350.0	3.45-9.83
MPN	7	Coexistence of both genetic alterations at diagnosis	27-66 years	7.2-25.9	2.85-8.3
MPN	2	Presence of JAK2 at diagnosis, and subsequent development of the BCR-ABL1 translocation	-----	19.7-20.0	7.5

Table 1: Summary of Cases Reported [4-19].

relapsed. In June 2013, the laboratory tests showed that the white cell count was  $17.2 \times 10^3/\text{ml}$ , while the differential blood count showed 82.8% neutrophils, 9.41% lymphocytes, 4.01% monocytes 3.49% eosinophils and 0.30% basophils. The red blood count was  $6.23 \times 10^6/\text{ml}$ , the hemoglobin 13.2 mg/ml, the HCT 43.43% and a platelets count was  $7.39 \times 10^5/\text{ml}$ . Because of the absence of a hematologic response to therapy, and the complete molecular response of *BCR/ABL1* presented by the patient, it was thought that another genetic alteration might coexist.

Because of the results of the last hematology studies in which both the platelet and white blood cell counts were increased, it was believed that this patient had a myeloproliferative neoplasm which was *BCR-ABL1* positive in combination with a mutation in the *JAK2* gene. Therefore, we performed a test of the *JAK2* V617F mutation by allele-specific PCR, and a positive heterozygous result was obtained for this mutation. The *JAK2* mutation appeared after three years of having the *BCR-ABL1* translocation, when the patient relapsed (Figure 2).

## Discussion

The *JAK2* gene and the *ABL1* gene regulate hematopoietic function. The *JAK2* mutations leads to uncontrolled cell proliferation and produces clinical characteristic compatible with MPN. The *BCR-ABL1* translocation produces an imbalance in cell division, differentiation and apoptosis, this produce a CML. The two alterations are present in this patient. The high platelet counts show the presence of a MPN syndrome, secondary to the CML presentation.

As mentioned in the introduction, the *BCR-ABL1* transcript and *JAK2* mutation were previously believed to be mutually exclusive (according to the WHO criteria, 2008); and the presence of either one was used to classify the MPNs into two main groups: Philadelphia positive chronic myelogenous leukemia and Philadelphia negative MPNs, which are PV, ET, PMF. However a few cases have been reported in which there was a co-existence of two genetic alterations. In some of these the *BCR-ABL1* and *JAK2* mutations were found to co-exist [3-11].

This case is the first reported from Central American and one of the few reported internationally, where the patient was initially diagnosed as having Philadelphia positive chronic myeloid leukemia, and in the following years developed the presence of the *JAK2* V617F mutation. This is the first such case among 168 Guatemalan patients with Philadelphia positive chronic myeloid leukemia that we have studied.

As can be observed, our patient had a complete hematologic response only with a dose of 800 mg of Imatinib, although the recommended dose is half of that. After the patient remained well for three years, he relapsed. We discarded Imatinib resistance as an explanation of the relapse since the monitoring of *BCR-ABL1* quantification indicated a continuing complete molecular response. Therefore, it was thought that

the relapse was due to the presence of a secondary genetic alteration, which had subsequently developed in the presence of the *BCR-ABL1* transcript. For this reason, we investigated the presence of the *JAK2* V617F mutation and a heterozygous positive result was obtained. No other cytogenetic or molecular abnormalities were found.

We concluded that the alteration that caused the loss of complete hematologic response was the *JAK2* V617F mutation, secondary to the presence of *BCR-ABL1* transcript in a patient with chronic myeloid leukemia.

In Table 1, is shown a summary of the similar reported cases. There is a wide clinical variety of the co-existence of *BCR-ABL1* and *JAK2* mutations. Some patients present the both alterations at diagnosis and some present one first and then the other. Only four cases reported the initial diagnosis of chronic myeloid leukemia *BCR-ABL1* positive, have been initially treated with Imatinib, and after years of treatment have relapsed and subsequently shown the secondary *JAK2* mutation. This is the fifth case with these features.

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