

## Detection of JAK2 V617F mutation in Philadelphia chromosome-negative myeloproliferative neoplasms

### Detección de la mutación JAK2 V617F en neoplasias mieloproliferativas cromosoma Philadelphia negativas

Santos Cruz Alemán,<sup>I</sup> Adriana Larios Roque,<sup>I</sup> Uxmal Caldera Suazo,<sup>I</sup> Carlos Santamaria Quesada,<sup>II</sup> Ann-Christin Puller,<sup>I</sup> Allan Pernudy Ubau<sup>I</sup>

<sup>I</sup> Molecular Biology Laboratory 'M.A. Elmer Cisneros in Memoriam', Polytechnic Institute of Health, National Autonomous University of Nicaragua (UNAN). Managua, Nicaragua.

<sup>II</sup> Clinical Laboratory Department, Molecular Biology Laboratory, National Children's Hospital. San José, Costa Rica.

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#### ABSTRACT

**Introduction:** Myeloproliferative neoplasms (MPNs) comprise several hematological neoplasms such as polycythemia vera (PV), essential thrombocythemia (ET), and idiopathic myelofibrosis (IMF). Molecular lesions have been investigated as the underlying mechanism for the genesis of MPNs with the specific mutation in the Janus kinase 2 (JAK2) gene, JAK2 V617F, being most frequent. The variant JAK2 V617F protein is characterized by a substitution of valine for phenylalanine at position 617 (V617F) resulting in constitutively unregulated enzymatic activity.

**Objectives:** Currently, the diagnoses of MPNs in Nicaragua are based solely on clinical presentation of the patient and routine blood analyses. We aim for the implementation of the molecular diagnosis of JAK2 V617F in order to allow for a more specific classification and to provide targeted therapy for positive patients.

**Methods:** To detect the JAK2 V617F mutation in MPNs patients, a cross-sectional study was performed with 29 patients with clinical diagnosis of PV, ET, or IMF. DNA was extracted from whole peripheral blood using a commercial kit. A polymerase chain reaction (PCR) technique was carried out to detect the mutation at the genomic level using allele-specific primers.

**Results:** This study represents the first report of JAK2 V617F analysis in Nicaragua. The mutation was detected in 14 out of 29 MPNS patients. Following the recommendations of the World Health Organization (2016), we suggest the molecular testing for the JAK2 V617F mutation as part of the initial diagnostic portfolio for patients suffering myeloproliferative neoplasms in Nicaragua and in the rest of Latin American countries.

**Keywords:** JAK2 V617F; endpoint PCR; myeloproliferative neoplasms.

## **RESUMEN**

**Introducción:** las neoplasias mieloproliferativas (NMP) comprenden varias neoplasias hematológicas como la policitemia vera (PV), la trombocitemia esencial (TE) y la mielofibrosis idiopática (MI). Lesiones moleculares han sido investigadas como el mecanismo subyacente para la génesis de las NMP con la mutación específica en el gen Janus kinasa 2 (JAK2), siendo la mutación JAK2 V617F la más frecuente. La variante de la proteína JAK2 V617F se caracteriza por una sustitución de valina por fenilalanina en la posición 617 (V617F) dando como resultado una actividad enzimática constitutivamente no regulada. En la actualidad, los diagnósticos de NMP en Nicaragua se basan únicamente en la presentación clínica del paciente y análisis de sangre de rutina. El objetivo de este trabajo es la implementación del diagnóstico molecular de JAK2 V617F con el fin de permitir una clasificación más específica y proporcionar terapia dirigida a pacientes positivos.

**Métodos:** para detectar la mutación JAK2 V617F en pacientes con NMP, se realizó un estudio transversal con 29 pacientes con diagnóstico clínico de PV, TE o MI. El ADN se extrajo de sangre periférica usando un Kit comercial. Se llevó a cabo una técnica de reacción en cadena de la polimerasa (PCR) para detectar la mutación a nivel genómico usando cebadores alelos específicos.

**Resultados:** este estudio representa el primer informe del análisis JAK2 V617F en Nicaragua. La mutación se detectó en 14 de los 29 pacientes con NMP. Siguiendo las recomendaciones de la Organización Mundial de la Salud (2016), se sugiere la prueba molecular de la mutación JAK2 V617F como parte de la cartera diagnóstica inicial para pacientes con neoplasias mieloproliferativas en Nicaragua y en el resto de países latinoamericanos.

**Palabras clave:** JAK2 V617F; PCR punto final; neoplasias mieloproliferativas.

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## **INTRODUCTION**

Philadelphia chromosome-negative myeloproliferative neoplasms (MPNs) include several clonal hematological neoplasms, such as polycythemia vera (PV), essential thrombocythemia (ET) and idiopathic myelofibrosis (IMF), which are thought to arise from the transformation of a hematopoietic stem cell. The main clinical features of these diseases are overproduction of functional mature blood cells and a protracted clinical course.<sup>1</sup> The use of molecular techniques has allowed to uncover the pathogenesis of these neoplasms. Mutations in Janus kinase 2 (JAK2) have been identified that result in the constitutive activation of its kinase activity, thereby elevating the proliferative capacity and survival of the mutated cells.<sup>2</sup> In 2005, Jones and colleagues conducted a study with 480 patients diagnosed with MPNs, finding that 81 % of patients diagnosed with PV and 41 % of ET patients carried the JAK2 V617F mutation.<sup>3</sup> More recent reports confirm JAK2 V617F presence in 95 % of PV patients and 50-60 % of ET and IMF patients.<sup>4</sup> Thus, the detection of this JAK2 mutation is a pivotal diagnostic feature recognized in the World Health Organization (WHO) classification for MPNs since 2008, alongside the measurement of serum erythropoietin.<sup>6</sup> Furthermore, constitutively activated JAK2 kinase can be targeted by therapeutic inhibition, emphasizing the relevance of analyzing the presence of JAK2 mutations.<sup>7</sup>

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In Nicaragua, the detection of the JAK2 V617F mutation is not yet being performed. Our aim is the implementation of the PCR-based JAK2 V617F analysis for the first time in Nicaragua. The identification of JAK2 V617F-positive patients will have a strong impact on the diagnosis, classification, and treatment of MPNs.

## **METHODS**

The study was conducted in compliance with the ethical regulations and principles governing research.

A cross-sectional study was performed with 29 patients with clinical diagnosis of MPNs. Samples were obtained from the hematology services of the national referral hospital *Teaching Hospital "Roberto Calderón"* and the therapeutic phlebotomy service (*Blood Bank*) of the *Nicaraguan Red Cross* in order to detect JAK2 V617F.

A PCR assay for JAK2 V617F detection was implemented for the first time by Baxter and colleagues in 2005 in the United Kingdom.<sup>5</sup> The implementation of this technique was carried out in the *Molecular Biology Laboratory* of the *Polytechnic Institute of Health, POLISAL, UNAN-Managua*. DNA was extracted from 200 mL of whole peripheral blood in EDTA as anticoagulant using a commercial kit (QIAamp DNA Blood Mini Kit) following the manufacturer's recommendations. PCR experiments were conducted as previously described.<sup>5</sup> This PCR uses two forward primers, called JAK2\_F and JAK2\_F\_I/C and one common reverse primer (JAK2\_R). The JAK2\_F primer is specific for the mutant allele while the JAK2\_F\_I/C primer amplifies both the normal and the mutant allele, thus serving as an internal control of the PCR reaction (table).

**Table . Primers used for detection of JAK2 V617F**

Primer	Sequence
Reverse	5' CTGAATAGTCCTACAGTGTTTTTCAGTTTCA 3'
JAK2_F (allele specific)	5'AGCATTTGGTTTTAAATTATGGAGTATATT 3'
JAK2_F_I/C (internal control)	5' ATCTATAGTCATGCTGAAAGTAGGAGAAAG 3'

## **RESULTS**

Twenty-nine cases of MPNs were analyzed by allele-specific PCR including 18 patients with PV, ten with ET, and one patient with IMF. Of the 29 investigated cases, 14 were carriers of the JAK2 V617F mutation. Eleven presumptive PV patients were positive (61 %), as well as three diagnosed as ET patients (30 %). The only investigated IMF case was PCR-negative.

Figure depicts the electrophoresis of JAK2V617F-specific PCR amplicons for four PV patients of which two were positive for the mutation. We therefore demonstrate the successful establishment of the PCR-based JAK2 V617F detection in Nicaragua.

## **DISCUSSION**

The JAK2 V617F mutation was detected in approximately half of the analyzed 29 patients (14/29) with MPNs. Interestingly, only 61 % of the presumptive PV cases were PCR-positive although all presented clinical traits and even laboratory data suggesting the presence of PV. In the remaining cases, the presence of a related syndrome cannot be excluded. Especially in potential cases of PV which are PCR-negative for JAK2 V617F further studies of the *JAK2* gene should be indicated. For example, mutations affecting exon 12 of the *JAK2* gene were described, which range from point mutations to small insertions or deletions.<sup>8</sup> Most of these alterations are associated with PV, however, they only account for 4 % of the cases. It is also valid to indicate the possibility of a secondary polycythemia which shares clinical symptoms with primary polycythemia but shows increased erythropoietin levels and is negative for *JAK2* mutations.<sup>8,9</sup> The analysis of potential mutations in *JAK2* exon 12 and of erythropoietin levels will be addressed in future investigations. The importance of discriminating primary and secondary polycythemia is that patients with the secondary form do not need cancer treatment such as hydroxyurea which entails a number of side effects and might have a negative impact on the patients' quality of life. Detection of JAK2 V617F in patients with PV is relevant as targeted therapies directly inhibiting JAK2 kinase activity, such as ruxolitinib, dasatinib or nilotinib, result in minimal myelosuppression and reduced secondary toxicity.<sup>10</sup>

This study of the detection of the JAK2 V617F mutation in patients with clinical diagnosis of MPNs represents the first report of this kind in Nicaragua. In accordance with the WHO recommendations, the PCR-based analysis of JAK2 V617F can now be incorporated as a primary diagnostic test for patients with presumptive MPNs in this country.

Incorporating molecular techniques that facilitate medical diagnosis in the field of hematology is of great scientific value for the *Polytechnic Institute of Health* at the UNAN-Managua and will immediately influence the quality of the patients' life.

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## **CONFLICT OF INTEREST**

The authors declare no conflict of interest.

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*Dr. Allan Pernudy Ubau*. Molecular Biology Laboratory 'M.A. Elmer Cisneros in Memoriam', Polytechnic Institute of Health, National Autonomous University of Nicaragua (UNAN), Recinto Universitario 'Ruben Dario', de la Rotonda Universitaria 1 km al Sur, Managua, Nicaragua; Tel: +505-2277-0267, ext.: 5197.  
Correo electrónico: [pernudi@gmail.com](mailto:pernudi@gmail.com).