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JAK2, CALR, and MPL Mutation Profiles in Colombian patients with BCR-ABL Negative Myeloproliferative Neoplasms

Perfiles de Mutación JAK2, CALR y MPL en Pacientes Colombianos con Neoplasias Mieloproliferativas BCR-ABL Negativas

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Abstract

Background:

Among the chronic myeloproliferative neoplasms (MPNs) not associated with BCR-ABL mutations are polycythemia vera, primary myelofibrosis, and essential thrombocythemia. These diseases are caused by mutations in genes, such as the JAK2, MPL, and CALR genes, which participate in regulating the JAK-STAT signaling pathway.

Objective:

This study aimed to establish the frequencies of mutations in the JAK2, MPL, and CALR genes in a group of Colombian patients with a negative clinical diagnosis of BCR-ABL chronic myeloproliferative neoplasms.

Methods:

The JAK2 V617F and MPL W515K mutations and deletions or insertions in exon 9 of the CALR gene were analyzed in 52 Colombian patients with polycythemia vera, primary myelofibrosis, and essential thrombocythemia.

Results:

The JAK2V617F mutation was carried by 51.9% of the patients, the CALR mutation by 23%, and the MPL mutation by 3.8%; 23% were triple-negative for the mutations analyzed. In these neoplasms, 6 mutation types in CALR were identified, one of which has not been previously reported. Additionally, one patient presented a double mutation in both the CALR and JAK2 genes. Regarding the hematological results for the mutations, significant differences were found in the hemoglobin level, hematocrit level, and platelet count among the three neoplasms.

Conflict of interest:

The authors declare no conflicts of interest

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Conclusions:

Thus, this study demonstrates the importance of the molecular characterization of the JAK2, CALR and MPL mutations in Colombian patients (the genetic context of which remains unclear in the abovementioned neoplasms) to achieve an accurate diagnosis, a good prognosis, adequate management, and patient survival.

Resumen

Antecedentes:

Entre las neoplasias mieloproliferativas crónicas no asociadas con mutaciones BCR-ABL se encuentran la policitemia vera, la mielofibrosis primaria y la trombocitemia esencial. Estas enfermedades están causadas por mutaciones en genes, como los genes JAK2, MPL y CALR, que participan en la regulación de la vía de señalización JAK-STAT.

Objetivo:

Establecer las frecuencias de mutaciones en los genes JAK2, MPL y CALR en un grupo de pacientes colombianos con diagnóstico clínico negativo de NMP BCR-ABL.

Métodos:

Se analizaron las mutaciones y deleciones o inserciones JAK2 V617F y MPL W515K en el exón 9 del gen CALR en 52 pacientes colombianos con policitemia vera, mielofibrosis primaria y trombocitemia esencial.

Resultados:

La mutación JAK2V617F la portaban el 51.9% de los pacientes, la mutación CALR el 23.0% y la mutación MPL el 3.8%; El 23.0% fueron triple negativos para las mutaciones analizadas. En estas neoplasias se identificaron seis tipos de mutación en CALR, uno de los cuales no ha sido reportado previamente. Además, un paciente presentó una doble mutación tanto en el gen CALR como en el JAK2. En cuanto a los resultados hematológicos para las mutaciones, se encontraron diferencias significativas en el nivel de hemoglobina, el nivel de hematocrito y el recuento de plaquetas entre las tres neoplasias.

Conclusiones:

Así, este estudio demuestra la importancia de la caracterización molecular de las mutaciones JAK2, CALR y MPL en pacientes colombianos (cuyo contexto genético aún no está claro en las neoplasias antes mencionadas) para lograr un diagnóstico certero, un buen pronóstico, un manejo adecuado y una mejoría del paciente. supervivencia.

Remark

1) ¿Why was this study conducted?

This study was conducted due to the lack of molecular information related to the driver mutations in the genes JAK2, MPL and CALR in Colombian patients. These mutations are known to be associated with the diagnosis and prognosis of pathologies such as polycythemia vera, primary myelofibrosis, and essential thrombocythemia.

2) ¿What were the most relevant results of the study?

The most relevant results of this study can be summarized as follows: Despite the relatively small size of the population cohort, we were able to find a patient with mutations in both JAK2 and CALR genes. Furthermore, our investigation shows a mutation in the CALR gene that has not been reported before. Notably, our study stands as a pioneering effort to describe the mutational frequency of these three gene mutations in Colombian patients with myeloproliferative neoplasm.

3) ¿What do these results contribute?

To date, this study is the first to describe the mutational profile of these three genes in BCR-ABL1-negative chronic myeloproliferative neoplasms in Colombia which allow clinician an researchers to understand the importance of molecular profiling in the diagnosis and prognosis of patients with these pathologies.

Introduction

Chronic myeloproliferative neoplasms (MPNs) not associated with negative BCR-ABL or Philadelphia mutations are a cluster of diseases whose main characteristic is the clonal proliferation of myeloid cells with variable morphological maturity and hematopoietic efficiency. The most common neoplasms in this group are polycythemia vera, primary myelofibrosis, and essential thrombocythemia [1]. According to Shallis et al. [2], the incidence of negative BCR-ABL chronic myeloproliferative neoplasms is 0.44-5.87 cases per 100,000 inhabitants, with rates of 0.84, 1.03, and 0.47 cases per 100,000 inhabitants for polycythemia vera, essential thrombocythemia, and primary myelofibrosis, respectively [2].

Studies on BCR-ABL-negative neoplasms in Colombia are scarce. The only study to have investigated the clinical characteristics of these neoplasms was presented in the first report of the Colombian myeloproliferative neoplasms registry performed by the Colombian Association of Hematology and Oncology (ACHO), comprising 79 patients from different hospitals in the country from 2013 to 2017. In this group, essential thrombocythemia was the most frequent disease of the three pathologies with 93 patients (51.9%), followed by polycythemia vera with 55 patients (30.7%) and primary myelofibrosis with 31 patients (17.3%) [3]. However, no molecular studies of these neoplasms in the Colombian population have been published to date.

Mutations in genes regulating the JAK-STAT signaling pathway, like JAK2, MPL, and CALR genes, cause negative BCR-ABL chronic myeloproliferative neoplasms. For example, the tyrosine kinase JAK2 V617F missense mutation leads to an exchange of valine for phenylalanine at position 617, producing constitutive phosphorylation of JAK2 and Ba/F3 or Ba/F3-EpoR, allowing several cell lines to survive and proliferate independently of cytokines [4]. Likewise, mutations in the MPL gene, such as the W > L or W > K change in codon 515, cause spontaneous activation of the JAK-STAT pathway [5]. Moreover, somatic mutations have been informed by the CALR gene involving changes in the reading frame caused by deletions or insertions in exon 9 [6].

The main clinical characteristic of polycythemia vera is increased red blood cell production independent of the mechanisms that normally regulate erythropoiesis [7]. Ninety-five percent of patients with this pathology have the V617F mutation or another mutation in the JAK2 gene, resulting in the rapid increase of the erythroid lineage and other myeloid lines (panmyelosis) [1]. However, essential thrombocythemia primarily compromises the megakaryocytic lineage and is characterized by sustained thrombocytosis (platelet count >450 x 10^9/L) in the peripheral blood and a rising number of large mature megakaryocytes, which form clusters [8]. Patients with essential thrombocythemia have a JAK2 mutation in 50-60% of cases, CALR mutations in 30%, and MPL mutations in 3%; approximately 12% are triple-negative. None of these mutations is specific to TE, but their presence excludes reactive thrombocytosis (the main differential diagnosis of TE) [4]. Finally, primary myelofibrosis is characterized by the rapid increase of granulocytes and megakaryocytes in the bone marrow related to the deposition of fibrous connective tissue and extramedullary hematopoiesis in the late stages of the disease [9]. The JAK2 V617F mutation is observed in 50-60% of cases regardless of the disease stage, the CALR mutation in 24% of cases, and MPL mutations in 8%; approximately 12% of cases are triple negative [5].

Mutations in the JAK2, MPL, and CALR genes are fundamental for diagnosing, prognoses, and monitoring patients with chronic myeloproliferative neoplasms and h were proposed and reviewed by the WHO in 2008-2016 [10]. No publication has identified the frequency and type of these mutations in these three genes in patients with BCR-ABL-negative chronic myeloproliferative neoplasms in Colombia. This study aimed to establish the frequencies of mutations in the JAK2, MPL, and CALR genes in Colombian patients with a negative clinical diagnosis of BCR-ABL chronic myeloproliferative neoplasms. The genetic analysis enables the determination of a more precise molecular diagnosis in these Colombian patients to establish the most appropriate prognosis and treatment that impact their survival.

Materials and Methods

A descriptive, cross-sectional study was performed. The study population comprised a convenience sample of patients with a clinical and histopathological diagnosis of negative chronic myeloproliferative neoplasms BCR-ABL according to the criteria of the World Health Organization (WHO) [10] and older than 16 years old. Exclusion criteria were: diagnosis <15 year old, there were no molecular studies from whole blood samples and patients were not willing to participate in the study. Samples were obtained from patients at diagnosis and patients who relapsed in any of the three diseases considered in this study Clinical and laboratory parameters were obtained from the time of diagnosis or relapse by reviewing their clinical records. Samples were referred to the Medical Genetics Unit of the Universidad de Antioquia from three hospitals in Medellín-Colombia (Hospital San Vicente Fundación, Clínica León XIII, IPS Universitaria, and Hospital Pablo Tobón Uribe) between 2017 and 2021. After the patients provided signed informed consent, their peripheral blood samples were used for molecular biology analysis of the JAK2, MPL, and CALR genes. The study protocol and informed consent were approved by the Bioethics Committee for experimentation in humans of the Faculty of Medicine of Universidad de Antioquia.

Molecular analysis of the JAK2, MPL, and CALR genes

DNA extraction was performed using whole blood samples and the commercial QIAamp DNA Mini and Blood Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's recommendations.

The p.V617F mutation located in exon 14 of the JAK2 gene was detected using real-time PCR (qPCR) and the commercial JAK2 Mutation Detection Kit from Amoydx (Haicang District, Xiamen- China). The MPL W515L and MPL W515K mutations were detected using the commercial MPL W515L / K MutaScreen Kit from ipsogen, following the manufacturer's instructions. Samples with mutations were confirmed by direct, bidirectional Sanger sequencing using an ABI 3500 Applied Biosystems genetic analyzer and the primers MPL-F: TGGGCCGAAGTCTGACCCTTT and MPL-R: ACAGAGCGAACCAAGAATGCCTGT, as previously reported [11].

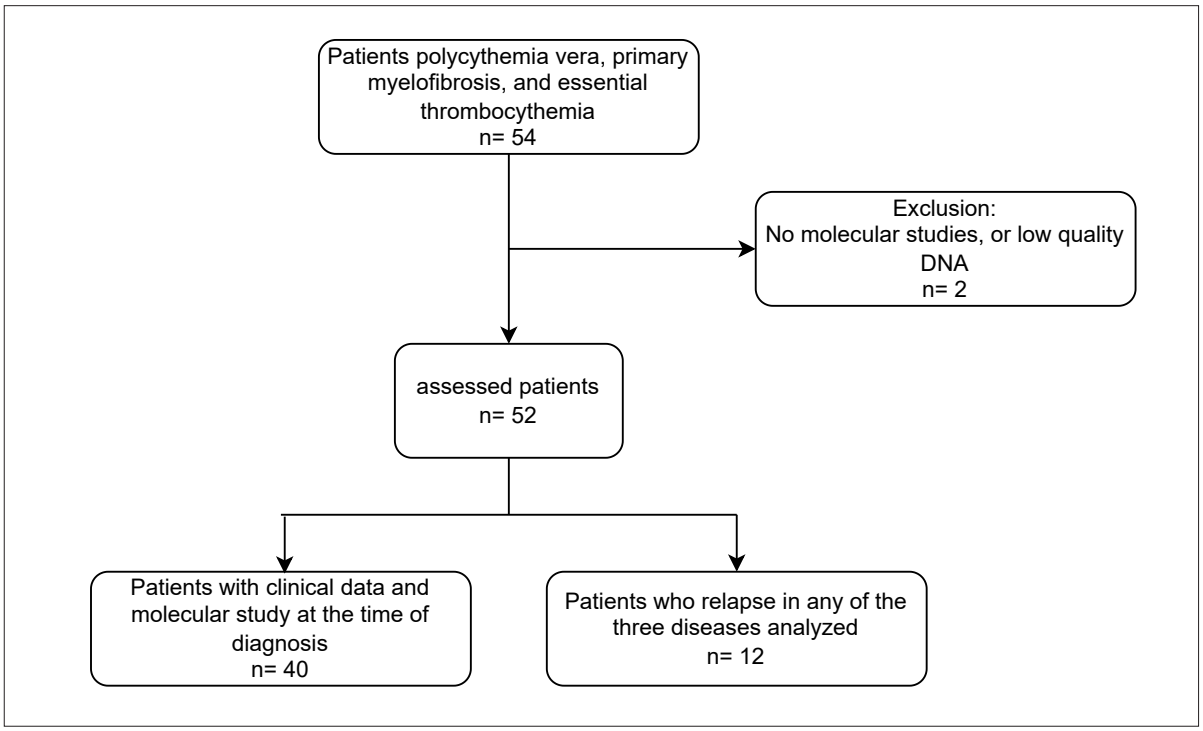

Figure 1. Flow diagram of selected patients.

For the CALR gene, the insertions/deletions (indels) in exon 9 were evaluated using by analyzing the size of the amplified fragments. The following primers were used for amplification: CALR-F: 5'GAGGTGTGTGCTCTGCCTG3' and CALR-R: 5'AGAGACATTATTTGGCGCGG3'. The forward allele was labeled with 6-fluorescein (6-FAM), and the normal allele had a size of 298 bp, according to Jones et al [12]. Fragment size analysis was performed using bidirectional Sanger sequencing and an ABI 3500 Applied Biosystems genetic analyzer using the Gene Mapper program. The samples positive for indels were sequenced by the Sanger method to confirm the results obtained by fragment analysis. Sequencing was performed using the following primers: CALR.SF: 5'ACAACTTCCTCATCACCAACG3' and CALR.SR: 5'GGCCTCAGTCCAGCCCTG3' following the conditions of Klampfl *et al*[13]. In their approach, amplification, subsequent purification, and sequencing of the products of PCR are required. The chromatograms obtained were analyzed using the Chromas Pro program, and the sequences were aligned with the reference sequence reported in GenBank using the Clustal Omega program.

Statistical analysis

The results are shown as absolute frequencies and percentages for categorical variables (sex, JAK2 mutation, CALR mutation, MPL mutation, and triple-negative mutation) and medians and interquartile ranges (25th percentile and 75th percentile) for quantitative variables (hemoglobin level, hematocrit level, platelet count, leukocyte count, lymphocyte count, neutrophil count). Comparisons among the JAK2, CALR, and triple-negative mutation groups and different types of neoplasms concerning hematological results were made using the Kruskal-Wallis test. Paired group comparisons were made using the Mann-Whitney U test. For all the analyses, the R environment (R Core team 2021) version 4.0.5 (2021-03-31) and Rstudio Version 1.4.1106 were used [14].

Table1. Demographic characteristics and hematological results of patients with BCR-ABL1-negative chronic myeloproliferative neoplasms according to diagnosis, sex, and age

	Polycythemia vera n= 13	Essential thrombocythemia n= 20	primary myelofibrosis n= 16	nonclassifiable BCR-ABL1-negative chronic myeloproliferative neoplasms n= 3
N	13	20	16	3
Age (mean (SD))	52.92 (15.98)	55.45 (16.12)	68.25 (9.90)	53.00 (21.38)
Female (%)	5 (38.5)	13 (65.0)	6 (37.5)	1 (33.3)
Male (%)	8 (30.0)	7 (26.0)	10 (37.0)	2 (7.0)
Hemoglobin level (g/dL), (median [QR])	17.80 [16.00, 20.00]	12.90 [11.23, 13.70]	11.50 [9.55, 13.45]	14.50 [14.25, 14.75]
Hematocrit level (%) (median [QR])	54.00 [50.00, 60.00]	38.20 [33.45, 43.20]	36.15 [28.50, 40.62]	45.00 [45.00, 45.50]
Platelet count (number of platelets/µL) (median [QR])	376000.00 [288250.00, 534750.00]	792500.00 [503750.00, 1278000.00]	159000.00 [71250.00, 255000.00]	236000.00 [158500.00, 286000.00]
Leukocyte count (number of leukocytes/µL) (median [QR])	8900.00 [7700.00, 10760.00]	9300.00 [6730.00, 12435.00]	7820.00 [4210.00, 10700.00]	6327.00 [5848.50, 51963.50]
Lymphocyte count (number of lymphocytes /µL) (median [QR])	2090.00 [1733.75, 2700.00]	2231.00 [1504.70, 3050.00]	1120.00 [876.50, 1852.00]	2505.00 [2397.50, 2612.50]
Neutrophil count (number of neutrophils /µL) (median [QR])	6130.00 [3846.25, 7360.00]	5250.00 [3580.00, 7815.00]	6710.00 [3752.50, 8612.00]	3830.00 [3344.50, 11876.50]

Results

Fifty-four people with this pathology were included in the study, of whom two were excluded because there were no molecular studies because low quality of samples (Figure 1). Twenty-seven patients (51.9%) were men and 25 were women (48.1%). The average age was 58 years (SD: 15.8), with a minimum of 16 and a maximum of 87 years, where primary myelofibrosis presented at a later age of diagnosis (age value = 68.25 years) (Table 1). The most frequent diagnosis was essential thrombocythemia with 20 patients (38.46%), followed by primary myelofibrosis with 16 patients (30.76%) and polycythemia vera with 13 patients (25%). However, 3 (5.76%) patients could not be classified into one of the diagnoses and were classified as having non classifiable BCR-ABL1-negative chronic myeloproliferative neoplasms. Significant differences were found in the hemoglobin level, hematocrit level, hematocrit, and platelet count among the three neoplasms (p= <0.001). Patients diagnosed with polycythemia vera had the highest hemoglobin and hematocrit levels, essential thrombocythemia patients had the highest platelet count, and primary myelofibrosis patients had the lowest hemoglobin levels (Table 1).

Of the 52 patients analyzed, 51.9% had the JAK2 V617F mutation, 85% had polycythemia vera, 35% had essential thrombocythemia, 43.7% had primary myelofibrosis and 67% had non-classifiable *BCR-ABL1*-negative chronic myeloproliferative neoplasms. In addition, mutations in CALR exon 9 were found in 35% of essential thrombocythemia patients and 31% of primary myelofibrosis patients. Finally, the mutation frequency of the MPL was 12.5% of primary myelofibrosis. Furthermore, in the group of patients diagnosed with essential thrombocythemia, a double-positive case was found for JAK2 V617F and CALR, contributing doubly to the frequencies of these genes (Table 2).

Regarding the mutation in exon 10 of MPL, 2 two patients had the W515K mutation. Of the 12 patients with mutations in CALR exon 9, seven presented a 52 bp deletion (type I mutation- c.1092_1143del; L367fs*46), two presented a 5 five bp insertion (type II mutation - c1154_1155ins- TTGTC; K385fs*47) and two had type I "alike" mutations (del 34 bp and 46 bp). Notably, one patient had a triple mutation in the CALR gene: a deletion of 10p (NM _004343.4):c.1130_1139delAAGAGGAGGA); this location also had an insertion of 9 bp (NM_004343.4):c.1130_1131insGCCTCTGTC) and another 9 bp deletion ((NM_004343.4) c.1177_1185delGAGGAUGAG)-(CALR:p.E398_D400del)).

Table 2. Frequencies of mutations in the three genes analyzed in patients with BCR-ABL1-negative chronic myeloproliferative neoplasms

Total patients n=52 (%)	**JAK2**	**CALR**	**MPL**	**Triple Negative**
Total frequency n= 52 (%) positive n (%)	27 (51.9)	12 (23.0)	2 (3.8)	12 (23.0)
Frequency by disease				
Polycythemia vera n=13 (%)	11 (21.1)	0	0	2 (3.8)
Essential thrombocythemia n= 20 (%)	7 (13.5)	7 (13.5)	0	7 (13.5)
Primary myelofibrosis n= 16 (%)	7 (13.5)	5 (9.6)	2 (3.8)	2 (3.8)
nonclassifiable BCR-ABL1-negative chronic myeloproliferative neoplasms n= 3 (%)	2 (3.8)	0	0	1 (1.9)

Note: There is a patient who is double positive for CARL and JAK2 and therefore the number of mutations is 53.

Figure 2 describes the main clinical and hematological characteristics of patients main clinical and hematological characteristics with BCR-ABL1-negative chronic myeloproliferative neoplasms according to JAK2, CALR and triple-negative mutations in these genes. Patients with a mutation in MPL were excluded from the statistical analysis because of the small sample size (two patients). Regarding the hematological results, a significant difference was found between triple-negative patients and those with mutations in CALR in the counts of leukocytes (p= 0.019) and lymphocytes (p= 0.051). Patients positive for CALR mutations had the lowest counts for these two hematologic variables (Figure 2A and B.). Although patients with the JAK2 mutation had a lower platelet count than those with the CALR and triple negative mutations (Figure 2C), they had higher hemoglobin and hematocrit values (Figure 2D and E).

Discussion

The present study evaluated the frequencies of mutations in the JAK2, MPL and CALR genes in 52 Colombian patients. To date, this study is the first to describe the mutational profile of these three genes in *BCR-ABL1*-negative chronic myeloproliferative neoplasms in Colombia. Although the sample size was small, frequencies similar to those reported in other studies of Latin American populations, such as those in Argentina and Mexico were obtained (Table 3)[15],[16]. These similarities were likely because the populations share a common genetic background with a triethnic origin: European, Amerindian and African.

The genetic characterization of Colombian patients with *BCR-ABL1*-negative chronic myeloproliferative neoplasms whose genetic context is different from other populations is essential for an accurate diagnosis, good prognosis and adequate management and survival of the patient. The JAK2 V617F mutation is associated with an increased risk of arterial thrombosis and a decreased risk of post-arterial thrombocythemia myelofibrosis. In polycythemia vera, a higher frequency of the JAK2V617F mutant allele has been associated with pruritus and fibrotic transformation. In general, the JAK2V617F mutation is associated with older age, higher hemoglobin level, leukocytosis, and lower platelet level. In addition, JAK2 exon 12 mutation-positive patients generally have predominantly erythroid myelopoiesis, subnormal serum erythropoietin, and younger age at diagnosis, but their prognosis is similar to exon 14 JAK2V617F [5]. In essential thrombocythemia, the CALR mutation is associated with lower hemoglobin level, higher platelet count, lower white blood cell count, and a younger age compared to the JAK2V617F mutation. Similar features have been observed in patients with primary myelofibrosis. The CALR mutation is also associated with the male gender, slower risk of thrombosis, and better overall survival [8].

The JAK2 V617F mutation was the most common among the three neoplasms evaluated in our cohort, with frequencies similar to those reported in other populations [15,16,19]. However, few differences were observed in mutational frequencies given that the most common mutation associated with this pathology was the JAK2 V617F mutation studied herein [13]. This mutation activates three main receptors: the erythropoietin receptor, granulocyte colony-stimulating factor receptor and MPL. For this reason, JAK2V617F is associated with polycythemia vera, essential thrombocythemia and primary myelofibrosis [27]. However, the MPL W515K mutation was reported only in two patients with primary myelofibrosis. This mutation occurs at a low frequency, primarily in patients with ET or primary myelofibrosis, implying that it may favor determining the megakaryocytic lineage instead of the erythroid line [28].

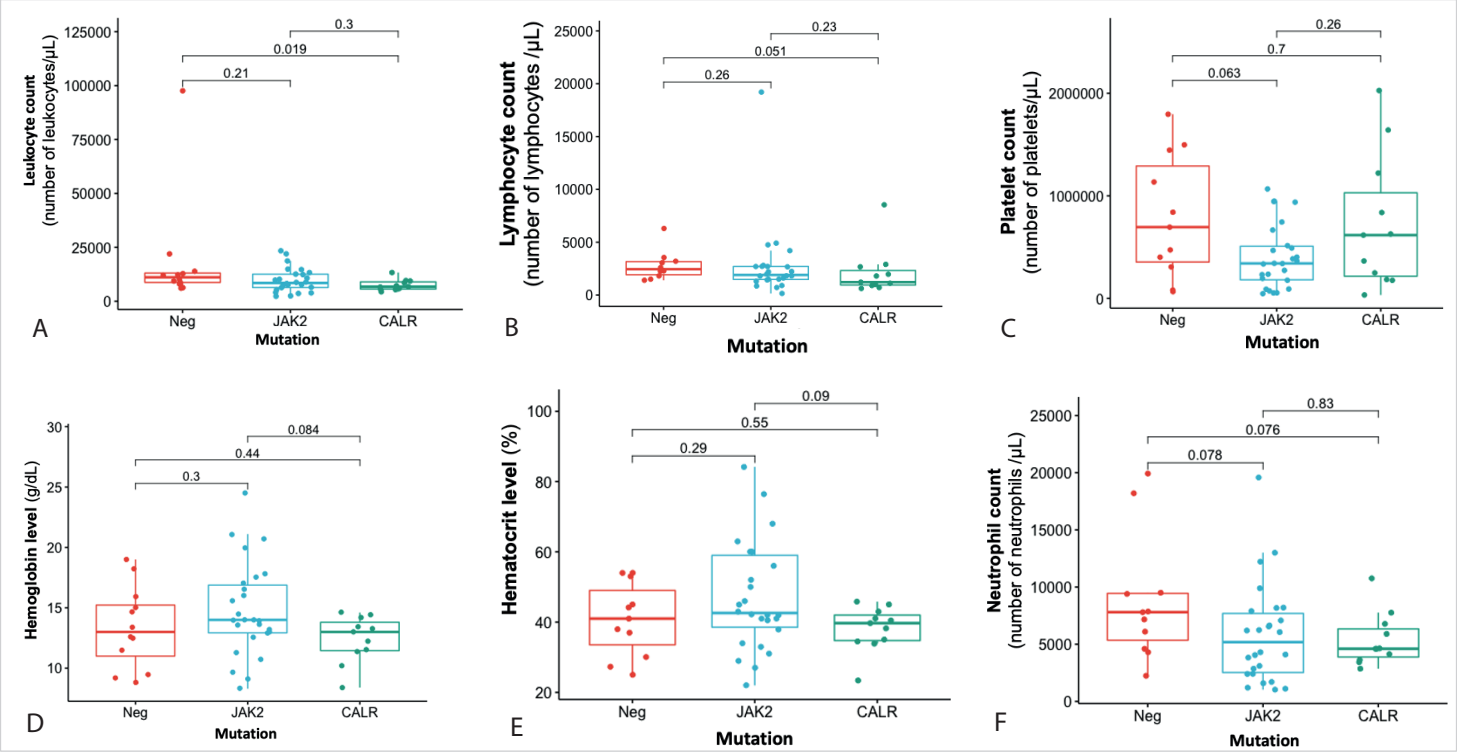

Figure 2. Relationship between the mutational profile of JAK2, CALR, and triple negative samples and the hematological characteristics of the patients. A. Leukocyte count, B. Lymphocyte count, C. Platelet count, D. Hemoglobin level, E. Hematocrit Level and F. Neutrophil count.

In this study, the frequency of mutations in CALR exon 9 was 35% for both essential thrombocythemia and primary myelofibrosis. Similar frequencies were in different studies, as reported in Table 3. Notably, in patients diagnosed with essential thrombocythemia, a double-positive case was found for JAK2 V617F and CALR. Recently, the cooccurrence of CALR and JAK2V617F mutations was reported in some cases of chronic *BCR-ABL1*-negative chronic myeloproliferative neoplasms [29-35], as well as JAK2 V617F and MPL W515L/S mutations [36]. Cooccurrence among these genes occurred in 5-15% of MPL cases. These findings contrast the hypothesis that mutations in JAK2, CALR and MPL are mutually exclusive(10). Furthermore, in this study, six types of mutations were found in exon 9 of CALR, primarily deletions; the most prevalent was the type I mutation, followed by type II [33-35].

Notably, a patient presented three mutations in the CALR gene: a deletion of 10 bp (NM _004343.4):c.1130_1139delAAGAGGAGGA); this location also had an insertion of 9 bp (NM_004343.4):c.1130_1131insGCCTCTGTC) and another downstream nine bp deletion ((NM_004343.4) c.1177_1185delGAGGAUGAG)- (CALR:p.E398_D400del)). The ten bp deletion was reported in refractory anemia as a pathogenic variant (COSV57120310), changing the reading frame to that of the more frequent CALR mutations [37]. The insertion of 9 bp at this point has not been reported to date and represents a striking finding in this patient. Finally, the third mutation is a nine bp deletion reported in a study of myeloproliferative neoplasms. This variant is annotated as a 9 bp polymorphism (rs550353351; NM_004343.4: c.1177_1185del- GAGGATGAG) that eliminates three amino acids (NP_004334.1: p. Glu398_Asp400del) that shifts the +1 bp reading frame, resulting in replacement of the normal C-terminal end by a new amino acid sequence [36]. The severity of these mutations could explain the patient's lack of a treatment response and subsequent death.

We could not compare the clinical and hematological characteristics stratified by disease and mutation, given the small sample size. However, an analysis of the clinical and hematological characteristics by mutation was performed in the total study population. Patients with JAK2

Table 3. Comparison of the frequencies of mutations in the JAK2, CALR and MPL genes in different populations

	Country	N	JAK2			CALR			MPL		
			PV (%)	ET (%)	PMF (%)	PV (%)	ET (%)	PMF (%)	PV (%)	ET (%)	PMF (%)
1	OMS [10]		>95	50-60	50-60.0	0.0	30.0	24.0	0	3.0	8.0
2	Argentina [15]	439	94.9	61.2	62.0	0.0	21.5	16.3	0	2.8	6.1
3	Mexico [16]	27	62.0	36.0	25.0	0.0	29.0	25.0	0	7.0	0
4	South Korea [17]	199	91.4	63.3	57.4	0.0	17.7	14.8	0	2.5	9.3
5	USA [18]	3023	100	61.0	67.0	0.0	15.0	3.0	0	13.3	6.0
6	Japan [19]	143	97.0	59.9	60.2	0.0	26.9	21.6	0	4.7	1.1
7	Italy [20]	1282	NA	62.0	62.0	NA	24.0	26.0	NA	4.0	5.0
8	Brazil [21]	73	100	37.0	62.0	0.0	41.0	33.0	NA	NA	NA
9	Thailand [22]	100	94.7	74.5	25.0	NA	35.7	33.3	NA	0	0
10	China [23]	492	61.5	40.4	44.2	0.0	27.9	15.4	0	1.3	0
11	India [24]	130	100	61.7	57.6	0.0	15.1	23.7	0	9.1	3.4
12	Brazil [25]	65	NA	52.0	52.0	NA	13.0	38.0	NA	4.0	0
13	Egypt [26]	200	48.9	44.1	32.5	0.0	19.1	17.5	0	0	0
14	This project (Colombia)	52	85.0	35.0	44.0	0.0	35.0	31.0	0	0	12.5

mutations had lower platelet counts than those with CALR and triple-negative mutations, findings that were similar to those published in different populations, such as those in Brazil and Thailand [22,25]. Patients with JAK2 mutations also presented high hemoglobin and hematocrit levels, are ported in the 2017 WHO guidelines and other studies [10,27,38]. However, the leukocyte and lymphocyte counts were lower in patients with CALR mutations than in triple-negative patients. This result is consistent with several populations in Latin America, Korea, and Italy and WHO reports [10,15,17,33,34]. Notably, the two patients with rare mutations (double positive for JAK2 and CALR and triple mutation in the CALR) had a diagnosis of essential thrombocythemia and presented low hemoglobin and hematocrit values compared with normal reference values in this patient type. The double-positive patient for JAK2 and CALR also had a low white blood cell count. The diagnosis of essential thrombocythemia in patients with rare mutations has also been reported in other studies of different populations [31,35,39].

Regarding the relationship between hematological characteristics and neoplasms, patients with polycythemia vera presented high hemoglobin and hematocrit values, a finding that is expected in this pathology associated with a hematological phenotype of isolated erythrocytosis because of a predominant alteration of the erythroid lineage. Furthermore, variable hyperplasia of the megakaryocytic/granulocytic lineages was observed [9,10,27,40]. Patients with essential thrombocythemia presented a high platelet count independent of their mutational profile, a finding that is consistent with what is reported in WHO guidelines [10,17,22,33,34]. Another characteristic of essential thrombocythemia was a higher frequency of female diagnosed patients (13 women vs. 7 men), a finding that has also been reported in other studies [41-43]. Finally, patients with primary myelofibrosis presented low hemoglobin levels, coinciding with other findings reported in the literature [4,18] and considered a minor diagnostic criterion in the WHO guidelines [10].

This study has a limitation in the sample size, decreasing the power to detect significant associations and increasing the risk of reporting false positives. However, the variants analyzed have been previously reported, substantially reducing the risk and increasing the reliability of the results. Therefore, future studies with a larger sample size are suggested, such as a prospective multicenter study to perform complete genetic analysis and validate our study results and the usefulness of prognosis in Colombian patients with *BCR-ABL1*-negative chronic myeloproliferative neoplasms.

References

1. Nann D, Fend F. Synoptic diagnostics of myeloproliferative neoplasms: Morphology and molecular genetics. Cancers. 2021;13(14):1-22. doi: 10.3390/cancers13143528

2. Shallis RM, Zeidan AM, Wang R, Podoltsev NA. Epidemiology of the Philadelphia Chromosome-Negative Classical Myeloproliferative Neoplasms. Hematology/Oncology Clinics of North America. 2021;35(2):177-89. DOI: 10.1016/j.hoc.2020.11.005

3. Abello V, Quintero G, Espinosa D, Solano MH, Casas CP, Saavedra D, et al. Descripción de las características clínicas de las neoplasias mieloproliferativas crónicas (NMPC) Description of the clinical characteristics of chronic myeloproliferative neoplasms (MPNs) First report of the colombian registry of MPNs. Acta Médica Colombiana. 2017;42(1):35-41.

4. Langabeer SE, Andrikovics H, Asp J, Bellosillo B, Carillo S, Haslam K, et al. Molecular diagnostics of myeloproliferative neoplasms. European J Haematol. 2015;95(4):270-9. DOI: 10.1111/ejh.12578

5. Ferreira Cristina S, Polo B, Lacerda JF. Somatic Mutations in Philadelphia Chromosome-Negative Myeloproliferative Neoplasms. Seminars in Hematology. 2018;55(4):215-22. DOI: 10.1053/j.seminhematol.2018.04.005

6. Araki M, Komatsu N. The role of calreticulin mutations in myeloproliferative neoplasms. International Journal of Hematology. 2020;111(2):200-5. DOI: 10.1007/s12185-019-02800-0

7. Vainchenker W, Dusa A, Constantinescu SN. JAKs in pathology: Role of Janus kinases in hematopoietic malignancies and immunodeficiencies. Seminars in Cell and Developmental Biology. 2008;19(4):385-93. DOI: 10.1016/j.semcdb.2008.07.002

8. Song J, Hussaini M, Zhang H, Shao H, Qin D, Zhang X, et al. Comparison of the Mutational Profiles of Primary Myelofibrosis, Polycythemia Vera, and Essential Thrombocytosis. American Journal of Clinical Pathology. 2017;147(5):444-52. DOI: 10.1093/ajcp/aqw222

9. Tefferi A, Pardanani A. Myeloproliferative neoplasms: A contemporary review. JAMA Oncology. 2015;1(1):97-105. DOI: 10.1001/jamaoncol.2015.89

10. Brown NA, Elenitoba-Johnson KSJ. Update from the 4th Edition of the World Health Organization Classification of Head and Neck Tumours: Hematolymphoid Tumours. Head and Neck Pathology. 2017;11(1):96-109. DOI: 10.1007/s12105-017-0802-5

11. Furtado LV, Weigelin HC, Elenitoba-Johnson KSJ, Betz BL. A Multiplexed fragment analysis-based assay for detection of JAK2 exon 12 mutations. Journal of Molecular Diagnostics. 2013;15(5):592-9. DOI: 10.1016/j.jmoldx.2013.04.006

12. Jones AV, Ward D, Lyon M, Leung W, Callaway A, Chase A, et al. Evaluation of methods to detect CALR mutations in myeloproliferative neoplasms. Leukemia Research. 2015;39(1):82-7. DOI: 10.1016/j.leukres.2014.11.019

13. Klampfl T, Gisslinger H, Harutyunyan AS, Nivarthi H, Rumi E, Milosevic JD, et al. Somatic Mutations of Calreticulin in Myeloproliferative Neoplasms. New England Journal of Medicine. 2013;369(25):2379-90. DOI: 10.1056/NEJMoa1311347

14. R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

15. Ojeda MJ, Bragós IM, Calvo KL, Williams GM, Carbonell MM, Pratti AF. CALR, JAK2 and MPL mutation status in Argentinean patients with BCR-ABL1- negative myeloproliferative neoplasms. Hematology. 2018;23(4):208-11. DOI: 10.1080/10245332.2017.1385891

16. Labastida-Mercado N, Galindo-Becerra S, Garcés-Eisele J, Colunga-Pedraza P, Guzman-Olvera V, Reyes-Nuñez V, et al. The mutation profile of JAK2, MPL and CALR in Mexican patients with Philadelphia chromosome-negative myeloproliferative neoplasms. Hematology/ Oncology and Stem Cell Therapy. 2015;8(1):16-21. DOI: 10.1016/j.hemonc.2014.12.002

17. Kim SY, Im K, Park SN, Kwon J, Kim JA, Lee DS. CALR, JAK2, and MPL mutation profiles in patients with four different subtypes of myeloproliferative neoplasms: Primary myelofibrosis, essential thrombocythemia, polycythemia vera, and myeloproliferative neoplasm, unclassifiable. American Journal of Clinical Pathology. 2015;143(5):635-44. doi: 10.30699/IJP.2021.136458.2495

18. Szuber N, Mudireddy M, Nicolosi M, Penna D, Vallapureddy R, Lasho TL, et al. 3,023 Mayo Clinic Patients with Myeloproliferative Neoplasms: Risk-Stratified Comparison of Survival and Outcomes Data Among Disease Subgroups. Blood. 2018;132(Supplement 1):3035-3035. DOI: 10.1016/j.mayocp.2018.08.022

19. Misawa K, Yasuda H, Araki M, Ochiai T, Morishita S, Shirane S, et al. Mutational subtypes of JAK2 and CALR correlate with different clinical features in Japanese patients with myeloproliferative neoplasms. International Journal of Hematology. 2018;107(6):673-80. DOI: 10.1007/s12185-018-2421-7

20. Pietra D, Rumi E, Ferretti V V., Di Buduo CA, Milanesi C, Cavalloni C, et al. Differential clinical effects of different mutation subtypes in CALR-mutant myeloproliferative neoplasms. Leukemia. 2016;30(2):431-8. DOI: 10.1038/leu.2015.277

21. Machado-Neto JA, de Melo Campos P, de Albuquerque DM, Costa FF, Lorand-Metze I, Olalla Saad ST, et al. Somatic mutations of calreticulin in a Brazilian cohort of patients with myeloproliferative neoplasms. Revista Brasileira de Hematologia e Hemoterapia. 2015;37(3):211-4. DOI: 10.1016/j.bjhh.2015.03.012

22. Singdong R, Siriboonpiputtana T, Chareonsirisuthigul T, Kongruang A, Limsuwanachot N, Sirirat T, et al. Characterization and Prognosis Significance of JAK2 (V617F), MPL, and CALR Mutations in Philadelphia-Negative Myeloproliferative Neoplasms. Asian Pacific Journal of Cancer Prevention. 2016;17(10):4647-53. doi: 10.22034/APJCP.2016.17.10.4647

23. Lang T, Nie Y, Wang Z, Huang Q, An L, Wang Y, et al. Correlation analysis between JAK2, MPL, and CALR mutations in patients with myeloproliferative neoplasms of Chinese Uygur and Han nationality and their clinical characteristics. Journal of International Medical Research. 2018;46(11):4650-9. doi: 10.1177/0300060518787719

24. Rabade N, Subramanian PG, Kodgule R, Raval G, Joshi S, Chaudhary S, et al. Molecular genetics of BCR-ABL1 negative myeloproliferative neoplasms in India. Indian Journal of Pathology and Microbiology. 2018;61(2):209-13. DOI: 10.4103/IJPM.IJPM_223_17

25. Nunes DPT, de Lima LT, Chauffaille M de L, Mitne-Neto M, dos Santos MT, Cliquet MG, et al. CALR mutations screening in wild type JAK2V617F and MPLW515K/L Brazilian myeloproliferative neoplasm patients. Blood Cells, Molecules, and Diseases. 2015;55(3):236-40. DOI: 10.1016/j.bcmd.2015.07.005

26. Soliman EA, El-Ghlban S, El-Aziz SA, Abdelaleem A, Shamaa S, Abdel-Ghaffar H. JAK2, CALR, and MPL Mutations in Egyptian Patients With Classic Philadelphia-negative Myeloproliferative Neoplasms. Clinical Lymphoma, Myeloma and Leukemia. 2020;20(10):e645-51. DOI: 10.1016/j.clml.2020.05.011

27. Vainchenker W, Kralovics R. Genetic basis and molecular pathophysiology of classical myeloproliferative neoplasms. Blood. 2017;129(6):667-79. DOI: 10.1182/blood-2016-10-695940

28. Pardanani AD, Levine RL, Lasho T, Pikman Y, Mesa RA, Wadleigh M, et al. MPL515 mutations in myeloproliferative and other myeloid disorders: A study of 1182 patients. Blood. 2006;108(10):3472-6. DOI: 10.1182/blood-2006-04-018879

29. Lundberg P, Karow A, Nienhold R, Looser R, Hao-Shen H, Nissen I, et al. Clonal evolution and clinical correlates of somatic mutations in myeloproliferative neoplasms. Blood. 2014;123(14):2220-8. DOI: 10.1182/blood-2013-11-537167

30. Mcgaffin G, Harper K, Stirling D, Mclintock L. JAK2 V617F and CALR mutations are not mutually exclusive; findings from retrospective analysis of a small patient cohort. British Journal of Haematology. 2014;167(2):276-8. DOI: 10.1111/bjh.12969

31. Jeong JH, Lee HT, Seo JY, Seo YH, Kim KH, Kim MJ, et al. Screening PCR versus sanger sequencing: Detection of CALR mutations in patients with thrombocytosis. Annals of Laboratory Medicine. 2016;36(4):291-9. DOI: 10.3343/alm.2016.36.4.291

32. Chen CC, Gau JP, Chou HJ, You JY, Huang CE, Chen YY, et al. Frequencies, clinical characteristics, and outcome of somatic CALR mutations in JAK2-unmutated essential thrombocythemia. Annals of Hematology. 2014;93(12):2029-36. DOI: 10.1007/s00277-014-2151-8

33. Tefferi A, Lasho TL, Finke CM, Knudson RA, Ketterling R, Hanson CH, et al. CALR vs JAK2 vs MPL-mutated or triple-negative myelofibrosis: Clinical, cytogenetic and molecular comparisons. Leukemia. 2014;28(7):1472-7. DOI: 10.1038/leu.2014.3

34. Tefferi A, Wassie EA, Guglielmelli P, Gangat N, Belachew AA, Lasho TL, et al. Type 1 versus Type 2 calreticulin mutations in essential thrombocythemia: A collaborative study of 1027 patients. American Journal of Hematology. 2014;89(8):121-4. DOI: 10.1002/ajh.23743

35. Shirane S, Araki M, Morishita S, Edahiro Y, Takei H, Yoo Y, et al. JAK2, CALR, and MPL mutation spectrum in Japanese patients with myeloproliferative neoplasms. Haematologica. 2015; 100(2): e46-8. doi: 10.3324/haematol.2014.115113

36. Murugesan G, Guenther-Johnson J, Mularo F, Cook JR, Daly TM. Validation of a molecular diagnostic assay for CALR exon 9 indels in myeloproliferative neoplasms: Identification of coexisting JAK2 and CALR mutations and a novel 9 bp deletion in CALR. International Journal of Laboratory Hematology. 2016;38(3):284-97. DOI: 10.1111/ijlh.12484

37. Broséus J, Lippert E, Harutyunyan AS, Jeromin S, Zipperer E, Florensa L, et al. Low rate of calreticulin mutations in refractory anaemia with ring sideroblasts and marked thrombocytosis. Leukemia. 2014;28(6):1374-6. DOI: 10.1038/leu.2014.49

38. Cazzola M, Kralovics R. From Janus kinase 2 to calreticulin: The clinically relevant genomic landscape of myeloproliferative neoplasms. Blood. 2014;123(24):3714-9. DOI: 10.1182/blood-2014-03-530865

39. Kang MG, Choi HW, Lee JH, Choi YJ, Choi HJ, Shin JH, et al. Coexistence of JAK2 and CALR mutations and their clinical implications in patients with essential thrombocythemia. Oncotarget. 2016;7(35):57036-49. doi: 10.18632/oncotarget.10958

40. Barbui T, Finazzi G, Carobbio A, Thiele J, Passamonti F, Rumi E, et al. Development and validation of an International Prognostic Score of thrombosis in World Health Organization-essential thrombocythemia (IPSET-thrombosis). Blood. 2012;120(26):5128-33. DOI: 10.1182/blood-2012-07-444067

41. Barbui T, Thiele J, Passamonti F, Rumi E, Boveri E, Ruggeri M, et al. Survival and disease progression in essential thrombocythemia are significantly influenced by accurate morphologic diagnosis: A international study. Journal of Clinical Oncology. 2011;29(23):3179-84. DOI: 10.1200/JCO.2010.34.5298

42. Tefferi A, Vainchenker W. Myeloproliferative neoplasms: Molecular pathophysiology, essential clinical understanding, and treatment strategies. Journal of Clinical Oncology. 2011;29(5):573-82. DOI: 10.1200/JCO.2010.29.8711

43. Thiele J, Kvasnicka HM, Müllauer L, Buxhofer-Ausch V, Gisslinger B, Gisslinger H. Essential thrombocythemia versus early primary myelofibrosis: A multicenter study to validate the WHO classification. Blood. 2011;117(21):5710-8. DOI: 10.1182/blood-2010-07-293761