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Meta-analysis of the frequency of JAK2 in primary myelofibrosis according to the detection method used

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Abstract

Background and objective: in the global scientific literature, the frequency of JAK2 is highly heterogenous in chronic myeloproliferative neoplasms. The objective of this study was to analyze the prevalence of the JAK2 mutation in primary myelofibrosis (PMF) and compare it according to the detection method used, from 2007-2018.

Materials and methods: a systematic review with meta-analysis, using 21 searches in three multidisciplinary databases. The PRISMA guideline phases of identification, screening, selection and inclusion were applied. Reproducibility and evaluation of the methodological quality were ensured. The analyses were based on frequencies and meta-analysis for the prevalence of the mutation with its 95% confidence interval.

Results: twenty-nine studies with 744 patients were included, mainly from Korea, Brazil and China. The most commonly used technique was AS-PCR, and the prevalence of JAK2 with this technique ranged from 33.3 to 71.4%; with real-time PCR ranging from 42.9 to 77.3%, sequencing from 14.3-57.4%, and ARMS from 36.4-83.3%. The prevalence of JAK2 showed no statistically significant differences according to the type of diagnostic test used.

Conclusion: high frequencies of the JAK2V617F mutation are seen in PMF, which shows that this entity should not be diagnosed solely based on clinical and hematological characteristics, but also on the patients' genetic screening. **(Acta Med Colomb 2020; 45. DOI: https://doi.org/10.36104/amc.2020.1462).**

Key words: prevalence; mutation; JAK2; primary myelofibrosis; meta-analysis.

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Introduction

Primary myelofibrosis (PMF) is a Philadelphia-negative chronic myeloproliferative neoplasm (CMPN). The most recent World Health Organization classification has subclassified it into prefibrotic and fibrotic states, given the need to differentiate it from essential thrombocythemia. The estimated annual incidence for this disease is 0.5-1.5 cases per 100,000 persons; its prevalence is increasing due to improved diagnosis and survival (1, 2).

Just like the rest of the CMPNs, PMF is characterized by clonal expansion of hematopoietic stem cells which leads to uncontrolled production of mature cells, mainly megakaryocytes and granuloctytes. A characteristic that differentiates this entity from the rest of those in this group is reactive bone marrow fibrosis, with clinical manifestations such as severe anemia, splenomegaly, thrombosis and bleeding (3). It is important to mention that bone marrow fibrosis may be caused by things other than PMF, including reactive states and hematological entities such as some acute leukemias; in these cases, myelofibrosis is termed "secondary" (4).

A diagnostic resource which has permitted the differentiation of the types of myelofibrosis and has elucidated the pathogenesis of CMPNs, has been the detection of various mutations (4), classified as "drivers" and "other mutations". The latter are related to disease prognosis and progression. The driver mutations are used as diagnostic markers; for PMF, the detection of JAK2, CALR and MPL is recommended, with the first being the most relevant (1, 4).

JAK2 is a Janus kinase protein which participates in the JAK-STAT signaling pathway that regulates various cellular processes such as proliferation, differentiation, and apoptosis (5, 6). The JAK2 alteration identified in PMF is JAK2V617F, in which an amino acid substitution produces

an altered protein product responsible for the pathogenesis of the disease (5, 6).

Ever since JAK2 was identified in PMF, the objective of several studies has been to determine its frequency, with highly heterogeneous results, probably attributable to the type of study population, as well as to variability in the diagnostic validity parameters of the tests used to detect the marker. To that effect, there are studies with frequencies as low as 14.3%, reported by Jaradat in 2015, and as high as 80.0 and 83.3%, reported by Suzuki in 2007 and Park in 2013, respectively (7-9).

Based on this research background, the objective of this systematic review is to meta-analyze the prevalence of the JAK2 mutation in PMF and compare it according to the detection technique.

Materials and methods

Type of study: a systematic literature review with meta-analysis of indirect measures.

Search protocol and study selection according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guide (10).

Identification: a search was performed without time limits in the Medline PubMed, Scielo and ScienceDirect multidisciplinary databases, using the terms *primary myelofibrosis, JAK2, chromosome Ph, Philadelphia chromosome, Philadelphia -Ph- chromosome, Philadelphia translocation* and BCR ABL Negative. It should be clarified that the restriction of the window of time to 2007 and later was done *a posteriori, based on the oldest study found with the review protocol*.

Screening: articles were included which contained the search terms in the title, abstract or key words; duplicate titles were eliminated. Subsequently, the inclusion criteria of studies related to the topic of interest (CMPN), studies reporting the frequency of the JAK2V617F mutation in PMF, and original articles and publications in humans or *in vivo,* were applied. Some of the syntaxes used were: on PubMed *(((JAK2[Title/Abstract]) AND primary myelofibrosis[Title/Abstract]); chromosome ph[Title/Abstract]; BCR-ABL Negative[Title/Abstract]; on ScienceDirect: TITLE-ABSTR-KEY(BCR ABL Negative) or TITLE-ABSTR-KEY(Chromosome Ph OR Philadelphia chromosome OR Ph chromosome OR Philadelphia translocation); TITLE-ABSTR-KEY(BCR ABL Negative), and on Scielo: (ti:((ab:(JAK2 primary myelofibrosis))))*.

Selection: in the next phase, articles with a low number of patients (studies with 10 or fewer cases), studies with incomplete information which did not specify the type of diagnosis or did not report the frequency of mutation, experimental or clinical studies and studies which evaluated diagnostic tests were excluded.

Inclusion: the characterization of the studies was performed with extraction of the following variables: title, authors, type of study, main topic of the study, journal, publication year, first author, study country, number of patients evaluated, frequency of the JAK2V617F mutation, technique for detecting the mutation and description of the study subjects.

Analysis of reproducibility and assessment of methodological quality: the reproducibility of the study search and data extraction was evaluated using two researchers who applied the protocol independently, resolving discrepancies by consensus. The methodological quality was determined using the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) guideline, with the criteria being applied by two researchers, in order to ensure the reproducibility of this phase.

Data analysis

The study variables were described using absolute and relative frequencies. A meta-analysis of indirect measures by detection technique (a comparison of the prevalence of the mutation according to the diagnostic technique, but based on primary studies which do not make this comparison, but rather report the prevalence independently for each detection test analyzed) was used to analyze the frequency of the JAK2V617F mutation in PMF, through a proportion estimate with its 95% confidence interval and Z Test (confidence intervals for the difference in proportions).

Results

A total of 12,845 studies were obtained without applying limits. These were restricted to 1,909 results with the title, abstract and key word search; 253 duplicate articles, 1,482 articles that did not meet the inclusion criteria, and 145 that met the exclusion criteria were eliminated. In the end, 29 studies were selected for the qualitative and quantitative data synthesis (Figure 1).

The studies were published between 2007 and 2018; the countries with the most studies were Korea (n=5), Brazil (n=3) and China (n=3). An analysis by continent shows that the largest number of studies come from Europe and Asia, with 38% each, followed by America with 21% and, finally, Africa with 3%. In the studies which reported the subjects' average age, it was over 45 years, and most used the WHO diagnostic criteria (Table 1).

All the studies had excellent methodological quality, as they met more than 70% of the STROBE guideline criteria. However, most did not explicitly state the parameters used to estimate the sample size, nor discuss the limitations or possibility of generalizing the results (Figure 2).

Based on the use of AS-PCR, the prevalence of JAK2 ranged from 33.3 to 71.4%; with real-time PCR, the range was from 42.9 to 77.3%; with sequencing, it was 14.3-57.4%; and with ARMS, it was 36.4-83.3% (Figure 3). Two studies that used PCR-RFLP reported a prevalence of 72.7 and 40% (32, 32); in Takata's study with SNP it was 36.4%, for Vytrva with DHPLC it was 63.6%, for Wu Z with HRM it was 58.0%, and for Misawa with ABC-PCR it was 53.8% (34-37).

There was no statistically significant difference in the prevalence of JAK2 by type of diagnostic test; there was a prevalence of 64.6% (95%CI=54.7-74,6) using ARMS in 99 patients; 57.3% (95%CI=47.2-67.3) with real-time PCR in 103 patients; 51.2% (95%CI=44.1-58.3) in 205 patients evaluated with AS-PCR, and 51.0% (95%CI=40.6-61.4) in 98 patients analyzed by sequencing (Figure 4). However, it should be clarified that a comparison of the groups with the highest and lowest prevalence had a statistical power of 77.5%, which would indicate a high β error, evidencing the need to increase the number of studies, and patients per study, of this disease.

Discussion

The results of this review with 29 studies and 744 patients show that the main technique used was AS-PCR; the prevalence of JAK2 with this technique ranged from 33.3 to 71.4%. The prevalence with real-time PCR was between 42.9 and 77.3%, with sequencing it was 14.3-57.4%, and with ARMS it was 36.4-83.3%.

Most of the studies were carried out in Korea, Brazil and China, countries with significant development and research policy budget allocations (38). South America showed insufficient development in the search for mutations at the various hematological centers, despite JAK2 being included as a major diagnostic criterion in the 2016 WHO update. This prevents an accurate diagnosis of the disease which could keep it from being confused with other causes of medullary fibrosis, and at the same time prevents a comprehensive study of the disease (1, 39).

The high frequencies of JAK2 mutation found in this study evidence the need to migrate from the conventional prognostic systems based mainly on clinical characteristics, such as the *International Prognostic Scoring System* (IPSS) and *Dynamic International Prognostic Scoring System* (DIPSS), towards new systems which stratify patients' risk based on their genetic profile, mainly the detection of the JAK2 mutation (40-45).

In this vein, the low number of studies in Latin American countries could suggest a lack of adherence to the prognostic scoring criteria of the new international systems based on genetic and molecular characteristics, such as the *Genetically Inspired Prognostic Scoring System for Primary Myelofibrosis* (GIPSS) and *Mutation-Enhanced International Prognostic Scoring System* (MIPSS), which provide accurate stratification of patients' risk, both at diagnosis as well as during follow-up, in terms of survival and risk of progression to leukemia (44, 45).

The prevalence of JAK2 mutations showed no statistically significant difference according to the technique used. These results could be affected by a low statistical power (77.5%) caused by a low number of studies and patients analyzed per each of the techniques. In fact, in an evaluation of methodological quality, only 17% describe how they reached the sample size, which affects

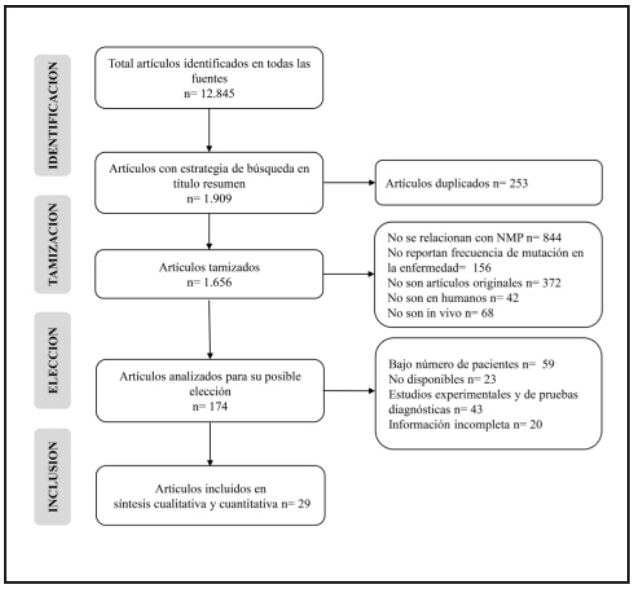

Figure 1. *Flowchart of the study search and selection.*

the estimation of prevalence. This could be explained by the difficulty in including patients with PMF due to its low occurrence and the active search for cases worldwide

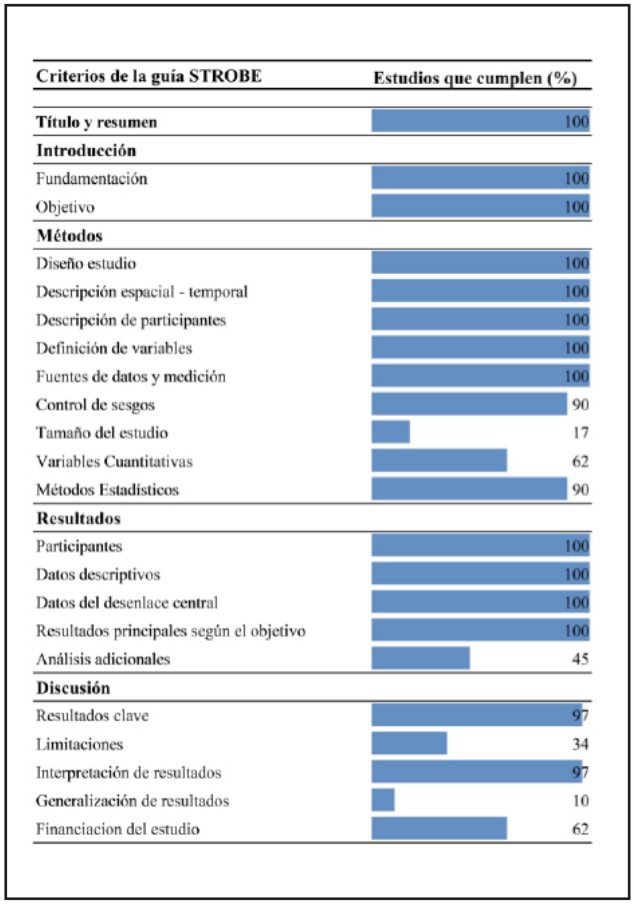

Figure 2. *Assessment of the methodological quality of the studies.*

Table 1. Study description according to year, country, age and diagnostic standard.

Author	Year	Country	Age	Diagnostic standard
AS-PCR				
Speletas M (11)	2007	Greece	61.0 [a]	Italian diagnostic criteria
Lucia E (12)	2008	Italy	No data Sin dato	WHO 2001
Pardanani A (13)	2008	United States	58.0 [a]	WHO 2001
Xu W (14)	2008	China	48.0 [b]	WHO 2001
Bang S (15)	2009	Korea	No information	WHO 2001
Medinger M (16)	2009	Switzerland	54.0 [a]	Not specified
Kim JT (17)	2010	Korea	58.3 [b]	WHO 2008
Benmoussa A (18)	2011	Morocco	56.83 [a]	Not specified
Vadikolia CM (19)	2011	Greece	66.5 [a]	WHO 2008
Ha J (20)	2012	Korea	67.3 [a]	WHO 2008
Zhang XY (21)	2012	China	No information	Not specified
Real-time PCR				
Boveri E (22)	2008	Italy	58.0 [a]	WHO 2001
Dos Santos L (23)	2011	Brazil	59.3 [a]	WHO 2008
Payzin KB (24)	2014	Turkey	62.8 [a]	WHO 2008
Azevedo AP (25)	2017	Portugal	No information	WHO 2008
Sequencing				
Jaradat SA (7)	2015	Jordan	No information	Not specified
Kim S (26)	2015	Korea	61.5 [a]	WHO 2008
Lekovic D (27)	2017	Serbia	62.0 [a]	WHO 2008
ARMS				
Trifa AP (28)	2010	Romania	> 60.0 [a]	WHO 2001
Park SH (9)	2013	Korea	62.0 [a]	Experts (histopathology)
Borowczyk M (29)	2015	Poland	56.0 [a]	WHO 2008
Ojeda MJ (30)	2018	Argentina	No information	WHO 2008
PCR-RFLP				
da Silva R (31)	2012	Brazil	No information	Clinical diagnosis
Didone A (32)	2016	Brazil	62.3 [b]	WHO 2008
Others				
Tefferi A (RT-PCR) (33)	2009	United States	50.5 [a]	WHO 2001
Takata Y (SNP) (34)	2014	Japan	69.3 [a]	WHO 2008
Vytrva N (DHPLC) (35)	2014	Austria	72.9 [a]	WHO 2001
Wu Z (HRM) (36)	2014	China	No information	WHO 2008
Misawa K (ABC-PCR) (37)	2018	Japan	60.0 [a]	WHO 2008

[a] Mean age of patients with PMF. [b] Mean age of patients with CMPN, without specifying that of PMF.

(46). This situation could be improved by carrying out multicenter studies which would allow the inclusion of more patients and, in this way, improve the estimate of prevalence or increase the statistical power of the comparisons in different subgroups.

The amplification resistant mutation system (ARMS) and allele-specific PCR (AS-PCR) are simple methods for detecting mutations which involve changes in a single base or small deletions. Both can have a high sensitivity, even with a very small number of mutated cells. However, this parameter may be affected by the type of mutation and correct primer design (47). On the other hand, real-time PCR is highly sensitive and specific, with very short processing times. Unlike the described methods, it allows a reproducible quantification of the genetic material; however, this technique may produce a large quantity of inaccurate data when there is not a strict control of the quality of the analytical variables, such as the quality of the standards and the correct selection of the housekeeping gene (48).

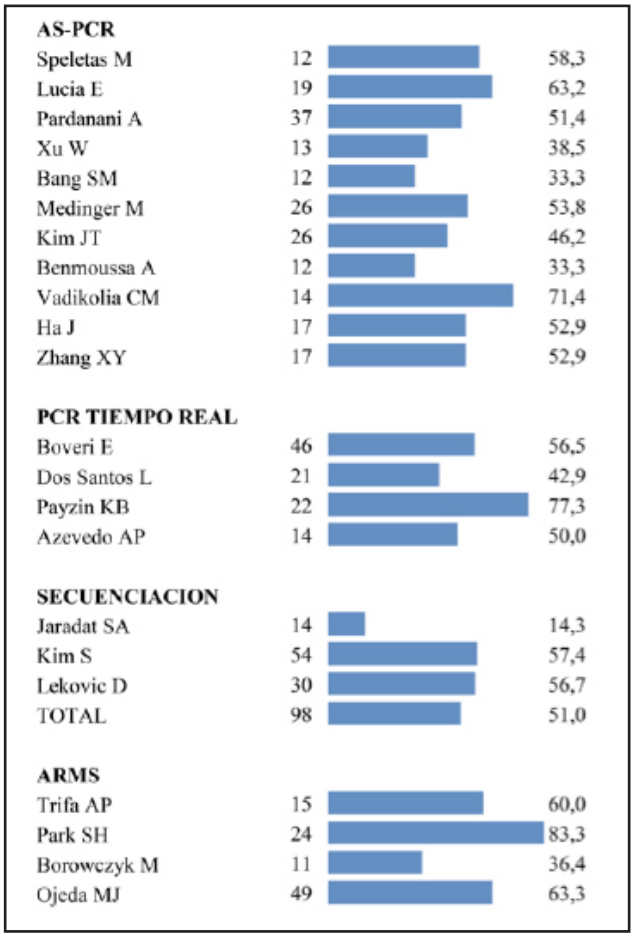

Figure 3. *Prevalence of JAK2 in studies using PCR, sequencing and ARMS.*

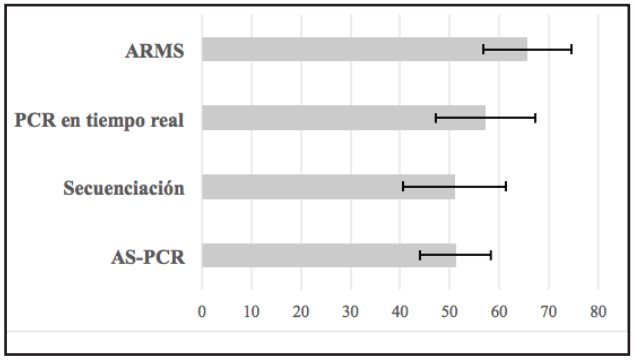

Figure 4. *Meta-analysis of indirect measures for the prevalence of JAK2 according to the use of PCR, sequencing and ARMS.*

Sequencing is a molecular technique which enables the specific modifications in gene sequences to be pinpointed exactly. However, unlike the previous techniques, its main limitation, when Sanger sequencing is used, is its low analytical sensitivity and the high concentration of DNA required (49).

In line with these characteristics, and despite the fact that this study did not find differences between the techniques employed due to the low statistical power of the comparisons, the use of sequencing methods other than Sanger must be recommended, in order to achieve greater sensitivity in the genetic screening of PMF patients.

The limitations of the current study include a low sample size in the included studies, which shows the need to carry out new studies on the topic. In addition, due to the number of studies and patients in each technique, a robust meta-analysis was not carried out in the statistical evaluation of heterogeneity, publication bias or the sensitivity of the summary measures. This could be related to the language restrictions used and the search strategies in each of the sources consulted. In this vein, subsequent studies should improve the comprehensiveness of article selection in this field by increasing the search languages, and broadening the number of terms and sources consulted, among other strategies to minimize potential selection bias.

With regard to the techniques employed in the various studies, most have high sensitivity: however, according to the GEMFIN group and Sociedad Española de Hematología y Hemoterapia recommendations, the use of methods such as Sanger sequencing, pyrosequencing and PCR-RFLP is not recommended, as they have low sensitivity which is limited to a high number of mutated clones (50).

Conclusion

A high frequency of JAK2 mutations was seen, showing that the diagnosis of PMF should not be made only by clinical and hematological characteristics, but also by the search for specific molecular markers.

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