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DETERMINATION OF *JAK2, CALR, MPL* GENE MUTATIONS IN 763 SUSPECTED CASES OF PHILADELPHIA CHROMOSOME-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS

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Abstract

Introduktion. *Myeloproliferative neoplasms (MPNs) have a high prevalence and genetic mutations play a role in their occurrence. MPNs are associated with clonal hematopoiesis, genomic instability, hemostasis dysregulation, and an altered immune response. Among the MPNs not associated with BCR-ABL1 mutations (Ph-negative/BCR-ABL1-negative MPNs) are polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). These diseases are caused by mutations in genes, such as the JAK2, CALR, and MPL genes, which participate in regulating the JAK-STAT signaling pathway. These mutant clones induce an inflammatory immune response that leads to immuno-thrombosis. Despite differences in disease type, MPNs are characterized by shared clinical, pathological, and molecular features. Clinical complications include an increased risk of arterial and venous thrombosis, varying degrees of constitutional symptoms caused by pro-inflammatory cytokines, splenomegaly, and a higher risk of progression to acute myeloid leukemia.*

The aim *of this study was to ascertain the prevalence of JAK2 V617F, CALR exon 9 Type 1 (52 bp deletion) and Type 2 (5 bp insertion), MPL W515A/L/K/R-S mutations among a cohort of Ukrainian patients diagnosed with BCR-ABL1-negative MPNs. Determination of these mutations can be valuable in the screening, diagnosis, and treatment of patients.*

Materials and methods. *The present study included DNA samples from 763 patients with suspected BCR-ABL1-negative myeloproliferative diseases (including 430 cases of PV, 182 cases of ET, and 151 cases of PMF), admitted to the SI "Institute of Blood Pathology and Transfusion Medicine of NAMS of Ukraine" (Lviv, Ukraine) for genetic analysis between 2022 and 2024.*

Mutations of JAK2 V617F, CALR, and MPL were analyzed using allele-specific polymerase chain reaction (PCR). GeneMAP™ kits manufactured by Genmark (Turkey) were utilized in the study. Molecular genetic analyses were performed using the CFX96™ Real-Time System (Bio-Rad, USA) and the geneMAP™ Viewer, according to established methodologies.

Results. *According to our data, 44.04 % (336/763) of patients with Ph-negative MPNs had the JAK2 V617F mutation. The overall frequency of mutations in the CALR gene was 14.03 % (31/221): 9.50 % Type 1 CALR mutation (21/221) and 4.52 % Type 2 CALR mutation (10/221). MPL mutations were much less common: 2.11 % (4/190): 1.58 % MPL W515L (3/190) and 0.53 % MPL W515K (1/190).*

Consistent with our studies, the JAK2 V617F frequencies in PV, ET, and PMF were 52.09 %, 35.16 %, and 31.79 %, respectively. The percentage of CALR *mutant was 9.34 % in ET and 9.27 % in PMF, while the percentage of MPL mutant was 0.55 % in ET and 1.99 % in PMF.*

Conclusions. *Our results showed that the positive rate of driver mutations in patients with PV, ET, PMF was 52.09 %, 45.05 %, and 43.05 %, respectively, which significantly improved the diagnostic*

rate, especially in ET and PMF. Subsequently, these results were employed by hematologists for diagnostic purposes.
The present study offers significant insights into the characteristics of JAK2, CALR, and MPL mutations in BCR-ABL1-negative MPNs, underscoring the necessity of genetic characterization to optimize the clinical management of these diseases.
Keywords: *JAK2 V617F; CALR; MPL; chronic myeloproliferative neoplasms; essential thrombocythemia; polycythemia vera; primary myelofibrosis.*

Introduction

Myeloproliferative neoplasms (MPNs) are characterized by the clonal proliferation of hematopoietic precursors in the bone marrow. According to the WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues, these neoplasms are classified as chronic myeloid leukemia (CML), *BCR-ABL1*-positive; chronic neutrophilic leukemia (CNL); polycythemia vera (PV); essential thrombocythemia (ET); primary myelofibrosis (PMF); chronic eosinophilic leukemia (CEL); or MPN-unclassifiable (MPN-U) [1, 2]. The diagnostic criteria for PV, ET, and PMF also include the exclusion of *BCR-ABL1*. These three conditions have overlapping morphological features and are therefore referred to collectively as *BCR-ABL1*-negative or Philadelphia chromosome-negative myeloproliferative neoplasms (Ph-negative MPNs). *JAK2* (Janus kinase 2) V617F, *CALR* (calreticulin) exon 9, and *MPL* (receptor for thrombopoietin) exon 10 mutations have been identified as the primary drivers of the majority of Ph-negative MPNs. These mutations have been observed to impact sequential phases of proliferative signal transduction. Consequently, the emergence of a specific mutation is hypothesized to preclude subsequent mutations from acquiring selective advantages for clonal expansion [2, 3].

The identification of *JAK2* in *BCR-ABL1*-negative MPNs has substantially improved our understanding of the molecular and genetic basis of these diseases [4, 5]. Subsequently, the identification of *CALR* and *MPL* mutations has further advanced our understanding and diagnostic criteria [6–9].

The genetic hallmark of Ph-negative MPNs is the constitutive activation of the JAK-STAT signaling pathway. Hematopoietic stem cells (HSCs) can acquire somatic driver mutations, which leads to the formation of mutant proteins that affects the

JAK-STAT pathway and results in uncontrolled cell proliferation in all three lineages. *JAK2* is associated with cytokine receptors such as erythropoietin receptor (EpoR), thrombopoietin receptor (TpoR), and granulocyte-colony-stimulating factor receptor (G-CSFR). TpoR is encoded by the myeloproliferative leukemia virus oncogene (*MPL*) and G-CSFR by Colony-Stimulating Factor 3 Receptor (*CSF3R*). Driver mutations that affect this pathway are predominantly *JAK2* V617F and *JAK2* exon 12 mutations, *MPL* mutations, *CALR* exon 9 mutations, and mutations in *CSF3R.*

BCR-ABL1-negative MPNs consist of three entities: ET, PV, and PMF. These entities are characterized by the overproduction of differentiated cells of various lineages that can typically be identified in the peripheral blood. All three entities carry an increased risk of thromboembolic complications, hemorrhage, and progression to acute myeloid leukemia. Although these neoplasms have distinct characteristics, there is much overlap clinically, which makes diagnosis challenging. Both PV and ET can progress to secondary myelofibrosis [2, 10].

The most common *JAK2* mutation encountered in PV is the V617F somatic mutation in exon 14. This mutation results in the substitution of valine for phenylalanine at position 617 in the pseudokinase domain. This mutation causes a conformational change in *JAK2* and triggers cytokine-independent activation of the JAK-STAT pathway. This signaling pathway then activates downstream targets, resulting in uncontrolled clonal proliferation and an increase in predominantly normal red blood cells [11–13].

The *JAK2* V617F mutation has been included in the list of major diagnostic criteria for *BCR-ABL1*-negative MPNs since 2008. It is recommended that a qualitative determination of the *JAK2* V617F mutation be performed during the initial examination of patients with suspected Ph-negative MPNs. Subsequent assessments of patients with detected *JAK2* V617F mutations allow for the quantification of allelic burden to monitor therapy efficacy.

The *JAK2* V617F mutation is present in more than 95% of PV cases. However, 3–4% of cases are associated with *JAK2* exon 12 mutations. Rare *JAK2*-negative cases harbor either driver mutations that activate the JAK-STAT pathway or non-driver mutations involved in DNA methylation. A small percentage of *JAK2*-negative cases have shown mutations in genes involved in histone modification [3].

Genetically, 50–60% of ET patients harbor the *JAK2* V617F (or functionally similar) mutations, while 15–30% patients present with *CALR* and 1–4% have the *MPL* mutations. However, up to 12% of cases are triple-negative for these driver mutations [2, 3].

According to the most recent research, approximately 50–60% of PMF cases harbor *JAK2* V617F mutations, 20–24% have *CALR* mutations, and 8–10% have *MPL* mutations. However, about 12% of cases have none of these mutations and are considered "triple-negative" [3, 11, 14, 15].

Aim. Presently, genetic testing is not widely implemented to confirm the presence of MPNs. Therefore, the objective of this study was to determine the presence of *JAK2* V617F, *CALR* Type 1/Type 2 and *MPL* W515A/L/K/R-S mutations in 763 suspected cases of *BCR-ABL1*-negative MPNs and to evaluate their diagnostic value.

Materials and methods

The procedure of molecular genetic analysis was based on two major processes: the extraction of DNA and the PCR amplification using TaqMan probes and primers with a real-time PCR machine.

Our study included DNA samples from 763 patients with suspected *BCR-ABL1*-negative MPNs, admitted to the SI "Institute of Blood Pathology and Transfusion Medicine of NAMS of Ukraine" (Lviv, Ukraine) for genetic analysis between 2022 and 2024.

Peripheral blood samples were collected in 3-mL vacuum tubes containing K2 EDTA. DNA was isolated from fresh whole blood, taken at the time of suspected diagnosis, using GeneAll® Exgene™ Blood SV kit (Korea) following the

manufacturer's instructions. The concentration and purity of DNAs were assessed spectrophotometrically.

Mutations of *JAK2* V617F, *CALR*, and *MPL* were analyzed using allele-specific PCR. GeneMAP™ kits manufactured by Genmark (Turkey) were utilized in the aforementioned study.

Initially, the detection of the *JAK2* V617F mutation was performed using the "geneMAP™ Somatic Mutation Detection Kit" (Genmark, Turkey) according to the manufacturer's instructions. Subsequent studies were conducted in *JAK2* V617F-negative patients with suspected ET and PMF to detect mutations in the *CALR* gene Type 1 (del 52 b.p.) / *CALR* gene Type 2 (ins 5 b.p.) and subsequently mutations in the *MPL* gene (W515A/L/K/R-S), following established methodologies. The assessment of *CALR* mutations were conducted using the "geneMAP™ *CALR* Screening Kit" (Genmark, Turkey), which was designed for the qualitative detection of 36 *CALR* somatic mutations and the identification of the two predominant *CALR* mutations, classified as Type 1 and Type 2. The quantification of *MPL* exon 10 mutations was performed using the "geneMAP™ *MPL* W515A/L/K/R-S Detection Kit" (Genmark, Turkey).

For PCR amplification, a CFX96™ Real-Time System (Bio-Rad, USA) was used. Automated analysis was performed using the geneMAP™ Viewer for CFX96™ Real-Time System. The multiplex real-time PCR assay facilitates the amplification of target nucleic acids of mutated alleles and endogenous control (EC). Allele 1 (EC) was labeled with a VIC fluorophore, and Allele 2 (the mutant alleles) were detected by the FAM channel. The sensitivity of allele specific PCR assays was 0.2% of mutant allele over wild type allele background according to the manufacturer's instructions.

Results and discussions

In our research 763 cases of *BCR-ABL1*-MPNs comprising of 430 cases of PV, 182 cases of ET and 151 cases of PMF were enrolled.

The *JAK2* V617F mutation was defined in 763 patients. The age of the patients ranged from 21 to 76 years (median: 53 years). The conducted studies revealed that the *JAK2* V617F mutation was present in 336 patients, 174 of whom were women and 162 of whom were men.

In total, 44.04 % patients (336/763) were detected with *JAK2* V617F mutation in 224 cases of PV, 64 cases of ET and 48 cases of PMF. For *JAK2* V617F-negative patients with PV (206 cases), testing for mutations in the *CALR* and *MPL* genes was not established.

Subsequent studies were performed to detect mutations in the *CALR* and *MPL* genes in *JAK2* V617F-negative cases diagnosed (118 ET and 103 PMF, respectively).

A total of 14.03 % (31/221) of patients harbored *CALR* mutations. Type 1 *CALR* mutations (del 52 b.p.) were detected in 13 of 118 and 8 of 103 of *JAK2* V617F-negative ET and PMF cases, respectively. Type 2 *CALR* mutations (ins 5 b.p.) were identified in 4 of 118 and 6 of 103 of *JAK2* V617F-negative ET and IMF cases, respectively.

Furthermore, *MPL* mutations were detected in 2.11 % (4/190) of *JAK2* V617F/*CALR*-negative patients. One patient with suspected ET was identified the *MPL* W515L mutation. Two patients with suspected PMF were identified *MPL* W515L mutations and one patient was identified with *MPL* W515K mutation.

According to our data, 44.04 % (336/763) of patients with Ph-negative MPNs had the *JAK2* V617F mutation. The overall frequency of mutations in the *CALR* gene was 14.03 % (31/221): 9.50 % Type 1 *CALR* mutation (21/221) and 4.52 % Type 2 *CALR* mutation (10/221). *MPL* mutations were much less common: 2.11 % (4/190): 1.58 % *MPL* W515L (3/190) and 0.53 % *MPL* W515K (1/190).

Conclusions

1. The presence of mutations in the *JAK2* V617F, *CALR*, and *MPL* genes serves to confirm the diagnosis of *BCR-ABL1*-negative MPNs.

2. In total, a combined group of patients (763) with suspected diagnoses of PV, ET, and PMF was examined for the presence of *JAK2* V617F, *CALR* Type 1/Type 2 and *MPL* W515A/L/K/R-S gene mutations.

3. Consistent with our studies, the *JAK2* V617F frequencies in PV, ET, and PMF were 52.09 %, 35.16 %, and 31.79 %, respectively. The percentage of *CALR* mutant was 9.34 % in ET and 9.27 % in PMF, while the percentage of *MPL* mutant was 0.55 % in ET and 1.99 % in PMF.

4. Our results showed that the positive rate of driver mutations in patients with PV, ET, PMF was 52.09 %, 45.05 %, and 43.05 %, respectively, which significantly improved the diagnostic rate, especially in ET and PMF. Subsequently, these results were employed by hematologists for diagnostic purposes.

5. Considering the lack of a definitive diagnostic method in Ph-negative MPNs, the results of this study showed that molecular studies, including *JAK2* V617F, *CALR*, and *MPL* mutations can be useful and effective in the diagnosis of MPNs.

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Conflict of interest. The authors declare that they have no competing interests.

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