



Evaluation of the Relationship between Janus Kinase 2 Mutational Burden and Clinical Findings in Adult Myeloproliferative Neoplasm Patients

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Abstract

Aim: Philadelphia-negative chronic myeloproliferative neoplasms (Ph-negative MPNs) are associated with various genetic abnormalities. The *JAK2* V617F mutation is the most common one and plays a crucial role in diagnosis. We aimed to evaluate the relationship between *JAK2* mutational burden and clinical parameters of MPN patients.

Methods: The present cross-sectional study was conducted on patients with MPN referred to our clinic for *JAK2* mutation screening between January 2019 and December 2020. The clinical information of the patients was obtained from the hospital automation system and records in the hematology and medical genetics departments. We evaluated 143 *JAK2* positive patients diagnosed with polycythemia vera, primary myelofibrosis (PMF) and essential thrombocythemia.

Results: The mean age of the patients was 60.29 (standard deviation:14.81). The mutational burden was correlated with spleen size and lactate dehydrogenase (LDH) level, particularly in PMF ($p=0.002$, $p=0.003$, respectively). There was no significant difference in age, gender, mutation burden and laboratory findings in patients with and without thrombosis and bleeding.

Conclusion: Clinical parameters and *JAK2* mutational burden are related, but this relationship differs based on the MPN types. The spleen size in MPN, particularly massive splenomegaly and high LDH levels, may be correlated with the *JAK2* mutational burden. This relationship is more pronounced for PMF. There is no significant relationship between *JAK2* mutational burden and vascular complications such as thrombosis and bleeding.

Keywords: Myeloproliferative disorders, Janus kinaz 2, primary polycythemia, essential thrombocythemia, primary myelofibrosis

Introduction

Philadelphia-negative chronic myeloproliferative neoplasms (Ph-negative MPNs) consist mainly of polycythemia vera (PV), primary myelofibrosis (PMF) and essential thrombocythemia (ET) (1). Ph-negative MPNs are associated with various genetic abnormalities. One of these abnormalities is Janus kinase 2 (*JAK2*), a protein tyrosine kinase gene. It is associated with cellular growth and proliferation. The most common genetic mutation found in Ph-negative MPNs is the *JAK2* V617F mutation, wherein phenylalanine replaces valine due to a point mutation in Codon 617. The *JAK2* gene is located on the short arm of chromosome 9. This mutation increases tyrosine phosphorylation activity, resulting in hypersensitivity of

hematopoietic progenitor cells to growth factors (2,3). Treatment options for MPN patients include acetylsalicylic acid (ASA), phlebotomy, and hydroxyurea. Where hydroxyurea cannot be used due to its side effects, interferon-alpha ($IFN-\alpha$), *JAK2* inhibitors (ruxolitinib) or busulfan are recommended (4). *JAK2* inhibitors such as ruxolitinib are more effective for splenomegaly and clinical symptoms than clonal effects. Studies on target molecules except *JAK2* are ongoing (5).

The *JAK2* V617F mutation occurs in approximately half of ET and PMF patients and in 90% of PV patients. Other common genetic abnormalities in MPNs include mutations in *CALR* and *MPL*. *JAK2*, *CALR* and *MPL* mutations have been listed under World Health Organization's MPN diagnostic criteria (6). With the NGS (next-generation

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sequencing) technique, all of these mutations can be viewed at once (7). Testing for the *JAK2* V617F mutation in MPN needs to be performed routinely for diagnosis as well as for prognosis. In addition, it is opined that the mutation burden may be associated with complications related to the disease (8). The clinical course of myeloproliferative diseases is known to be quite heterogeneous. It has been stated that *JAK2* V617F positivity is associated with clinical heterogeneity (9-11). Thrombosis risk in MPN patients is considered to be in low- and high-risk groups. Patients over 60 years of age and/or the presence of arterial or venous vascular complications are considered to be a high-risk group in PV. *JAK2* positivity for ET is also a factor for the high-risk category (12). MPN and clonal hematopoiesis are clinical conditions that may evolve into each other, and *JAK2* positivity is also seen in clonal hematopoiesis. There is a correlation between the burden of *JAK2* and other genomic changes and clinical severity (13). In the literature, the clinical findings of MPN patients with and without *JAK2* mutations were evaluated.

In this study, we aimed to evaluate the relationship between *JAK2* mutational burden and clinical parameters of the MPN patients referred to our clinic. We evaluated the laboratory findings, spleen size, thrombosis status and treatment options of the patients.

Methods

Study Design

Written informed consent was obtained from the participants. Our study complies with the principles of the Helsinki Declaration. This study was approved by the Ataturk University Clinical Research Ethic Committee (decision number: B.30.2.ATA.0.01.00/129).

The present cross-sectional study was conducted on patients with MPN referred to our clinic for *JAK2* mutation screening between January 2019 and December 2020. We identified 143 (16.94%) *JAK2* positive patients among the 844 patients who were examined. The clinical information

of the MPN patients was obtained from the hospital automation system and records in the hematology and medical genetics departments.

We evaluated the laboratory parameters, clinical follow-up, thrombotic complications and *JAK2* mutational burden of these *JAK2* positive patients.

JAK2 Genetic Analysis

DNA isolation from patient blood samples was performed using the EZ1 DNA Blood 200 µL (Qiagen) kit. DNA quality was standardized through measurement with nanodrop technology. A real-time polymerase chain reaction (PCR) study was performed with 25 ng of purified DNA. The Ipsogen *JAK2* MutaQuant Kit (Qiagen) was used for the detection and quantification of the *JAK2* V617F/G1849T somatic mutation in the Rotor Gene-Q PCR system. Mutation analysis was performed with PCR software.

Statistical Analysis

The statistical analysis of laboratory results and patients' data was carried out using SPSS version 20.0. Categorical data was compared using the Pearson chi-square test. To determine the distribution of continuous variables, the Kolmogorov-Smirnov and Shapiro-Wilk tests were used; normally distributed data were presented as the mean and SD, while non-normally distributed data were presented as the median and interquartile range. The Mann-Whitney U test and the Kruskal-Wallis H test were performed for independent samples. The threshold for statistical significance was set at $p=0.05$.

Results

There were 90 (62.9%) PV, 34 (23.8%) ET and 19 (13.3%) PMF patients among a total of 143 patients. Demographic features were presented in Table 1.

When laboratory findings were evaluated according to the diagnosis, white blood cell (WBC), red blood cell (RBC), hemoglobin (Hgb), hematocrit (Htc), neutrophil (neu), erythropoietin and *JAK2* mutational burden showed

Table 1. *JAK2* mutational burden and patient demographics

	Diagnosis			Total	Mean age (SD)
	PV	ET	PMF		
<i>JAK2</i> (1-49%)	53 (58.9%)	30 (88.2%)	9 (47.4%)	92 (64.3%)	
<i>JAK2</i> (50-100%)	37 (41.1%)	4 (11.8%)	10 (52.6%)	51 (35.7%)	
Total	90 (100%)	34 (100%)	19 (100%)	143 (100%)	
	Diagnosis			Total	Mean age (SD)
	PV	ET	PMF		
Male	56 (62.2%)	14 (41.2%)	13 (68.4%)	83 (58%)	59.47 (SD: 14.99)
Female	34 (37.8%)	20 (58.8%)	6 (31.6%)	60 (42%)	60.37 (SD: 15.78)
Total	90 (100%)	34 (100%)	19 (100%)	143 (100%)	60.29 (SD: 14.81)

PV: Polycythemia vera, ET: Essential thrombocythemia, PMF: Primary myelofibrosis, SD: Standard deviation, *JAK2*: Janus kinase 2

significant differences between groups (Table 2). The JAK2 mutational burden was significantly different in terms of diagnosis (p<0.05) (Figure 1).

Based on JAK2 mutational burden, two groups were formed: ≥50% and <50%. When laboratory findings were evaluated based on these groups, WBC, Hgb, neu, platelet (plt), and lactate dehydrogenase (LDH) values showed significant differences between groups. WBC, neu, LDH levels (Figure 2), spleen size (Figure 3) and median age were higher in the high mutational burden group (p<0.05). Gender was not a significant factor (p>0.05) (Table 3). In addition, we compared the laboratory parameters according to JAK2 mutational burden in three different MPN diagnosis groups. However, when divided into subgroups, the number of samples was unevenly insufficient and significant results could

not be obtained. Significant differences in WBC, Hgb, neu, LDH, uric acid, and spleen size were detected only in the PV group because of the relatively higher sample number (p<0.05). Thrombosis occurred in 26 (18.2%) patients out of 143, while gastrointestinal bleeding occurred in three (2.1%) patients. Among the patients with thrombosis, four had cerebrovascular events, two had pulmonary thromboembolism, ten had myocardial infarction, four had pulmonary vein thrombosis, two had splenic vein thrombosis, one had deep vein thrombosis and one had digital arterial thrombosis. When the groups with and without thrombosis were compared, there was

Table 2. Laboratory findings, demographics and diagnosis				
	PV	ET	MF	p-value
	Median (IQR)	Median (IQR)	Median (IQR)	
Hgb (g/dL)	16.10 (3.67)	15 (3.38)	13.90 (9.30)	0.000*
Htc (%)	50.15 (11.38)	44.65 (10.53)	38.40 (4.8)	0.000*
RBC (x10 ¹² /L)	6.26 (2.19)	5.32 (1.49)	4.60 (0.95)	0.000*
WBC (x10 ⁹ /L)	12.44 (8.70)	10.15 (3.38)	13.90 (9.30)	0.018*
Neu (x10 ⁹ /L)	9.06 (7.88)	7.08 (3.08)	9.69 (9.86)	0.002*
Plt (x10 ⁹ /L)	570 (452.25)	641 (242)	288 (354)	0.000*
Lymp (x10 ⁹ /L)	2.01 (1.23)	2.25 (0.85)	1.70 (0.94)	0.068
Uric acid (mg/dL)	6.10 (2.30)	5.60 (2.08)	5.75 (3.18)	0.159
LDH (U/L)	350 (207)	286 (153)	461 (352)	0.248
Erythropoietin (mU/mL)	3.03 (3.65)	4.89 (2.83)	20.31 (36.20)	0.000*
JAK2%	33.50 (35)	22.50 (18)	50 (45)	0.000*
Spleen size (mm)	136.5 (51)	140 (51)	184.5 (80)	0.009*
Age	64 (24)	61 (23)	65 (13)	0.473
	N (%)	N (%)	N (%)	Total
Patients	90 (62.9%)	34 (23.8%)	19 (13.3%)	143 (100%)
Female	34 (37.8%)	20 (58.8%)	6 (31.6%)	60 (42%)
Male	56 (62.2%)	14 (41.2%)	13 (68.4%)	83 (58%)

Htc: Hhematocrit, Hgb: Hemoglobin, RBC: Red blood cell, WBC: White blood cell, neu: Neutrophilia, plt: Platelet, lymph: Lymphocyte, LDH: Lactate dehydrogenase, IQR: Interquartile range
 *significant p-values, detected by the Kruskal-Wallis test

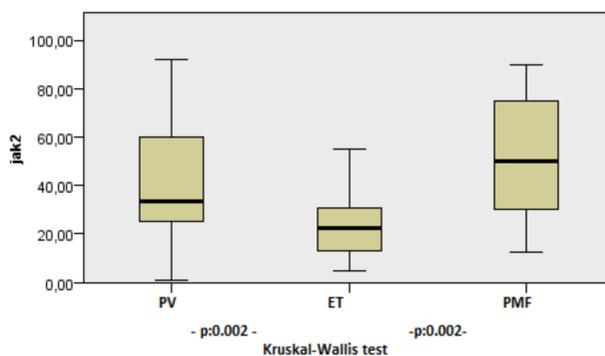


Figure 1. JAK2 mutational burden in terms of diagnosis PMF: Primary myelofibrosis, PV: Polycythemia vera, ET: Essential thrombocythemia, JAK2: Janus kinase 2

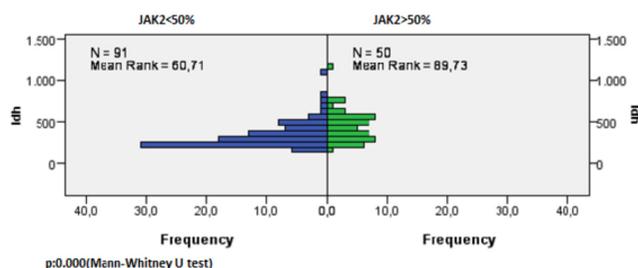


Figure 2. LDH levels in terms of JAK2 mutational burden JAK2: Janus kinase 2, LDH: Lactate dehydrogenase

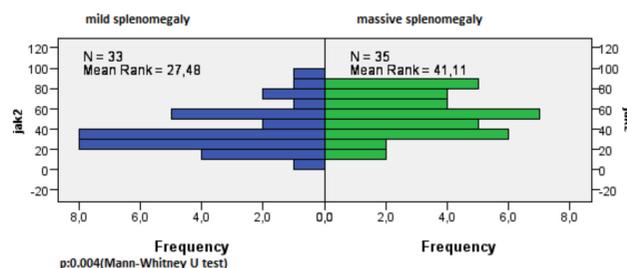


Figure 3. JAK2 mutational burden in terms of splenomegaly category JAK2: Janus kinase 2

no statistical difference in age, *JAK2* mutational burden, spleen size and laboratory findings. Only the lymphocyte count was lower ($p < 0.05$). Gender was not a significant factor ($p > 0.05$). Eighteen PV, seven ET patients, and one PMF patient were part of the thrombosis group.

Cases with a spleen size ≥ 160 mm in the ultrasonography were considered as massive splenomegaly (SM) and those with a spleen size of 130-160 mm were considered as mild splenomegaly. Laboratory findings were evaluated according to mild and massive SM groups (Table 4). The SM rate was 57.1% in the group having a *JAK2* $\geq 50\%$ mutation burden. Splenomegaly was found to be associated with *JAK2* mutation burden ($p = 0.002$) (Table 2). The SM rates were 48.7% in PV, 35.3% in ET and 83.3% in PMF patients. The PMF group was significantly different from the other groups ($p = 0.009$).

We also evaluated the treatment protocols of the patients. Most of the patients were treated with ASA+hydroxyurea (59.4%); 64.7% of the patients using this treatment were PV patients, 22.4% were ET, and 12.9% were PMF patients. The second most common treatment options were ASA (14.7%) and ASA+phlebotomy (14.7%). 71.4% of those treated with

ASA were ET patients, 23.8% were PV patients, and 4.8% were PMF patients. 100% of the patients treated with ASA+phlebotomy were PV patients. The other treatment protocols included ASA+ruxolitinib in 4.2%, ASA+IFN- α in 3.5%, ruxolitinib in 1.4%, clopidogrel+hydroxyurea in 0.7%, hydroxyurea in 0.7% and allogenic bone marrow transplantation in 0.7% of the patients. Htc and lymph levels were significantly higher in lower-aged patients treated with ASA+phlebotomy than in patients treated with ASA+hydroxyurea. The *JAK2* mutational burden, mean age and LDH level were significantly higher in patients treated with ASA+hydroxyurea than in those treated with ASA alone. The patients treated with ASA+hydroxyurea were older than the patients treated with ASA and ASA+phlebotomy. Twenty-one thrombosis patients were treated with ASA+hydroxyurea, three with ASA+IFN- α , one with ASA+ruxolitinib, and one with clopidogrel+hydroxyurea.

Discussion

Based on laboratory findings, thrombosis status, and treatments, we evaluated the *JAK2* V617F positive MPN patients. There have been studies evaluating MPN patients with and without *JAK2* V617F mutation in only

Table 3. Laboratory findings in terms of *JAK2* mutational burden

	JAK2% (1-49)	JAK2% (50-100)	p-value
	Median (IQR)	Median (IQR)	
Hgb (g/dL)	15.65 (3.20)	13.50 (4.60)	0.005*
Htc (%)	49.30 (9.60)	47.10 (16.10)	0.141
RBC ($\times 10^{12}/L$)	5.82 (1.63)	5.15 (3.11)	0.677
WBC ($\times 10^9/L$)	10.325 (4.98)	16.130 (10.52)	0.000*
Neu ($\times 10^9/L$)	7.26 (4.28)	12.54 (8.87)	0.000*
Plt ($\times 10^9/L$)	644 (397.5)	439. (364)	0.001*
Lymp ($\times 10^9/L$)	2.11 (1.03)	2.14 (1.25)	0.066
Uric acid (mg/dL)	5.72 (1.84)	6.76 (2.50)	0.3
LDH (U/L)	287 (159)	436.5 (241)	0.003*
Eritropoetin (mU/mL)	4.31 (4.54)	3.34 (4.37)	0.5
Age	61 (22)	65 (16)	0.003*
Spleen size (mm)	133 (48)	162 (76)	0.002*
	N (%)	N (%)	
Patients	92 (64.3%)	51 (35.7%)	0.8
Female	38 (63.3%)	22 (36.7%)	
Male	54 (65.1%)	29 (34.9%)	

Htc: Hematocrit, Hgb: Hemoglobin, RBC: Red blood cell, WBC: White blood cell, neu: Neutrophil, plt: Platelet, lymph: Lymphocyte, LDH: Lactate dehydrogenase, IQR: Interquartile range
*significant p-values, detected by the Mann-Whitney U test and means the distribution of the parameters was not the same across categories of *JAK2* mutational burden

Table 4. Laboratory findings in terms of splenomegaly groups

	Spleen size (130-159 mm)	Spleen size (≥ 160 mm)	p-value
	Median (IQR)	Median (IQR)	
Hgb (g/dL)	15.30 (4.05)	12.60 (4.30)	0.003*
Htc (%)	48.40 (11.90)	40.20 (13.20)	0.006*
RBC ($\times 10^{12}/L$)	5.91 (2.20)	4.80 (2.22)	0.008*
WBC ($\times 10^9/L$)	11.02 (8.14)	11.46 (12.43)	0.830
neu ($\times 10^9/L$)	7.67 (7.39)	8.80 (8.38)	0.480
Plt ($\times 10^9/L$)	623 (304.5)	391 (336)	0.005*
Lymp ($\times 10^9/L$)	2.19 (1.18)	1.41 (1.00)	0.018*
Uric acid (mg/dL)	5.90 (2.60)	6.60 (2.85)	0.299
LDH (U/L)	315 (216)	504 (224.75)	0.007*
Eritropoetin (mU/mL)	3.65 (3.55)	4.04 (4.31)	0.716
JAK2 %	30 (29)	52 (40)	0.004*
Age	64 (17)	64 (25)	0.917
Spleen size (mm)	138.30 (14)	180 (54)	0.000*
	N (%)	N (%)	
Patients	33 (48.53%)	35 (51.47%)	

Htc: Hematocrit, Hgb: Hemoglobin, RBC: Red blood cell, WBC: White blood cell, neu: Neutrophil, plt: Platelet, lymph: Lymphocyte, LDH: Lactate dehydrogenase, IQR: Interquartile range
*significant p-values, detected by the Mann-Whitney U test and means the distribution of the parameters was not the same across categories of spleen size

PV (11), in only ET (9,14-17), both in PV and ET (18-20), in ET and PMF (1), and in PV, ET and PMF (21). In these studies, within the *JAK2* positive group, Hgb (18,22), Htc (1,14,15,19), RBC (20) levels were significantly higher, and plt (1,14,15,18,20) levels were significantly lower. In various other studies, WBC (1,18-20,22) and neu (9) were also significantly higher. In our study, we evaluated PV, ET and PMF patients who already had the *JAK2* mutation.

There are studies evaluating the *JAK2* mutational burden in MPN patients, which are similar to our study. In a study by Tefferi et al. (11), *JAK2* heterozygous and homozygous PV patients were compared, and no statistical difference was found in age, gender, leukocyte or platelet counts, or thrombosis or bleeding cases, at the time of diagnosis. However, in homozygous patients, a significantly higher hemoglobin level at the time of diagnosis, increased pruritus, and a higher rate of fibrotic transformation were found (11). In the study by Zhou et al. (20), the *JAK2* V617F mutational burden was found to be significantly higher in PV compared to ET and PMF patients, similar to our study. It was reported that the *JAK2* mutational burden was positively correlated with WBC and plt counts in ET patients, and with WBC and RBC counts in PV patients (20). In the study by Liu et al. (21), high to low *JAK2* mutation burden was determined in patients with PV, PMF and ET, respectively. Patient age and WBC in PV, WBC in ET, and Hgb, WBC, and plt count in PMF were significantly correlated with higher mutation burden (21). In our study, Htc, Hgb, and RBC were significantly higher in the PV group compared to the ET and PMF groups, which is consistent with previous studies (18,20,23); while WBC, neu were significantly higher in the PV group compared to the ET group. In contrast to our study, Vannucchi et al. (19), indicated that age, Htc, and WBC levels were not different in terms of the diagnosis of PV or ET. In some studies, LDH levels were not different in *JAK2* mutant patients (1,9,17,24), while in other studies, LDH was different, consistent with our study (25).

In the study by Kittur et al. (14), it was stated that *JAK2* mutational burden was associated with splenomegaly and male gender in ET patients. Several studies have found a link between splenomegaly and *JAK2* mutations in ET patients (1,15,18,19). In our study, SM and *JAK2* mutational burden were related, but gender was not related, as in various other studies (16,26). According to our study, patients with SM may have a higher *JAK2* mutational burden.

There is conflicting information in the literature about the relationship between the *JAK2* mutation and thrombosis. Several studies have found that *JAK2* mutations are linked to thrombosis in MPN patients (9,14,16,19,27,28). According to another study, MPN patients with an allele

burden of $\geq 50\%$ *JAK2* patients had an increased risk of vascular complications, and they account for the majority of PV and a small proportion of ET (27). Thrombosis or bleeding risk, on the other hand, was not found to be associated with the *JAK2* mutation in myeloproliferative diseases in various studies (1,11,17,22,29). In our study, there was no significant difference between the groups with and without thrombosis, in terms of age, gender, *JAK2* mutational burden and laboratory parameters except lymph count. The lymph count was significantly higher in the group without thrombosis. This result may be due to the small number of patients with thrombosis. Across studies, low/high-risk of thrombosis groups are defined for PV (30) and ET patients (12,30). ASA is recommended in low-risk patients to prevent venous and arterial thrombosis. Phlebotomy is performed in both high and low-risk groups to keep the hematocrit values below 45% (12). Low-dose ASA, phlebotomy and hydroxyurea are recommended for patients in the high-risk category. Hydroxyurea regulates the plt count and reduces the risk of vascular complications. IFN- α , *JAK2* inhibitor or Busulfan are used when hydroxyurea cannot be used due to its side effects (4). In our study, consistent with the literature, ASA+hydroxyurea, ASA+phlebotomy and ASA were the common treatment options and early preventive treatments may be protective for thrombosis.

Study Limitations

Although our total number of patients was sufficient, when we evaluated the three different groups of MPNs, the number of samples was low in the ET and PMF groups, in particular. When the subgroups were classified according to *JAK2* mutational burden, a strong comparison could not be made because the numbers were further reduced. This is the limited aspect of our study. Despite these limitations, there have been few articles on the effects of *JAK2* mutational burden in all three MPNs, and we believe that this article will contribute to the literature.

Conclusion

Clinical parameters and *JAK2* mutational burden are related but this relationship differs based on the MPN types. The spleen size in MPN, particularly SM and high LDH level may be correlated with the *JAK2* mutational burden. This relationship is more pronounced for PMF. There is no significant relationship between *JAK2* mutational burden and vascular complications such as thrombosis and bleeding.

Ethics

Ethics Committee Approval: This study was approved by Ataturk University Clinical Research Ethic Committee (decision number: B.30.2.ATA.0.01.00/129).

Informed Consent: Retrospective study.

Authorship Contributions

Concept: C.Y.K., G.S., A.T., Design: C.Y.K., G.S., A.T., Data Collection or Processing: C.Y.K., G.S., Analysis or Interpretation: C.Y.K., G.S., A.T., Literature Research: C.Y.K., G.S., Writing: C.Y.K., G.S.

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