RESEARCH ARTICLE

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Clinical Impact of *JAK2V617F* Allele Burden in Philadelphia-Negative Myeloproliferative Neoplasms

Philadelphia-Negatif Kronik Myeloproliferatif Neoplazide *JAK2V617F* Allel Yükünün Klinik Önemi

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Abstract

Objective: The impact of *JAK2V617F* allele burden on clinical course in Philadelphia-negative (Ph-negative) myeloproliferative neoplasms (MPNs) is not clear. We analyzed the clinical impact of *JAK2V617F* allele burden in a relatively large series of patients with Ph-negative MPNs and long-term follow-up.

Materials and Methods: A total of 228 patients with Ph-negative MPNs, including 118 with essential thrombocythemia (ET), 84 with primary myelofibrosis (PMF), and 26 with polycythemia vera (PV), were analyzed. The *JAK2* MutaScreen assay was used to quantify *JAK2V617F* allele burden in genomic DNA.

Results: In PV cases, high *JAK2V617F* allele burden was associated with a trend towards inferior overall survival. In ET, high *JAK2V617F* allele burden was associated with lower hemoglobin and hematocrit levels, higher lactate dehydrogenase (LDH) levels, larger spleen size, and increased bleeding and mortality rates. In PMF, high *JAK2V617F* allele burden was associated with higher leukocyte counts and larger spleen size. In the entire cohort, high allele burden was associated with higher leukocyte and lower platelet counts, higher LDH levels, larger spleen size, higher percentage of bleeding events, higher death rate, and inferior overall survival.

Conclusion: Our results suggest that high *JAK2V617F* allele burdens are associated with more severe disease in PV and ET. In PMF, high *JAK2V617F* allele burdens were associated with more pronounced myeloproliferative phenotypes. In Ph-negative MPNs, high allele burdens were associated with more aggressive phenotypes. Our data with a long follow-up period support the possibility of *JAK2V617F* allele burden being used as a marker for predicting clinical phenotype in cases of Ph-negative MPNs.

Keywords: *JAK2V617F* allele burden, Philadelphia-negative myeloproliferative neoplasms, *JAK2* MutaScreen assay

Öz

Amaç: Philadelphia-negatif myeloproliferatif neoplazilerde (Ph-negatif MPN) *JAK2V617F* allel yükünün klinik seyir üzerine etkisi net değildir. Uzun takip süresi olan bu çalışmada, Ph-negatif MPN'de *JAK2V617F* allel yükünün rölatif olarak büyük hasta serisi üzerindeki klinik etkisinin araştırılması amaçlanmıştır.

Gereç ve Yöntemler: İki yüz yirmi sekiz Ph-negatif MPN tanılı olguda -118 esansiyel trombositemi (ET), 84 primer myelofibroz (PMF) ve 26 polisitemia vera (PV)- *JAK2V617F* allel yükü analiz edilmiştir. Genomik DNA'da *JAK2V617F* allel yükünün kantitatif ölçümü için "*JAK2* MutaScreen assay" kullanılmıştır.

Bulgular: PV olgularında yüksek *JAK2V617F* allel yükü kötü sağkalıma eğilim yaratmıştır. ET'de yüksek *JAK2V617F* allel yükü, düşük hemoglobin ve hematokrit seviyeleri, yüksek laktat dehidrojenaz (LDH) düzeyi, dalak boyutunda artış, kanama ve ölüm sıklığında artışla ilişkili bulunmuştur. PMF tanılı olgularda yüksek *JAK2V617F* allel yükü, yüksek lökosit değeri ve dalak boyutunda artışla ilişkilidir. Tüm Ph-negatif MPN grubunda yüksek *JAK2V617F* allel yükü, yüksek lökosit değeri, düşük trombosit değeri, yüksek LDH düzeyi, dalak boyutunda artış, artmış kanama sıklığı, yüksek ölüm oranı ve kötü sağkalım ile ilişkili bulunmuştur.

Sonuç: Bu çalışmaya göre yüksek *JAK2V617F* allel yükünün PV ve ET tanılı olgularda kötü seyirle ilişkili olduğu ve PMF tanılı olgularda daha belirgin bir myeloproliferatif fenotiple ilişkili olduğu söylenebilir. Bunun yanında tüm Ph-negatif MPN grubunda, yüksek allel yükü daha agresif fenotiple ilişkili bulunmuştur. Uzun takip süreli bu çalışma, *JAK2V617F* allel yükünün Ph-negatif MPN tanılı olgularda klinik fenotipi öngörmek için kullanılabilecek bir belirteç olabileceğini desteklemektedir.

Anahtar Kelimeler: *JAK2V617F* allel yükü, Philadelphia-negatif myeloproliferatif neoplazi, *JAK2* MutaScreen assay

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Introduction

Polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) are classified as Philadelphia-negative (Ph-negative) myeloproliferative neoplasms (MPNs) [1]. ET is usually associated with low *JAK2V617F* allele burden. Most patients with PMF display intermediate-high levels of mutant allele burden while most PV patients have a higher mutant allele burden [2]. Studies investigating the correlation between *JAK2V617F* allele burden and clinical phenotype in Ph-negative MPNs have yielded inconsistent results [3,4,5,6,7,8,9,10,11]. We investigated the impact of *JAK2V617F* allele burden on clinical and laboratory characteristics and outcomes in a total of 228 patients with Ph-negative MPNs (118 ET, 84 PMF, and 26 PV patients).

Materials and Methods

Study Population

The study group was a cohort of 228 patients with Ph-negative MPNs diagnosed according to the 2016 criteria of the World Health Organization between May 1995 and September 2019 [12]. Data obtained at the time of study enrollment included demographic factors, blood counts, and lactate dehydrogenase (LDH) levels at diagnosis and bleeding and thrombosis as clinical complications. The presence of cardiovascular disease risk factors including hypertension, diabetes mellitus, smoking, and dyslipidemia was investigated. Spleen size on abdominal ultrasound examination was recorded.

Genomic Analysis and Quantification of *JAK2V617F* Allele Burden

Samples obtained from 118 ET, 84 PMF, and 26 PV patients for the assessment of *JAK2V617F* allele burden were processed in the Molecular Hematology Laboratory of İstanbul University. The High Pure Polymerase Chain Reaction (PCR) Template Preparation Kit (Roche Diagnostics, Mannheim, Germany) was used to extract genomic DNA from peripheral blood granulocytes. A NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA) was used to measure DNA concentration. *JAK2V617F* mutational load was analyzed by *JAK2* MutaScreen assay (Ipsogen, Luminy Biotech, Marseille, France) using TaqMan allelic discrimination [13]. The assay involved the simultaneous use of two specific TaqMan probes and the measurement of the respective fluorescence of the two alleles (FAM for V617F and VIC for wild-type) to differentiate the amplification of each allele. The following PCR conditions were used to perform the reactions: initial denaturation step of 95 °C for 15 min, followed by 50 cycles of amplification consisting of denaturation at 95 °C for 15 s, annealing at 60 °C for 1 min, and a final extension step of 60 °C for 20 s. A Rotor-Gene 3000 real-time PCR instrument (Corbett Research, Sydney, Australia) was used for the quantitation of mutant and wild-type alleles. The mutant allele load was measured as percentage of total *JAK2* represented by *JAK2V617F* (i.e., *JAK2V617F* + *JAK2* wild type). The mutant allele burden was estimated with six scaled standards of the *JAK2V617F* mutant allele (2%, 5%, 12.5%, 31%, 50%, and 78%). *JAK2V617F* mutant allele loads of >50% and ≤50% were reported as high and low *JAK2V617F* allele burdens, respectively.

Statistical Analysis

The *JAK2V617F* allele burden was evaluated as an ordered categorical variable with respect to the following groups: 0% (*JAK2* wild-type), *JAK2V617F*-positive with mutant allele burden in the lower quartile ranges (allele burden of ≤50%), and *JAK2V617F*-positive with mutant allele burden in the upper quartile ranges (allele burden of >50%). IBM SPSS Statistics 20 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. Numerical variables were summarized as mean and standard deviation (SD). Comparisons of categorical variables between the three groups were performed by chi-square test. The Kruskal-Wallis test was used to make comparisons between categorical and continuous variables. The Mann-Whitney U test was used to compare differences between two groups. A two-tailed p-value of <0.05 was regarded as statistically significant. Estimations of overall survival (OS) and leukemia-free survival (LFS) were performed by Kaplan-Meier analysis. The log-rank test was used to investigate whether there were differences in OS or LFS between the groups.

Results

The *JAK2V617F* mutation was found to be positive in 135 of 228 patients (59.2%), including 12 PV patients (46.2%), 59 PMF patients (70.2%), and 64 ET patients (54.2%). The frequencies of *JAK2V617F*-positive patients with mutant allele burdens in the upper quartile ranges among the PMF, PV, and ET cases were 21.4%, 15.4%, and 4.2%, respectively (p=0.001). The frequency of *JAK2V617F*-positive patients with mutant allele burdens in the upper quartile ranges was higher for PV than ET (15.4% and 4.2%, respectively; p=0.045). The frequency of *JAK2V617F*-positive patients with high allele burdens was higher for PMF than ET (21.4% and 4.2%, respectively; p=0.001). Compared to PV, PMF showed a higher yet not statistically significantly different rate of *JAK2V617F*-positive patients with high allele burdens (21.4% and 15.4%, respectively; p=0.08). The rate of myelofibrotic transformation among the total of 144 ET and PV patients with a mean follow-up duration of 100.6 months (SD: 68.7) was 6.9% (n=10) (8/118 among ET and 2/26 among PV cases).

Comparison of Ph-Negative Myeloproliferative Neoplasms According to *JAK2V617F* Allele Burden Data

The mean follow-up duration of the Ph-negative MPN patients was 89.6 months (SD: 66.3). The mean ages of the total cohort

(52.1% female) at diagnosis and at the time of data collection were 53.2 (SD: 14.9) and 61.2 (SD: 14.5) years, respectively.

Ph-negative MPNs were divided into three groups according to the burden of the mutated *JAK2V617F* allele (Table 1). Patients with high allele burdens had higher leukocyte counts, lower platelet counts, and higher LDH levels at diagnosis compared to the other two groups (p=0.001, p=0.011, and p=0.001, respectively).

The mean spleen size was larger in patients with *JAK2V617F* allele burdens of >50% compared to the other two groups (p=0.001). The percentage of bleeding events in patients with high mutation loads was significantly higher compared to patients with low mutation loads and wild-type *JAK2* (p=0.02). The mortality rate was higher in patients with high mutation loads compared to the other two groups (p=0.002).

Kaplan-Meier Survival Curves of Patients with Ph-Negative Myeloproliferative Neoplasms According to *JAK2V617F* Allele Burden Data

Kaplan-Meier plots demonstrated significantly inferior OS in patients with mutant allele burdens in the upper quartile ranges (n=27) compared to patients with burdens in the lower quartile ranges (n=108) and *JAK2V617F* mutation-negative patients (n=93) (p=0.002) (Figure 1a). However, LFS did not differ between the three groups (p=0.979) (Figure 1b).

Kaplan-Meier Survival Curvesof Patients with Polycythemia Vera According to *JAK2V617F* Allele Burden Data

There was a trend towards inferior OS in patients with high mutation loads (n=4) compared to patients with low mutation loads (n=8) and *JAK2V617F* mutation-negative PV patients (n=14) (p=0.056) (Figure 2).

Comparison of Essential Thrombocythemia Patients According to *JAK2V617F* Allele Burden Data

ET patients with high allele burdens had lower hemoglobin and hematocrit levels and higher LDH levels at diagnosis compared to the other two groups (p=0.001, p=0.001, and p=0.008, respectively) (Table 2). There was a trend towards lower platelet counts in ET patients with *JAK2V617F* allele burdens in the upper quartile ranges (p=0.061).

The mean spleen size was larger in ET patients with *JAK2V617F* allele burdens of >50% (p=0.034). The frequency of bleeding events in ET patients with high mutation loads was significantly higher compared to that of patients with low mutation loads or wild-type *JAK2* (p=0.002). The mortality rate was higher among ET patients with high mutation loads than in the other two groups (p=0.007). The rate of myelofibrotic transformation was higher among patients with high mutation loads compared to patients with wild-type *JAK2* or low mutation loads, although the difference was not statistically significant (20%, 7.4%, and 5.1%; respectively; p=0.431).

Table 1. Clinical and laboratory characteristics of patients with Ph-negative myeloproliferative neoplasms according to *JAK2V617F* allele burden data (n=228).

Ph-negative myeloproliferative neoplasms	*JAK2* wild-type (mean [SD])	Low *JAK2* allele burden (≤50%) (mean [SD])	High *JAK2* allele burden (>50%) (mean [SD])	p
Number of patients	93	108	27	-
Female patients (%)	49 (52.7%)	56 (51.9%)	14 (51.9%)	0.992
Age at diagnosis (years)	52.51 [15.53]	52.29 [14.88]	59 [11.60]	0.074
Age at time of data collection (years)	60.47 [15.04]	60.54 [14.68]	66 [11.07]	0.157
Leukocytes at diagnosis (mm^3)	9987 [4818]	10469 [6159]	21811 [17176]	0.001
Hgb at diagnosis (g/dL)	12.76 [3.11]	12.87 [2.64]	12.3 [2.35]	0.635
Hct at diagnosis (%)	38.14 [8.61]	38.35 [8.14]	37.74 [7.95]	0.941
Platelets at diagnosis (mm^3)	833860 [524500]	667590 [400200]	560370 [356800]	0.011
LDH at diagnosis (U/L)	475.1 [266.7]	561.6 [301.8]	792.8 [473.6]	0.001
Spleen size at diagnosis (mm)	140.39 [33.26]	158.78 [45.75]	195.33 [56.64]	0.001
Follow-up duration (months)	90.18 [65.68]	93.64 [68.62]	71.26 [58.4]	0.266
Risk factors for cardiovascular diseases	62 (66.7%)	78 (72.2%)	21 (77.8%)	0.472
Bleeding	8 (8.6%)	18 (16.7%)	8 (29.6%)	0.02
Thrombosis	27 (29%)	35 (32.4%)	6 (22.2%)	0.572
Leukemic transformation	3 (3.3%)	4 (3.7%)	1 (3.7%)	0.982
Death	12 (12.9%)	26 (24.1%)	12 (44.4%)	0.002

Ph: Philadelphia chromosome, SD: standard deviation, Hgb: hemoglobin, Hct: hematocrit, LDH: lactate dehydrogenase.

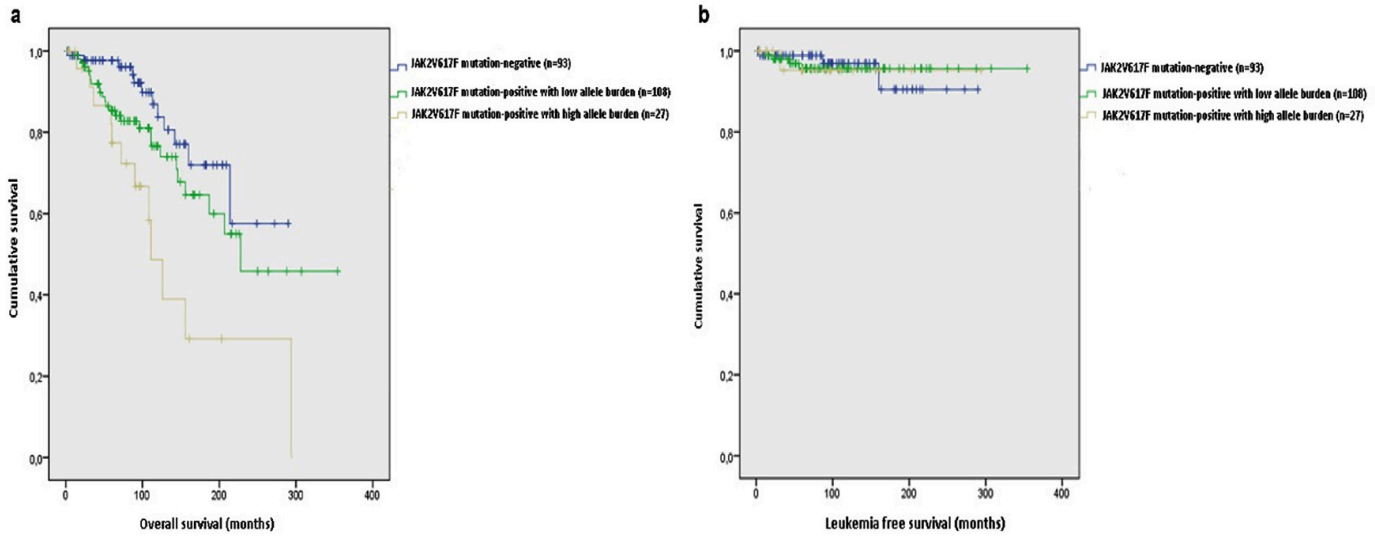

Figure 1. Overall survival and leukemia-free survival among patients with Ph-negative myeloproliferative neoplasms (n=228). a) Kaplan-Meier plot showing survival in Ph-negative myeloproliferative neoplasm cases stratified by *JAK2V617F* allele burden. Patients with high mutation loads had inferior overall survival compared to patients with low mutation loads and wild-type *JAK2V617F* (mean: 151 months, 95% confidence interval: 62-153; mean: 232 months, 95% confidence interval: 193-271; mean: 229 months, 95% confidence interval: 198-260, respectively; p=0.002). b) Comparison of LFS in patients with wild-type *JAK2V617F* and low and high *JAK2V617F* allele burden quartiles (mean: 274 months, 95% confidence interval: 256-293; mean: 340 months, 95% confidence interval: 326-353; mean: 281 months, 95% confidence interval: 258-305, respectively; p=0.979).

Table 2. Clinical and laboratory characteristics of patients with essential thrombocythemia (ET) according to *JAK2V617F* allele burden data (n=118).

ET	*JAK2* wild-type (mean [SD])	Low *JAK2* allele burden (≤50%) (mean [SD])	High *JAK2* allele burden (>50%) (mean [SD])	p
Number of patients	54	59	5	-
Female patients (%)	26 (48.1%)	34 (57.6%)	4 (80%)	0.299
Age at diagnosis (years)	52.13 [15.47]	49.71 [14.63]	53.2 [19.07]	0.679
Age at time of data collection (years)	60.48 [14.99]	59.9 [14.54]	63 [19.85]	0.829
Leukocytes at diagnosis (mm^3)	9897 [3355]	9848 [3666]	14240 [7279]	0.317
Hgb at diagnosis (g/dL)	12.46 [1.97]	13.95 [1.6]	11.16 [0.93]	0.001
Hct at diagnosis (%)	37.2 [5.44]	41.49 [4.85]	34.8 [4.76]	0.001
Platelets at diagnosis (mm^3)	1058630 [478690]	883951 [320372]	798600 [312966]	0.061
LDH at diagnosis (U/L)	423.6 [171.3]	426.5 [128.8]	718.2 [178.6]	0.008
Spleen size at diagnosis (mm)	129.79 [21.97]	136.78 [28.1]	181 [74]	0.034
Follow-up duration (months)	97.54 [69.52]	116.32 [67.64]	83.6 [89.92]	0.27
Risk factors for cardiovascular diseases	35 (64.8%)	46 (78%)	5 (100%)	0.110
Bleeding	4 (8.6%)	6 (10.2%)	3 (60%)	0.002
Thrombosis	19 (35.2%)	25 (42.4%)	2 (40%)	0.735
Myelofibrotic transformation	4 (7.4%)	3 (5.1%)	1 (20%)	0.431
Leukemic transformation	2 (3.7%)	0	0	0.303
Death	5 (9.3%)	8 (13.6%)	3 (60%)	0.007

SD: Standard deviation, Hgb: Hemoglobin, Hct: hematocrit, LDH: lactate dehydrogenase.

Kaplan-Meier Survival Curves of Essential Thrombocythemia Patients According to *JAK2V617F* Allele Burden Data

JAK2V617F mutation-negative ET patients and *JAK2V617F*-positive patients with mutant allele burdens in the lower quartile and upper quartile ranges showed no significant differences in OS (mean: 252 months, 95% confidence interval: 221-282; mean: 250 months, 95% confidence interval: 217-284; mean: 187 months, 95% confidence interval: 79-295, respectively; p=0.266).

Comparison of Primary Myelofibrosis Patients According to *JAK2V617F* Allele Burden Data

Women were significantly more frequent among the patients with the *JAK2* wild-type mutation compared to the other two groups (p=0.005) (Table 3). PMF patients with high mutation burdens were significantly more likely to have higher leukocyte counts at diagnosis (p=0.001). Mean spleen size was also larger in PMF patients with high mutation burdens compared to the other two groups (p=0.018).

Kaplan-Meier Survival Curves of Primary Myelofibrosis Patients According to *JAK2V617F* Allele Burden Data

OS did not differ among the *JAK2V617F* mutation-negative PMF patients, *JAK2V617F*-positive patients with low allele burdens, and *JAK2V617F*-positive patients with high allele burdens (mean: 162 months, 95% confidence interval: 111-213; mean: 132 months, 95% confidence interval: 76-190; mean: 117 months, 95% confidence interval: 81-153, respectively; p=0.266). LFS showed no difference between the three groups (mean: 239

Table 3. Clinical and laboratory characteristics of patients with primary myelofibrosis (PMF) according to *JAK2V617F* allele burden data (n=84).

PMF	*JAK2* wild-type (mean [SD])	Low *JAK2* allele burden (≤50%) (mean [SD])	High *JAK2* allele burden (>50%) (mean [SD])	p
Number of patients	25	41	18	-
Female patients (%)	21 (84%)	19 (46.3%)	8 (44.4%)	0.005
Age at diagnosis (years)	52.88 [15.69]	56.76 [15.01]	61.22 [9.5]	0.168
Age at time of data collection (years)	60 [14.91]	62.6 [15.32]	68.3 [7.7]	0.136
Leukocytes at diagnosis (mm^3)	10337 [7771]	11572 [8906]	26216 [19374]	0.001
Hgb at diagnosis (g/dL)	10.54 [2.59]	10.82 [2.46]	11.76 [1.93]	0.241
Hct at diagnosis (%)	32.53 [8.01]	32.18 [7.9]	35.5 [6.11]	0.289
Platelets at diagnosis (mm^3)	574904 [415328]	385368 [341131]	526778 [367514]	0.089
LDH at diagnosis (U/L)	674.7 [376.4]	798.3 [352.9]	921.5 [508.8]	0.222
Spleen size at diagnosis (mm)	171 [40.5]	195 [44]	213 [46]	0.018
Risk factors for cardiovascular diseases	13 (52%)	26 (63.4%)	12 (66.7%)	0.556
Bleeding	2 (8%)	11 (26.8%)	5 (27.8%)	0.152
Thrombosis	4 (16%)	8 (19.5%)	2 (11.1%)	0.726
Leukemic transformation	1 (4%)	4 (9.8%)	1 (5.6%)	0.653
Follow-up duration (months)	75.36 [55.33]	65.1 [61.98]	76.38 [52.26]	0.408
Death	6 (24%)	18 (43.9%)	9 (50%)	0.162

SD: Standard deviation, Hgb: Hemoglobin, Hct: hematocrit, LDH: lactate dehydrogenase.

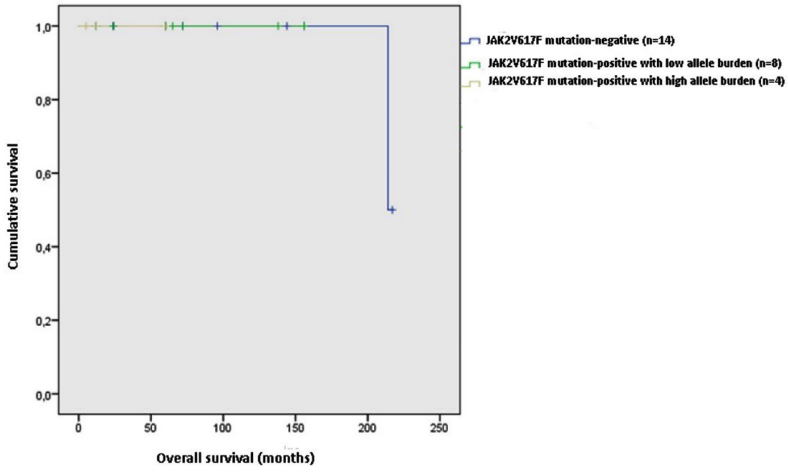

Figure 2. Overall survival for 26 patients with polycythemia vera stratified by wild-type and *JAK2V617F* allele burden quartiles. There was a trend towards inferior overall survival in patients with high mutation loads compared to patients with low mutation loads and patients with no *JAK2V617F* mutation (mean: 46 months, 95% confidence interval: 22-69; mean: 137 months, 95% confidence interval: 102-171; mean: 215 months, 95% confidence interval: 213-217, respectively; p=0.056).

months, 95% confidence interval: 221-258; mean: 308 months, 95% confidence interval: 265-351; mean: 192 months, 95% confidence interval: 172-213, respectively; p=0.538).

Discussion

This study has investigated the impact of *JAK2V617F* allele burden on disease phenotype and complications in a relatively large cohort of patients with Ph-negative MPNs with long follow-up durations. Several studies reported the frequency of the *JAK2V617F* mutation in ET and PMF as 60%-65% [14,15]. The *JAK2V617F* mutation was found to be present in approximately 95% of PV patients [16]. In one systematic review of 52 studies, the frequency of the *JAK2V617F* mutation ranged from 46.7% to 100% in PV, 31.3% to 72.1% in ET, and 25% to 85.7% in PMF cases [17]. These discrepancies in the results for the frequency of the *JAK2V617F* mutation are the result of different methods used to detect the mutation [17]. More recent studies suggest that droplet digital PCR (ddPCR) is a more accurate, sensitive, and reproducible assay with sensitivity of 0.01% compared to real-time quantitative PCR (qPCR) [18,19]. The present study, which used the *JAK2* MutaScreen assay, a qPCR assay with sensitivity of 2% [13], found the *JAK2V617F* mutation to be significantly more frequent in PMF than ET and PV (70.2%, 54.2%, and 46.2%, respectively). Compared to previous reports, we observed a lower frequency of the *JAK2V617F* mutation in PV. The lower frequency of *JAK2V617F* mutation in our PV cases may be attributed to the small number of PV patients included and it does not reflect the real frequency of the mutation in this population. Moreover, since our center is a reference center for MPN patients, the number of confirmed cases of *JAK2V617F*-negative PV may have been higher in our cohort.

Homozygosity for the *JAK2V617F* mutation (mutant allele burden of >50%) is reported to be present in 25%-30% of PV and PMF patients and in 2%-4% of ET patients [4,5,7]. Similar to previous data, the frequency of mutant allele burdens in the upper quartile ranges (allele burdens of >50%) was higher in PMF than ET (21.4% and 4.2%, respectively) in our study. In agreement with the literature, *JAK2V617F*-positive patients with high allele burdens were more common among the PV cases than the ET cases (15.4% and 4.2%, respectively). Consistent with previous data, the frequency of mutant allele burden in the upper quartile ranges was similar between PV and PMF. In our study, quantification of *JAK2V617F* allele burden was performed at variable times during the disease course. Several studies reported that *JAK2V617F* allele burden is relatively constant and remains stable over many years in ET and PMF [20,21,22]. In PV, however, it was reported that the incidence of homozygosity for the *JAK2V617F* mutation increases during the disease course [23]. The difference in the frequency of high mutation loads observed in our PV patients may be attributed to the possibility of performing tests for mutation load at variable times throughout the disease course and the small number of PV patients.

Previous studies proposed different cut-off values for *JAK2V617F* allele load for the stratification of ET cases [2,4,5,7,8,24,25,26,27,28]. In our study, ET patients with high mutation loads of >50% showed lower hemoglobin levels, in line with some studies yet in contrast with most previous data [5,7,8,27,28]. In agreement with some previous studies but contrary to some others [4,5,7,8,27,28], we found no association with high mutation load or leukocyte count. We observed a trend towards lower platelet counts in ET patients with high mutation loads in line with the report published by Pich et al. [27] yet contrary to most previous data. We found higher LDH levels in ET patients with high mutation loads, consistent with previous reports [27,28]. Confirming previous observations [4,7,24,26,27,28], our ET patients with high mutation loads had a larger mean spleen size. Contrary to some previous reports yet consistent with the findings of previous Turkish research [28], the prevalence of major thrombotic events did not significantly differ among our ET patients according to *JAK2* allele burden. As opposed to some previous reports yet in agreement with the study by Vannucchi et al. [4], our ET patients with high *JAK2V617F* allele burdens had more prevalent bleeding events. In contrast to Vannucchi et al. [4], however, there was no correlation between *JAK2V617F* allele burden and the presence of cardiovascular risk factors in our ET patients. Our study did not confirm the results of another group of investigators who reported a significant association between high *JAK2V617F* allele burden and myelofibrotic transformation in ET [4,29].

Different cut-off values for *JAK2V617F* allele load have been proposed to classify PMF patients [8,9,10,11,28,30,31,32]. In line with some previous reports but contrary to others [9,11,28,30], our study demonstrated no correlation between age and mutant allele burden. Contrary to some previous reports [11,30], we found an association between male gender and high mutation load in PMF. Consistent with previous data [8,9,10,11,28,30,32], however, our PMF patients with high mutation loads had higher leukocyte counts. As opposed to some previous reports yet consistent with others [8,10], we observed no difference in hemoglobin levels in PMF subgroups according to *JAK2* mutation load. In contrast to a previous study, we found no correlation between hematocrit levels and mutation load in PMF. Consistent with previous reports yet in contrast to the study by Barosi et al. [9], our study showed no impact of the *JAK2V617F* allele burden on platelet counts in PMF. In accordance with previous data [9,11,28,30], we found no significant correlation between LDH levels and *JAK2* mutation loads in PMF. In line with some previous studies yet in contrast to the observations of Wang et al. [30], PMF patients with high mutation loads had larger spleen sizes. Consistent with a previous study [28], the presence of cardiovascular risk factors did not differ among PMF patients

according to *JAK2* allele load. Confirming previous observations [10,28], PMF patients stratified according to *JAK2* allele burden data did not show any differences in terms of the prevalence of thrombotic events and bleeding.

Previous studies have proposed different cut-off values for *JAK2V617F* allele load to classify Ph-negative MPNs [3,6,31]. Consistent with a previous study [6], higher LDH levels were observed in our patients with high allele burdens. In contrast to the report by Bertozzi et al. [31], however, we detected no correlation between age and *JAK2* allele burden. In our study, in line, with an observation by Bertozzi et al. [31], Ph-negative MPN with high allele burden was associated with higher leukocyte counts. In our study, in contrast to the study by Bertozzi et al. [31], there was no correlation between hematocrit level and mutation load. In our study, Ph-negative MPN patients with high allele burdens displayed lower platelet counts as opposed to the study by Bertozzi et al. [31]. Contrary to some previous reports [3,31], high allele burdens of *JAK2V617F* were not associated with thrombosis. In agreement with the study by Bertozzi et al. [31], we found a higher percentage of bleeding events among patients with high allele burdens.

Few previous reports have highlighted the impact of *JAK2V617F* allele burden on outcomes in PV, ET, PMF, and entire cohorts of Ph-negative MPN patients [7,9,10,11,31,33,34]. Hjelmgren et al. [33] reported that PV patients with *JAK2* allele burdens of >50% had inferior OS compared to those with burdens of ≤50%. In agreement with the aforementioned report, there was a trend towards inferior OS in our PV patients with high mutation loads. Zhao et al. [7] reported that *JAK2V617F* allele burden did not affect OS in ET patients. In one previous Turkish study, ET patients showed no difference in OS following stratification according to *JAK2* allele burden data [34]. Similarly, in our ET patients, *JAK2V617F* allele burden did not affect OS. In our study, the mortality rate was higher among ET patients with high mutation loads, but the rate of leukemic transformation did not differ between the groups. This higher mortality rate may be attributed to the increased rate of bleeding complications in our ET patients with high mutation loads. In the study by Tefferi et al. [10], OS and LFS were shorter for the lower quartile compared to *V617F*-negative patients and those in the middle and upper quartiles. Similarly, Guglielmelli et al. [11] reported shorter OS for PMF patients in the lower quartile. In the same study, the mortality rate of patients in the lower quartile was significantly higher than that of patients in the upper quartiles [11]. In that study, *JAK2V617F* allele burden quartiles showed no significant differences for the percentage of leukemic transformation or LFS [11]. In the present study, PMF patients stratified according to *JAK2V617F* allele burden showed no significant differences in terms of leukemic transformation or mortality. Our findings are contrary to those of Barosi et al. [9], who reported increased risks of leukemic transformation and death for *JAK2V617F* mutation-positive patients. In contrast to some previous data yet consistent with an earlier Turkish study [10,11,34], OS was similar for PMF patients stratified by *JAK2V617F* allele burden quartiles. In line with several studies yet contrary to the study by Tefferi et al. [10], our PMF patients stratified according to *JAK2* allele burden showed no difference in LFS. To our knowledge, only one previous study investigated the impact of *JAK2* allele burden on mortality rate and OS in a total cohort of Ph-negative MPN patients [31]. In that study, allele burden was found to influence OS and patients in the highest quartile were shown to have a significantly higher mortality rate compared to the lower quartiles [31]. In our study, the total cohort showed no differences in LFS or the rate of leukemic transformation when stratified by *JAK2V617F* allele burden data. In line with the findings of Bertozzi et al. [31], the mortality rate was higher and OS was inferior in our patients with high mutation loads. Reduced OS in our Ph-negative MPN patients with high mutation loads may be attributed to the higher rate of bleeding complications in this subgroup.

Study Limitations

The current study has some limitations to be acknowledged. First, the study design was retrospective. Second, the number of PV patients included was limited. The third limitation concerns the characteristics of the PV population. Our PV population included the patients of a reference center; therefore, the number of confirmed cases of *JAK2V617F*-negative PV may be higher than usual. Thus, our PV population can be considered biased and our results cannot be extended to the entire population of PV patients. Different methods used to quantify the *JAK2V617F* mutation may have also accounted for the heterogeneous results obtained. Generally speaking, it is difficult to obtain an accurate study population, a problem that all studies of *JAK2V617F* allele burden in Ph-negative MPNs may encounter. In the present study, the *JAK2V617F* allele burden, which was reported to differ over time, especially for PV patients, was measured at variable times during the disease course. Differences in the stratification of mutational loads among various studies probably reflect ambiguous results in terms of the phenotypic characteristics of PV, ET, PMF, and entire cohorts of Ph-negative MPN patients. High-risk molecular mutations (*ASXL1*, *SRSF2*, *IDH1/2*, *EZH2*) and their associations with *JAK2V617F* allele burden were not analyzed in our PMF patients; this will be the subject of a future study.

Conclusion

Our results suggested that in a small series of PV patients, high *JAK2V617F* allele burden was associated with more severe disease with a trend towards inferior OS. In ET, our findings indicated that a high *JAK2V617F* allele burden was associated with more severe disease with lower hemoglobin and hematocrit levels,

higher LDH levels, larger spleen size, and increased bleeding and mortality rates. Moreover, in our study, ET patients with high allele burdens displayed a trend towards lower platelet counts. In PMF, our results suggested that high *JAK2V617F* allele burden was associated with a more pronounced myeloproliferative and severe phenotype with higher leukocyte counts and larger spleen size. In this study, conducted with a large number of Ph-negative MPN patients, our findings suggested that high allele burdens are associated with more aggressive disease phenotypes with higher leukocyte and lower platelet counts, higher LDH levels, larger spleen size, higher percentages of bleeding events, higher mortality rates, and inferior OS.

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Ethics

Ethics Committee Approval: This study was approved by the local ethics committee (study number 1284-07/11/2019).

Informed Consent: All participants provided written informed consent.

Authorship Contributions

Design- İ.Y.H.; Data Collection or Processing- İ.Y.H., A.D.A.; Analysis or Interpretation- E.Ş., F.H.; Writing- İ.Y.H., E.Ş., F.H., A.D.A., M.N.

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