RESEARCH ARTICLE

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Impact of *CALR* and *JAK2V*617F Mutations on Clinical Course and Disease Outcomes in Essential Thrombocythemia: A Multicenter Retrospective Study in Turkish Patients

Esansiyel Trombositemide *CALR* ve *JAK2V*617F Mutasyonlarının Klinik Seyir ve Hastalık Sonuçlarına Etkisi: Türk Hastalarda Geriye Dönük Çok Merkezli Çalışma

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Abstract

Objective: In this study, we investigated the effects of calreticulin (*CALR*) and *JAK2*V617F mutational status on clinical course and disease outcomes in Turkish patients with essential thrombocythemia (ET).

Materials and Methods: Seventeen centers from Türkiye participated in the study and *CALR*- and *JAK2*V617F-mutated ET patients were evaluated retrospectively.

Results: A total of 302 patients were included, of whom 203 (67.2%) and 99 (32.8%) were *JAK2*V617F- and *CALR*-positive, respectively. *CALR*-mutated patients were significantly younger (51 years vs. 57.5 years, p=0.03), with higher median platelet counts ($987x10^9$/L vs. $709x10^9$/L, p<0.001) and lower median hemoglobin levels (13.1 g/dL vs. 14.1 g/dL, p<0.001) compared to *JAK2*V617F-mutated patients. Thromboembolic events (TEEs) occurred in 54 patients (17.9%), 77.8%

Öz

Amaç: Bu çalışmada Türk esansiyel trombositemi (ET) hastalarında *CALR* ve *JAK2*V617F mutasyon durumunun klinik seyir ve hastalık sonuçlarına etkilerini araştırdık.

Gereç ve Yöntemler: Çalışmaya Türkiye'den 17 merkez katılmış olup, *CALR* ve *JAK2*V617F mutasyonu pozitif olan ET hastaları geriye dönük olarak değerlendirilmiştir.

Bulgular: Çalışmaya toplam 302 hasta dahil edildi. Bunların 203'ü (%67,2) *JAK2*V617F ve 99'u (%32,8) *CALR* pozitifti. *CALR* mutasyonlu hastalar *JAK2*V617F pozitif olanlara göre daha gençti (sırasıyla; 51 yaş, 57,5 yaş, p=0,03), daha yüksek ortanca trombosit sayısına (sırasıyla; $987x10^9$/L, $709x10^9$/L, p<0,001) ve daha düşük ortanca hemoglobin düzeylerine (sırasıyla; 13,1 g/dL, 14,1 g/dL, p<0,001) sahipti. Tromboembolik olaylar (TEO) 54 hastada (%17,9) meydana geldi ve bunların %77,8'i arteriyeldi. *CALR* mutasyonu ile karşılaştırıldığında

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Abstract

of which were arterial. Compared to *CALR* mutation, *JAK2*V617F was associated with a higher risk of thrombosis (8.1% vs. 22.7%, p=0.002). Rates of transformation to myelofibrosis (MF) and leukemia were 4% and 0.7%, respectively, and these rates were comparable between *JAK2*V617F- and *CALR*-mutated cases. The estimated overall survival (OS) and MF-free survival of the entire cohort were 265.1 months and 235.7 months, respectively. OS and MF-free survival durations were similar between *JAK2*V617F- and *CALR*-mutated patients. Thrombosis-free survival (TFS) was superior in *CALR*-mutated patients compared to *JAK2*V617F-positive patients (5-year TFS: 90% vs. 71%, respectively; p=0.001). Age at diagnosis was an independent factor affecting the incidence of TEEs.

Conclusion: In our ET cohort, *CALR* mutations resulted in higher platelet counts and lower hemoglobin levels than *JAK2*V617F and were associated with younger age at diagnosis. *JAK2*V617F was strongly associated with thrombosis and worse TFS. Hydroxyurea was the most preferred cytoreductive agent for patients with high thrombosis risk.

Keywords: *CALR* mutation, Essential thrombocythemia, *JAK2*V617F mutation, Myeloproliferative neoplasm

Öz

JAK2 V617F daha yüksek tromboz riski ile ilişkiliydi (%8,1'e karşı %22,7, p=0,002). Miyelofibroz (MF) ve lösemiye dönüşüm oranları sırasıyla %4 ve %0,7 idi ve bu oranlar *JAK2*V617F ve *CALR* mutasyonlu olgular arasında benzerdi. Tüm kohortta tahmini toplam sağkalım (OS) ve MF'siz sağkalım sırasıyla 265,1 ay ve 235,7 aydı. *JAK2*V617F ve *CALR* mutasyonlu hastalar arasında OS ve MF'siz sağkalım benzerdi. *CALR* mutasyonlu vakalarda trombozsuz sağkalım (TFS), *JAK2*V617F pozitif hastalara göre daha üstündü (5 yıllık TFS sırasıyla; %90, %71 [p=0,001]). Tanı yaşı TEO insidansını etkileyen bağımsız bir faktördü.

Sonuç: ET kohortumuzda *CALR* mutasyonları, *JAK2*V617F'ye göre daha yüksek trombosit sayısı, daha düşük hemoglobin düzeyi ve tanı anında daha genç yaşla ilişki bulundu. *JAK2*V617F, tromboz ve daha kötü TFS ile güçlü bir şekilde ilişkiliydi. Hidroksiüre yüksek tromboz riski olan hastalarda en çok tercih edilen sitoredüktif ilaçtı.

Anahtar Sözcükler: *CALR* mutasyonu, Esansiyel trombositemi, *JAK2*V617F mutasyonu, Miyeloproliferatif neoplazm

Introduction

Essential thrombocythemia (ET) is a Philadelphia-negative myeloproliferative neoplasm (MPN) characterized by high platelet counts due to the clonal proliferation of the megakaryocytic lineage within the bone marrow. Patients with ET may display molecular markers such as Janus kinase 2 (*JAK2*; 9p24), calreticulin (*CALR*; 19p13.2), or myeloproliferative leukemia virus (*MPL* oncogene; 1p34) in a mutually exclusive manner [1,2]. The valine-to-phenylalanine (V617F) alteration constitutively activates *JAK2*, resulting in overproduction of myeloid cells. The *JAK2*V617F mutation is present in approximately 55% of patients with ET, 25% of patients with *CALR*, and 3% of patients with *MPL* [1,3]. The statuses of these driver mutations are relevant not only for their diagnostic contribution but also for their prognostic significance [4].

Frameshift mutations in the *CALR* gene encoding molecular chaperones in the endoplasmic reticulum are the second most common somatic mutation in ET. Two mutations of the *CALR* gene, type 1 (c.1092_1143del; L367fs*46) and type 2 (c.1154_1155insTTGTC; K385fs*47), represent more than 80% of *CALR* mutations [1,2,5]. The mutant CALR protein interacts with the thrombopoietin receptor, MPL, via its extracellular domain, activating the downstream JAK-STAT pathway and resulting in cytokine-independent growth [6,7].

In patients with ET, the presence of *JAK2*V617F is associated with an increased risk of thrombosis and a lower risk of post-ET myelofibrosis (MF) [8]. Compared to *JAK2*V617F, mutant *CALR* is associated with younger age, male sex, higher platelet count, lower hemoglobin level, lower leukocyte count, and lower incidence of thrombotic events. In addition, patients with the type 2 *CALR* mutation tend to have higher platelet counts than patients with type 1 [9,10].

Previous studies have shown that in patients with ET, median survival is approximately 20 years [11,12], although life expectancy in ET is inferior to that of the general population, regardless of mutational status [12]. Young ET patients have clearly longer survival compared to their older counterparts, which requires appropriate action during patient management [13]. Risk factors for survival in ET are advanced age, leukocytosis, and thrombosis history. Mutational status does not seem to affect survival in patients with ET [14].

The prevalence of *JAK2*V617F and *CALR* mutations in Turkish patients with ET and the relationships of these driver mutations with clinical outcomes remain undetermined. In this study, we aim to investigate the effects of *CALR* and *JAK2*V617F mutation status on the clinical course and disease outcomes of Turkish ET patients.

Materials and Methods

*JAK2*V617F- and *CALR*-mutated ET patients aged ≥18 years were included in the study. *MPL*-mutated and triple-negative ET patients were excluded. Demographic data, clinical and laboratory characteristics, treatment modalities, and disease outcomes were evaluated retrospectively. Bone marrow

aspiration and biopsy were performed for all patients to exclude those with pre-fibrotic MF according to the 2016 revision of the World Health Organization's classification of myeloid neoplasms and acute leukemia [15].

*JAK2*V617F mutations were detected by quantitative polymerase chain reaction and *CALR* mutations were detected by next-generation sequencing of *CALR* exon 9. The 52-bp deletion (p.L367fs*46) was defined as *CALR* type 1 and the 5-bp TTGTC insertion (p.K385fs*47) as *CALR* type 2, while the others were grouped as "other." *CALR* subgroup analyses were performed, excluding patients with unknown *CALR* type.

At diagnosis, the International Prognostic Score of Thrombosis in Essential Thrombocythemia (IPSET-thrombosis) and the revised IPSET-thrombosis were used to define the risk of thromboembolic events (TEEs). The revised IPSET-thrombosis defines four risk categories according to three adverse variables (thrombosis history, age >60 years, and *JAK2*V617F): very low (no adverse features), low (presence of *JAK2*V617F), intermediate (age >60 years), and high (presence of thrombosis history or presence of both advanced age and *JAK2*V617F) [3,16]. Deep vein thrombosis (DVT), pulmonary thromboembolism (PTE), pulmonary arterial hypertension (PAH) due to chronic PTE, and TEEs occurring in any venous vessel were considered as venous-type TEEs. Myocardial infarction (MI), angina pectoris (AP), transient ischemic attack, ischemic stroke or cerebrovascular accident (CVA), thrombosis in the carotid artery, peripheral arterial occlusive disease, and TEEs in any arterial vessel were classified as the arterial type.

All procedures were performed in accordance with the ethical standards of the relevant institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Ethical approval was obtained from the Local Ethics Committee of İzmir Bozyaka Training and Research Hospital with approval number 2022/127 on August 10, 2022.

Statistical Analysis

IBM SPSS Statistics 22 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. The conformity of the variables to normal distribution was examined using visual (histogram and probability graphs) and analytical (Kolmogorov-Smirnov/Shapiro-Wilk test) methods. Descriptive statistics were presented as mean ± standard deviation, median (minimum-maximum), frequency distribution, and percentage. Comparisons between groups were made by chi-square, Fisher exact, and Mann-Whitney U tests as appropriate. Two major outcomes were assessed accordingly: overall survival (OS) and MF-free survival, calculated from the time of development of fibrosis in the bone marrow. Survival estimations were assessed by Kaplan-Meier method and the log-rank test was used for comparisons of survival distributions among groups. Thrombosis-free survival (TFS) was calculated from the date of diagnosis to date of thrombosis (uncensored) or last contact (censored). A Cox proportional hazard regression model was used for multivariable analysis. Values of $p<0.05$ were considered statistically significant.

In a one-way analysis of variance (ANOVA) study, sample sizes of 53, 33, and 9 were obtained from the 3 *CALR*-positive groups whose means were to be compared. According to the one-way ANOVA test, the total sample of 95 subjects achieved 100% power to detect differences among the means versus the alternative of equal means using an F-test with a 0.05000-significance level.

Results

Entire Patient Cohort

Patients with ET from 17 centers in Türkiye, diagnosed between January 1999 and July 2022, were included in the study. A total of 302 patients, 203 of whom had *JAK2*V617F-mutated ET and 99 of whom had *CALR*-mutated ET, were analyzed. While 174 (57.6%) of the patients were female, 128 (42.4%) were male. The median age was 55 years (range: 20-88 years). Median follow-up was 41.7 months (range: 0.17-278.3 months). Two hundred and three (67.2%) and 99 (32.8%) patients were *JAK2*V617F- and *CALR*-positive, respectively. At the time of diagnosis, 24.8% (n=74) of the patients had splenomegaly and 4 patients (1.3%) had splenectomy secondary to trauma. Median platelet count was $784x10^9$/L (range: $452-2635x10^9$/L), median hemoglobin level was 13.8 g/dL (range: 8.2-17.8 g/dL), and median leukocyte count was $9.6x10^9$/L (range: $3.4-33.4x10^9$/L) (Table 1).

According to the IPSET-thrombosis risk score, 84 (27.8%) patients were in the low-risk group, 81 (26.8%) patients were in the intermediate-risk group, and 137 (45.4%) patients were in the high-risk group. When we evaluated the revised IPSET-thrombosis risk score, 70 (23.2%) patients were in the very low-risk group, 99 (32.8%) were in the low-risk group, 25 (8.3%) were in the intermediate-risk group, and 108 (35.7%) were in the high-risk group.

Including the period before diagnosis, the time of diagnosis, and follow-up, TEEs occurred in 17.9% (n=54) of the patients, 77.8% of which were in arterial sites, while 14.8% were in venous sites and 7.4% were in both venous and arterial sites. Thirty-two patients (59.3%) had MI or AP, 2 patients (3.7%) experienced both MI and PTE, and thrombosis in the carotid artery occurred in 2 cases (3.7%). While 2 patients (3.7%) had both CVA and PAH due to chronic PTE, 7 (13%) had CVA. DVT occurred in 4 cases (7.4%), cerebral venous sinus thrombosis in 3 cases (5.6%), PTE in 1 case (1.9%), and portal vein thrombosis in 1 case (1.9%).

During the follow-up of 258 patients with no history of TEEs prior to or at the time of diagnosis, the TEE rate was 3.9%

Table 1. Demographic and biological features of patients with essential thrombocythemia.

	All patients, n=302 (100%)	*JAK2*V617F-positive, n=203 (67.2%)	*CALR*-positive, n= 99 (32.8%)	p
Median age (years)	55 (20-88)	57.5 (20-87)	51 (20-88)	0.03
Median follow-up time (months)	41.7 (0.17-278.3)	40.9 (0.47-240.3)	48.6 (0.17-278.3)	0.07
Sex				
Female	174 (57.6%)	120 (59.1%)	54 (54.5%)	0.4
Male	128 (42.4%)	83 (40.9%)	45 (45.5%)	
Splenomegaly				
Yes	75 (24.8%)	52 (25.6%)	23 (23.2%)	0.7
No	223 (73.8%)	149 (73.4%)	74 (74.7%)	
Splenectomy	4 (1.3%)	2 (1.0%)	2 (2.0%)	
At diagnosis (median)				
PLT (10^9/L)	784 (304-2635)	709 (304-2635)	987 (343-2449)	**<0.001**
Hb (g/dL)	13.8 (8.2-17.8)	14.1 (8.3-17.8)	13.1 (8.2-17.2)	**<0.001**
WBC (10^9/L)	9.6 (3.4-33.4)	9.8 (3.9-33.3)	8.9 (3.4-20.6)	0.06
Thromboembolic events				
Yes	54 (17.9%)	46 (22.7%)	8 (8.1%)	**0.002**
No	248 (82.1%)	157 (77.3%)	91 (91.9%)	
Type of thrombosis				
Arterial	42 (77.8%)	35 (76.0%)	7 (87.5%)	0.7
Venous	8 (14.8%)	7 (15.2%)	1 (12.5%)	
Venous and arterial	4 (7.4%)	4 (8.8%)	0 (0%)	
Comorbidities				
None	136 (45.0%)	84 (41.4%)	52 (52.5%)	0.2
CVD and/or metabolic	132 (43.7%)	97 (47.8%)	35 (35.4%)	
Neuropsychiatric	13 (4.3%)	9 (4.4%)	4 (4.0%)	
Others	21 (7.0%)	13 (6.4%)	8 (8.1%)	
IPSET-thrombosis				
Low	84 (27.8%)	0 (0%)	84 (84.8%)	**<0.001**
Intermediate	81 (26.8%)	70 (34.5%)	11 (11.1%)	
High	137 (45.4%)	133 (65.5%)	4 (4.0%)	
Revised IPSET-thrombosis				
Very low	70 (23.2%)	0 (0%)	70 (70.7%)	**<0.001**
Low	99 (32.8%)	99 (48.8%)	0 (0%)	
Intermediate	25 (8.3%)	0 (0%)	25 (25.3%)	
High	108 (35.7%)	104 (51.2%)	4 (4.0%)	
Treatments				
Only ASA	67 (22.2%)	51 (25.1%)	16 (16.2%)	**<0.001**
HU	178 (58.9%)	130 (64.0%)	48 (48.5%)	
Anagrelide	24 (7.9%)	8 (3.9%)	16 (16.2%)	
HU + anagrelide	17 (5.6%)	7 (3.4%)	10 (10.1%)	
IFN-α	16 (5.3%)	7 (3.4%)	9 (9.1%)	
MF transformation				
Yes	12 (4.0%)	8 (3.9%)	4 (4.0%)	0.9
No	290 (96.0%)	195 (96.1%)	95 (96.0%)	
Leukemic transformation				
Yes	2 (0.7%)	1 (0.5%)	1 (1.0%)	0.6
No	300 (99.3%)	202 (99.5%)	98 (99.0%)	

JAK2: Janus kinase 2; *CALR:* calreticulin; PLT: platelet count; Hb: hemoglobin; WBC: white blood cell count; CVD: cardiovascular disease; IPSET-thrombosis: International Prognostic Score of Thrombosis in Essential Thrombocythemia; ASA: acetylsalicylic acid; HU: hydroxyurea; IFN-α: interferon alpha; MF: myelofibrosis.

(n=10). Six of those patients were *JAK2*V617F-mutated and in the high IPSET-thrombosis risk group at the time of diagnosis, while 4 of them were *CALR*-mutated and in the low-risk group. Multivariate analysis was performed for analyzing the effects of hemoglobin levels, platelet counts, and age at diagnosis on TEE occurrence. Only age was found to be a factor independently affecting the cumulative TEE incidence (p=0.008) (Table 2).

Sixty-five (21.5%) female patients were of reproductive age and a total of 3 pregnancies occurred in 2 of those cases. While 2 of them resulted in delivery with the use of interferon alpha (IFN-α), 1 pregnancy resulted in intrauterine fetal death in the 6th gestational week. Demographic and biological features of patients according to mutational status are presented in Table 1.

Table 2. Multivariate analysis for factors that may influence the incidence of thromboembolic events.

	Thrombosis-free survival	
Variables	**HR (95% CI)**	**p**
Age at diagnosis (≤60 years and >60 years)	0.48 (0.28-0.83)	**0.008**
Hemoglobin level (g/dL)	0.31 (0.02-3.43)	0.34
PLT count (x10^9/L)	1.1 (1.0-2.81)	0.99
WBC count (x10^9/L)	1.0 (0.07-2.12)	0.95

PLT: Platelet; WBC: white blood cell; HR: hazard ratio; CI: confidence interval.

Characteristics and Treatments of the *JAK2*V617F- and *CALR*-Mutated Subgroups

CALR-mutated patients were significantly younger (median age at diagnosis: 51 years [range: 20-88 years] vs. 57.5 years [range: 20-87 years], p=0.03) than patients harboring the *JAK2*V617F mutation. Compared to *JAK2*V617F-mutated cases, patients with *CALR* mutations had higher median platelet counts (987x10^9/L [range: 458-2449x10^9/L] vs. 709x10^9/L [range: 452-2635x10^9/L], p<0.001) and significantly lower hemoglobin levels (13.1 g/dL [range: 8.2-17.2 g/dL] vs. 14.1 g/dL [range: 8.3-17.8 g/dL], p<0.001). The leukocyte counts of patients with *CALR* mutations were lower than those of patients with *JAK2*V617F mutations, but the difference did not reach statistical significance (8.9x10^9/L [range: 3.4-20.6x10^9/L] vs. 9.8x10^9/L [range: 3.9-33.3x10^9/L], p=0.06).

Regarding the IPSET-thrombosis risk stratification, 34.5% of *JAK2*V617F-mutated patients were in the intermediate-risk group and 65.5% in the high-risk group, while 84.8% of the *CALR*-mutated patients were in the low-risk group, 11.1% in the intermediate-risk group, and 4% in the high-risk group (p<0.001). According to the revised IPSET-thrombosis risk score, 48.8% of *JAK2*V617F-mutated patients were in the low-risk group and 51.2% in the high-risk group, while 70.7% of the *CALR*-mutated patients were in the very low-risk group, 25.3% in the intermediate-risk group, and 4% in the high-risk group (p<0.001). Compared to *CALR* mutations, *JAK2*V617F mutations were associated with a higher incidence of TEEs (8.1% vs. 22.7%, p=0.002). The incidence of thrombosis in arterial sites was higher than the incidence of thrombosis in venous sites in both groups, although the difference was not statistically significant (Table 1).

All patients received acetylsalicylic acid (ASA) for thromboprophylaxis. The percentage of patients treated with ASA alone was significantly higher in the *JAK2*V617F-mutated group compared to patients with *CALR* mutations (25.1% vs. 16.2%, p<0.001). Similarly, hydroxyurea (HU) therapy was found to be more commonly used for patients with *JAK2*V617F than *CALR*-mutated patients (64% vs. 48.5%, p<0.001). Other treatment modalities including anagrelide monotherapy, IFN-α, and the combination of HU and anagrelide were more common among *CALR*-mutated patients than patients harboring *JAK2*V617F (p<0.001) (Table 1).

There was no difference between the two groups in terms of median follow-up duration, sex distribution, splenomegaly rate, median leukocyte count, comorbidities, or rates of MF progression and leukemia (Table 1).

Patients with *CALR* Mutations

CALR-mutated patients were further analyzed according to subgroups as type 1, type 2, and others. There was no difference between cases of type 1, type 2, and other *CALR* mutations in terms of sex, splenomegaly, hemoglobin, platelet and leukocyte counts, comorbidities, IPSET-thrombosis score, revised IPSET-thrombosis score, treatment modalities, incidence of thrombotic events, or leukemic transformation (Table 3).

The median ages of patients with type 1, type 2, and other *CALR* mutations were 54.5, 50, and 37 years, respectively (p=0.005). The rate of progression to MF was higher in the others group than in cases of type 1 and type 2 *CALR* mutations (22.2%, 1.9%, and 0%, respectively; p=0.02). Similarly, the median follow-up of the others group was significantly longer than the median follow-up durations of patients with type 1 and type 2 mutations (68.5, 49.1, and 45 months, respectively; p=0.04) (Table 3).

Survival

Progression to MF was observed in 4% (n=12) of the entire cohort and leukemic transformation occurred in 0.7% (n=2) of the patients. One patient with leukemic transformation harbored a *TP53* mutation with a complex karyotype.

The estimated median OS for the entire cohort was 265.1 months (range: 255.8-274.3 months). There was no difference in OS between *CALR*- and *JAK2*V617F-mutated ET cases (254.9 months [range: 235.1-274.7 months] vs. 234.6 months [range: 228.1-241.2 months], p=0.1) (Figure 1). Estimated MF-free survival was 235.7 months (range: 205.7-265.8 months). Like OS, the MF-free survival duration was also similar between the *CALR*- and *JAK2*V617F-mutated groups (247.5 months [range: 213.9-281.2 months] vs. 199.7 months [range: 165.7-233.7 months], p=0.4) (Figure 1). In *CALR*-mutated patients, OS and MF-free survival were similar between the subgroups established according to *CALR* type (p=0.4 and p=0.2, respectively) (Figure 2). OS and MF-free survival were also similar between men and women (p=0.4 and p=0.06, respectively).

The 2-year and 5-year TFS rates were respectively 93% and 90% in *CALR*-mutated patients, while TFS was 77% and 71% at 2 years and 5 years in *JAK2*V617F-mutated patients, respectively. *CALR*-mutated patients thus had significantly longer TFS

Table 3. Clinical and biological features of patients with *CALR*-mutated essential thrombocythemia.

	All patients, n=95 (100%)	Type 1, n=53 (55.8%)	Type 2, n=33 (34.7%)	Others, n=9 (9.5%)	p
Median age (years)	52 (20-88)	54.5 (24-88)	50 (20-86)	37 (21-51)	**0.005**
Median follow-up time (months)	50.6 (0.2-278.3)	49.1 (0.2-278.3)	45.0 (3.7-161.3)	68.5 (35.4-236.2)	**0.04**
Sex					
Female	52 (54.7%)	28 (52.8%)	21 (63.6%)	3 (33.3%)	0.2
Male	43 (45.3%)	25 (47.2%)	12 (36.4%)	6 (66.7%)	
Splenomegaly					
Yes	22 (23.2%)	11 (20.8%)	6 (18.2%)	4 (44.4%)	
No	71 (74.7%)	40 (75.5%)	27 (81.8%)	5 (55.6%)	0.1
Splenectomy	2 (2.1%)	2 (3.7%)	0 (0%)	0 (0%)	
At diagnosis (median)					
PLT (x10^9/L)	994 (394-2449)	936 (394-2412)	1030 (343-2449)	865 (671-2442)	0.8
Hb (g/dL)	13.0 (8.2-17.2)	12.9 (8.2-16.4)	13.6 (10.2-17.2)	12.4 (10.2-15.0)	0.4
WBC (x10^9/L)	9.0 (3.4-20.6)	9.7 (3.4-18.4)	9 (3.7-15.9)	8.6 (4.2-20.6)	0.5
Thromboembolic events					
Yes	8 (8.4%)	4 (7.5%)	3 (9.1%)	1 (11.2%)	0.8
No	87 (91.6%)	49 (92.5%)	30 (90.9%)	8 (88.9%)	
Type of thrombosis					
Arterial	7 (87.5%)	3 (75.0%)	3 (100%)		
Venous	1 (12.5%)	1 (25.0%)	0 (0%)	-	0.6
Venous and arterial	0 (0%)	0 (0%)	0 (0%)		
Comorbidities					
None	50 (52.6%)	26 (49.1%)	17 (51.5%)	7 (77.8%)	
CVD and/or metabolic	34 (35.8%)	21 (39.6%)	12 (36.4%)	1 (11.1%)	0.7
Neuropsychiatric	5 (5.3%)	2 (3.8%)	2 (6.1%)	1 (11.1%)	
Others	6 (6.3%)	4 (7.5%)	2 (6.1%)	0 (0%)	
IPSET-thrombosis					
Low	80 (84.2%)	44 (83.0%)	27 (81.8%)	9 (100%)	
Intermediate	11 (11.6%)	7 (13.2%)	4 (12.1%)	0 (0%)	0.7
High	4 (4.2%)	2 (3.8%)	2 (6.1%)	0 (0%)	
Revised IPSET-thrombosis					
Very low	67 (70.2%)	34 (63.5%)	24 (72.7%)	9 (100%)	
Low	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0.2
Intermediate	24 (25.5%)	17 (32.7%)	7 (21.2%)	0 (0%)	
High	4 (4.3%)	2 (3.8%)	2 (6.1%)	0 (0%)	
Treatments					
Only ASA	18 (18.9%)	10 (18.9%)	1 (11.1%)	0 (0%)	
HU	45 (47.4%)	29 (54.7%)	2 (22.2%)	2 (28.6%)	0.5
Anagrelide	14 (14.7%)	5 (9.4%)	3 (33.3%)	3 (42.9%)	
HU + anagrelide	9 (9.5%)	4 (7.5%)	1 (11.1%)	1 (14.3%)	
IFN-α	9 (9.5%)	5 (9.4%)	2 (22.2%)	1 (14.3%)	
MF transformation					
Yes	3 (3.2%)	1 (1.9%)	0 (0%)	2 (22.2%)	**0.002**
No	92 (96.8%)	52 (98.1%)	33 (100%)	7 (77.8%)	
Leukemic transformation					
Yes	1 (1.1%)	1 (1.9%)	0 (0%)	0 (0%)	0.5
No	94 (98.9%)	52 (98.1%)	33 (100%)	9 (100%)	

CALR: Calreticulin; PLT: platelet count; Hb: hemoglobin; WBC: white blood cell count; CVD: cardiovascular disease; IPSET-thrombosis: International Prognostic Score of Thrombosis in Essential Thrombocythemia; ASA: acetylsalicylic acid; HU: hydroxyurea; IFN-α: interferon alpha; MF: myelofibrosis.

compared to *JAK2*V617F-mutated patients (p=0.001) (Figure 3).

Discussion

ET is a type of MPN characterized by an increased rate of TEEs, a varying burden of symptoms, and an intrinsic risk of progression to MF and acute leukemia; however, survival is only modestly reduced in most cases. In this study, we have presented the retrospective analysis of 302 patients with ET according to their *JAK2*V617F and *CALR* mutational statuses. Furthermore, we compared the clinical courses and disease outcomes of the *CALR*-mutated patients according to the *CALR* subtypes of type 1, type 2, and others.

There is growing evidence that *CALR*-mutated ET cases are phenotypically different from other molecular types of ET,

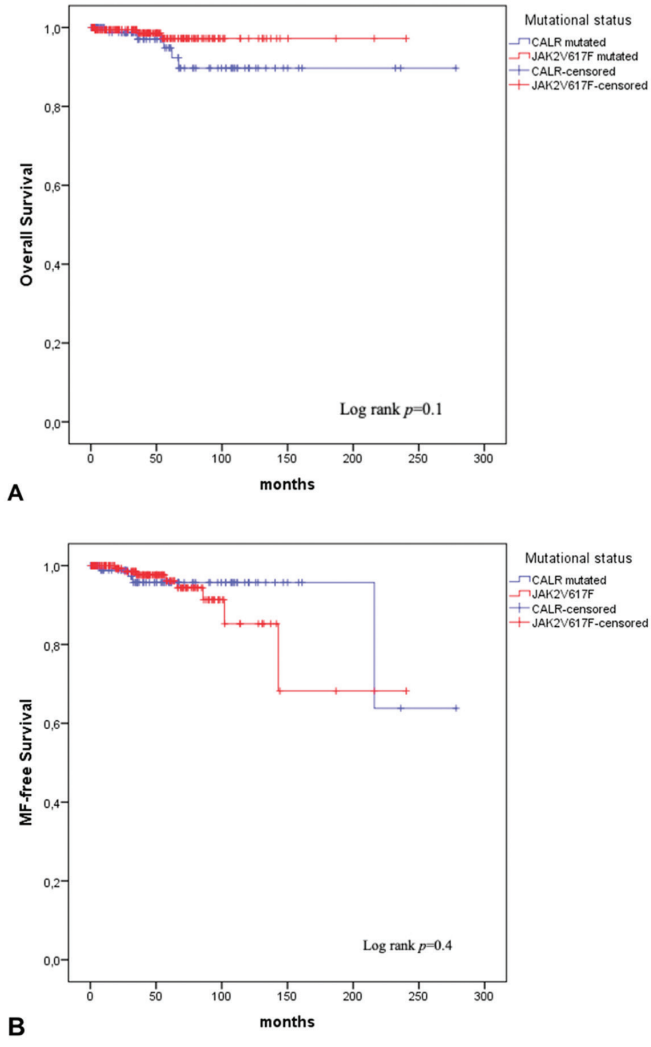

Figure 1. Overall (A) and myelofibrosis-free (B) survival according to the mutational statuses of the patients.

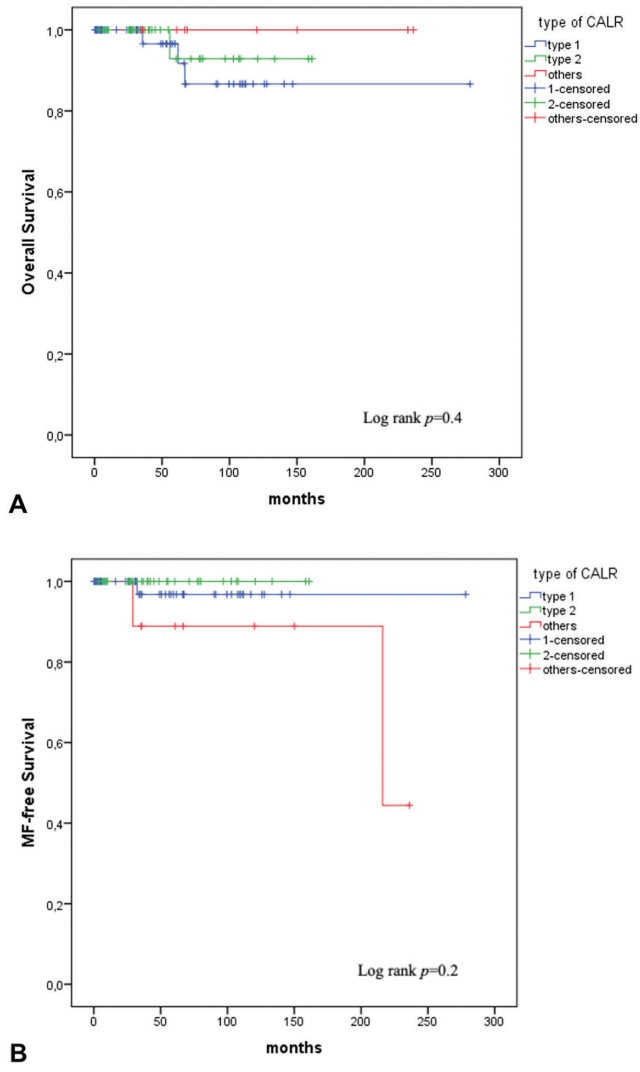

Figure 2. Overall (A) and myelofibrosis (MF)-free (B) survival of *CALR*-mutated patients according to *CALR* subtypes.

including *JAK2*V617F-mutated, *MPL*-mutated, and triple-negative cases, in terms of both clinical and hematological presentations and survival outcomes [17,18,19,20]. Moreover, Alvarez-Larrán et al. [17] underlined the need for new treatment modalities for *CALR*-mutated ET, arguing that conventional cytoreductive agents are less effective in these patients compared to other ET cases. However, in this study, we compared the outcomes of *CALR*-mutated patients with only those who were *JAK2*V617F-positive. Triple-negative ET is a heterogeneous group of diseases and may harbor additional non-driver mutations that affect disease outcome. For this reason, we excluded triple-negative patients from our study due to the heterogeneous nature of the disease and we also excluded *MPL* cases due to their low frequency.

Nangalia et al. [2] reported that patients with *CALR*-mutated MPNs presented with higher platelet counts and lower hemoglobin levels than patients carrying the *JAK2*V617F mutation. Among Han Chinese patients, *CALR* mutations

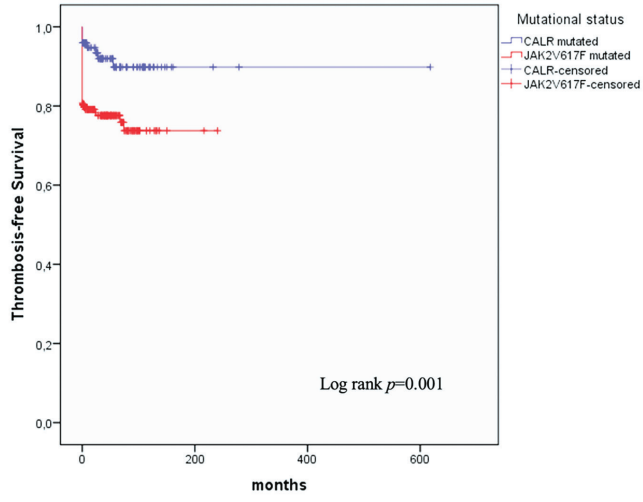

Figure 3. Thrombosis-free survival according to mutational status.

were associated with younger age and higher platelet counts compared to *JAK2*V617F mutations [21]. In addition, patients with *CALR* mutations in Tunisian and Belgian cohorts had higher platelet counts and lower hemoglobin levels and leukocyte counts than patients with *JAK2*V617F mutations [18,22]. Ceylan et al. [23] showed that patients with *CALR* mutations presented with higher platelet counts and lactate dehydrogenase levels compared to *JAK2*- and *MPL*-mutated patients. Similarly, in our patient cohort, *CALR*-mutated patients had significantly higher platelet counts and lower hemoglobin levels (p<0.001). In addition, these patients tended to have lower leukocyte counts (p=0.06).

In patients with ET, the risk for thrombotic complications may exceed 20% [7,24], and previous studies have shown that the risk of thrombosis is higher in *JAK2*V617F-mutated cases [25]. Among the 300 patients with ET reported by Gangat et al. [26], 106 (35%) experienced arterial (n=75) or venous (n=43) events. In univariate analysis, compared to *JAK2*V617F-mutated cases, *CALR*-mutated patients had better TFS (hazard ratio [HR]: 0.53, 95% confidence interval [CI]: 0.30-0.92). However, the authors concluded that the favorable effect of *CALR* mutations might be confined to younger patients [26]. Guglielmelli et al. [27] reported thrombosis (arterial or venous) in 152 (30%) of 502 ET patients, and of those cases, 96 (19%) were arterial and 82 (16%) were venous. In multivariate analysis, venous thrombosis risk at any time was significantly higher in *JAK2*V617F-mutated patients than that observed in *CALR*-mutated cases. This study confirmed the prothrombotic influence of *JAK2*V617F [28] as opposed to *CALR* mutations [27]. In a study that included 168 ET patients, 51 (30.35%) experienced thrombotic events, 60% of which were arterial [29], and *JAK2*V617F-mutated cases exhibited a 1.5-fold higher risk of developing thrombotic events. In our study, the total incidence of thrombotic complications was 17.9%, and most of them (77.8%) were arterial. In the literature, similar to our study, TEEs in ET patients are more common in arterial sites than venous sites, but the incidence of TEEs is generally reported to be higher than that of our patient cohort. Although many factors such as age, comorbidities, and smoking might have an impact on the incidence of TEEs in ET patients, the ASA use in all cases of our cohort might have contributed positively to the low incidence of thrombosis in our study. We also showed in multivariate analysis that age was the only factor independently affecting the incidence of TEEs. Since patients with *CALR* mutations were younger, age may have contributed positively to the lower incidence of TEEs in these patients. Like previous studies, in our cohort the incidence of all TEEs was significantly higher in *JAK2*V617F-mutated cases than *CALR*-mutated cases. Furthermore, *JAK2*V617F-mutated patients had high risk scores for both IPSET-thrombosis and the revised IPSET thrombosis scoring system compared to *CALR*-positive patients. The 2-year and 5-year TFS rates were superior in *CALR*-mutated patients compared to *JAK2*V617F-positive patients (p=0.001, HR: 0.71, 95% CI: 0.68-0.74). In a Japanese population, *JAK2*V617F mutations were related to more thrombotic events and more splenomegaly than *CALR* mutations [30]. In our population, the incidence of splenomegaly was similar between *JAK2*V617F- and *CALR*-mutated cases.

In ET, the leukemic transformation rates at 10 years are estimated to be <1% while those at 20 years average 5%, and the rates of developing MF are slightly higher [25]. Historical data about the probability of MF transformation in ET patients revealed rates of 2.7% (95% CI: 2.4-2.9) at 5 years, 8.3% (95% CI: 7.8-8.9) at 10 years, and 15.3% (95% CI: 6.1-24.5) at 15 years [31]. Barbui et al. [8] revealed that leukemic transformation rates at 10 and 15 years were 0.7% and 2.1%, respectively, and progression rates to overt MF at 10 and 15 years were 0.8% and 9.3%, respectively. They claimed that the absence of *JAK2*V617F was a risk factor for overt MF progression. The rate of leukemic transformation in our cohort (0.7%) is comparable to those mentioned in the literature, while the rate of progression to MF (4%) is slightly higher than the previously published values. The presence of *JAK2*V617F and *CALR* mutations had no impact on progression to MF and leukemic transformation in our study.

Among our patient cohort, OS and MF-free survival rates were similar between *JAK2*V617F- and *CALR*-mutated patients. In *CALR* subgroup analysis, the rates of MF occurrence were significantly higher in patients with other (non-type 1 and non-type 2) *CALR* mutations, but OS and MF-free survival were similar among all *CALR*-mutated patients. Our results support previous data suggesting that driver mutations do not have an impact on survival in ET [25]. In a study of 1494 ET patients, the independent adverse effect of male sex on survival was confirmed with multivariable analysis (HR: 1.6, 95% CI: 1.1-2.5, p=0.02), and in the context of the IPSET, the HRs (95% CI) were 1.6 (1.1-2.5) for male sex, 7.5 (3.1-18.3) for high-risk IPSET scores, and 4.1 (1.8-9.5) for intermediate-risk IPSET scores. Tefferi et al. [32] suggested that women with ET live longer than their male counterparts and that sex might supersede thrombosis history as a risk factor for OS. However, in our study, sex had no effect on survival outcomes, similar to previous findings in a Romanian cohort [29].

Recent studies revealed the adverse impact on survival of non-driver mutations such as *ASXL1*, *SF3B1*, *SRSF2*, and *TP53* [25,33]. *TP53* mutations were highly predictive for leukemic transformation in previous studies [33,34]. In our population, one of the two patients who experienced acute myeloid leukemia progression had *TP53* mutation with a complex karyotype.

The management of ET mainly focuses on reducing the risk of thrombosis, controlling myeloproliferation, and managing

disease-related symptoms and complications [35]. HU is recommended as a front-line cytoreductive therapy for patients with high-risk ET [25]. In the UK-PT1 study, HU was superior to anagrelide in reducing arterial thrombosis, major bleeding, and fibrotic progression [36]. However, in the ANAHYDRET study, HU and anagrelide were found to be similar in the prevention of thrombotic end points [37]. The majority of our patients (77.8%) were treated with cytoreductive agents. In our study, the overall cytoreduction rate was comparable to that of a Romanian cohort (77.8% vs. 76.8%, respectively), although approximately half of the Romanian patients received cytoreductive therapy without ASA/antiplatelets or anticoagulants [23]. HU was the most preferred cytoreductive treatment in both patient cohorts.

In our study, the percentage of patients receiving HU was higher among *JAK2*V617F-mutated patients than those with *CALR* mutations (64% vs. 48.5%), but the overall cytoreduction rate was higher among *CALR*-mutated patients (74.9% vs. 83.8%). The higher rate of HU use among patients with *JAK2*V617F was probably due to the higher incidence of thrombosis observed in this patient population. In addition, *CALR* mutations causing higher platelet counts seem to have prompted more cytoreductive therapy initiation. Although most of these patients had a low IPSET-thrombosis score, their exposure to cytoreductive treatment may be considered over-treatment in ET [38]. In contrast, extreme thrombocytosis has previously been associated with *CALR* mutations [10] and a lower risk of arterial thrombosis [39].

Guglielmelli et al. [27] confirmed the prothrombotic influence of *JAK2* as opposed to *CALR* mutations and suggested that extreme thrombocytosis might also play a part in contributing to the observed decreased risk of arterial thrombosis in *CALR*-mutated ET.

Study Limitations

Since our study was retrospective in nature, covering approximately 20 years, it has some limitations. The major limitations are the lack of comprehensive data regarding non-driver mutations and different median follow-up periods for different *CALR* mutation types. While acknowledging the limitations, we anticipate that our study may contribute to the literature as it includes a large number of Turkish patients and provides real-life data.

Conclusion

This is the first multicenter study investigating the disease characteristics and clinical courses of *CALR*-mutated ET patients in a Turkish population. Our results proved that *JAK2*V617F was strongly associated with thromboembolic complications and HU was the most preferred cytoreductive agent for patients with high thrombosis risk. Mutant *CALR* resulted in higher platelet counts and lower hemoglobin levels than mutant *JAK2*V617F and was related to younger ages at the time of diagnosis. We suggest that ET cases should be managed meticulously considering the disease characteristics caused by driver and non-driver mutations.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the Local Ethics Committee of İzmir Bozyaka Training and Research Hospital with approval number 2022/127 on August 10, 2022.

Informed Consent: Retrospective study.

Authorship Contributions

Surgical and Medical Practices: P.P., D.Ö., S.G., R.Ç., S.M., T.E., V.G., T.El., N.E., G.G., R.D.K., E.A.D., E.E.G., Y.İ., M.B., E.Y.; Concept: Z.N.Ö., P.P., D.Ö., S.G., R.Ç., S.M., T.E., V.G., T.El., G.E., G.G., R.D.K., E.A.D., E.E.G., Y.İ., M.B., E.Y., A.E.E.; Design: Z.N.Ö., P.P., D.Ö., S.G., R.Ç., S.M., T.E., V.G., T.El., G.E., G.G., R.D.K., E.A.D., E.E.G., Y.İ., M.B., E.Y., A.E.E.; Data Collection or Processing: Z.N.Ö., P.P., D.Ö., S.G., R.Ç., S.M., T.E., V.G., T.El., G.E., G.G., R.D.K., E.A.D., E.E.G., Y.İ., M.B., E.Y., A.E.E., V.K.; Analysis or Interpretation: Z.N.Ö, A.E.E.; Literature Search: Z.N.Ö, A.E.E.; Writing: Z.N.Ö, A.E.E.

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