Ankara Eğt. Arş. Hast. Derg., 2021 ; 54(3) : 429-433

Özgün Çalışma / Original Article **DOI: 10.20492/aeahtd.1015643**

ESANSİYEL TROMBOSİTOZLU HASTALARDA TROMBOEMBOLİZMİN MUTASYON DURUMUNA GÖRE DEĞERLENDİRİLMESİ; TEK MERKEZ DENEYİMİ*

EVALUATION OF THROMBOEMBOLISM ACCORDING TO MUTATION STATUS IN PATIENTS WITH ESSENTIAL THROMBOCYTOSIS; SINGLE-CENTER EXPERIENCE

Hikmettullah BATGI[1]

ABSTRACT

AIM: The presence of Janus kinase 2 (Jak2) mutation in essential thrombocytosis (ET) patients is associated with an increased risk of thrombosis, while the presence of Calreticulin (Calr) mutation is associated with a decrease in thrombosis risk. The aim of this study is to compare patients with mutation (Jak2, Calr, myeloproliferative leukemia virus oncogene [Mpl]) and non-mutation (triple-negative) patients in terms of the development of thromboembolism.

MATERIAL AND METHOD: 95 patients who were followed up with the diagnosis of ET between 2009 and 2020 were included in this study. The clinical characteristics, laboratory results, and mutation status of the patients were analyzed retrospectively, based on the patients' files. The patients in mutation-positive (Jak2, Calr) group A, only Jak2 mutation-positive group B, and triple-negative (Jak2, Calr negative) group C were compared.

RESULTS: The median age of ET patients was 53 years (18-91). The Jak2 mutation was found positive in 42% (n=40) of the patients with ET. Four patients (4%) were Calr mutation-positive, but Mpl mutation was not detected. Fifty-one patients (54%) were triple-negative. A total of 22 (23%) patients had a thrombotic event at diagnosis and follow-up. Thrombotic events were detected in 27.5% (11/44) of the patients with positive Jak2 mutation and in 21.5% (11/51) of the patients with triple-negative. No thrombotic event was detected in 4 patients with a positive Calr mutation. There was no statistically significant difference between the groups in terms of white blood cell count, platelet count and spleen size at the time of diagnosis($p = 0.7$). No statistically significant difference was found in terms of white blood cell count, thrombocyte count and spleen size examined at the time of diagnosis. When compared in terms of hemoglobin, age, and gender distribution (male / female), the difference was found statistically significant in those with positive mutation ($p = 0.001$ *, $p = 0.001$ *, $p = 0.03$*).

CONCLUSION: The risk of thrombosis in patients with triple-negative ET is similar to patients with Jak2V617F mutation and mutation (Jak2, Calr) positive patients. The most important limitation of the study was its small sample size, due to being a single-centre study. Further larger sample studies are required to investigate this subject and the relationships between the mentioned findings.

Keywords: Essential thrombocythemia, mutation, thrombosis

ÖZET

AMAÇ: Esansiyel trombositoz (ET) hastalarında Janus kinaz 2 (Jak2) mutasyonunun varlığı tromboz riskinde artış ile ilişkilendirilirken, kalretikülin (Calr) mutasyonunun varlığı tromboz riskinde azalma ile ilişkilidir. Bu çalışmanın amacı mutasyonlu (Jak2, Calr, myeloproliferative leukemia virus oncogene [Mpl]) ve mutasyonsuz (üçlü negatif) hastaları tromboemboli gelişimi açısından karşılaştırmaktır.

GEREÇ VE YÖNTEM: Bu çalışmaya 2009-2020 yılları arasında ET tanısı ile takip edilen 95 hasta dahil edildi. Hasta dosyalarına dayalı olarak hastaların klinik özellikleri, laboratuvar sonuçları ve mutasyon durumları geriye dönük olarak incelendi. Mutasyon pozitif (Jak2, Calr,) grup A, sadece Jak2 mutasyon pozitif grup B ve üçlü negatif (Jak2, Calr negatif) grup C'deki hastalar karşılaştırıldı.

BULGULAR: ET hastalarının ortanca yaşı 53 (18-91) idi. ET hastalarının %42'sinde (n=40) Jak2 mutasyonu pozitif bulundu. Dört hastada (%4) Calr mutasyonu pozitifti, ancak Mpl mutasyonu saptanmadı. Elli bir hasta (%54) üçlü negatifti. Toplam 22 (%23) hastada tanı ve takipte trombotik olay gelişti. Jak2 mutasyonu pozitif olan hastaların %27,5'inde (11/44) ve üçlü negatif olan hastaların %21,5'inde (11/51) trombotik olay tespit edildi. Calr mutasyonu pozitif olan 4 hastada trombotik olay saptanmadı. Üçlü negatif hastalarla karşılaştırıldığında mutasyon pozitif hastalarda trombotik olaylar açısından istatistiksel olarak anlamlı bir fark bulunmadı ($p= 0,7$). Tanı anında bakılan beyaz küre sayısı, trombosit sayısı ve dalak boyutu açısından gruplar arasında istatistiksel olarak anlamlı bir fark bulunmadı. Hemoglobin, yaş ve cinsiyet dağılımı (erkek/kadın) açısından karşılaştırıldığında, mutasyon pozitif olanlarda aradaki fark istatistiksel olarak anlamlı bulundu ($p=0,001$, $p=0,001$, $p=0,03$).

SONUÇ: Üçlü negatif ET'li hastalarda tromboz riski, Jak2V617F mutasyonlu ve mutasyon (Jak2, Calr) pozitif hastalardaki ile benzerdir. Bu çalışmanın en önemli kısıtlılığı tek merkezli olması nedeniyle örneklem büyüklüğünün küçük olmasıdır. Bu konuyu ve bahsedilen bulgular arasındaki ilişkileri araştırmak için daha geniş örneklemli çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Esansiyel trombositemi, mutasyon, tromboz

[1] Ankara Training and Research Hospital Hematology Clinic, Ankara, Turkey

Geliş Tarihi / Submitted : Ekim 2021 / Ekim 2021

Kabul Tarihi / Accepted : Aralık 2021 / December 2021

Sorumlu Yazar / Corresponding Author:
Hikmetullah BATGI
Ankara Training and Research Hospital Hematology Clinic, Ankara, Turkey Hacettepe Neigh. Ulucanlar street. No: 89, Altındağ, Ankara, Turkey
Gsm: +90 506 628 95 75
E-mail: batgih@gmail.com

Yazar Bilgileri /Author Information:
Hikmetullah BATGI (ORCID: 0000-0002-5993-1403)

Ankara Training and Research Hospital Scientific Research and Publication Ethics Committee (Decision number and date: 715/2021-29/07/2021).

INTRODUCTION

Essential thrombocythemia (ET) is a myeloproliferative neoplasm (MPN) characterized by an increased number of mature megakaryocytes in the bone marrow and persistent thrombocytosis in the peripheral blood (1). It is a clonal hematological disease characterized by overproduction of platelets that mainly affects the elderly and the average age of onset is 57 years (1).

Patients with ET display mutations in Jak2 (Janus kinase 2; located on chromosome 9p24) (55%), Calr (Calreticulin; located on chromosome 19p13.2) (15-24%) and Mpl (myeloproliferative leukemia virus oncogene; located on chromosome 1p34) (~4%) genes. According to literature, 15-35% of the patients with ET are triple-negative for these mutations (2,3,4).

Thromboembolism is an important cause of mortality and morbidity in ET, with a reported incidence of 7-22% (4,5,6). The presence of Jak2V617F mutation in essential thrombocytosis (ET) patients was associated with an increased risk of thrombosis (5), while the presence of Calreticulin mutation was associated with a decrease in thrombosis risk (6).

The aim of this study was to compare the demographic characteristics, clinical characteristics, and development of thromboembolism in patients with mutation (Jak2-Calr-Mpl) and patients without mutation (triple-negative).

MATERIAL AND METHOD

This retrospective study was conducted on 95 patients diagnosed with BCR-ABL negative ET in a tertiary care hospital's Hematology Clinic between 2009 and 2020. The diagnosis of MPN was made according to the World Health Organization diagnostic criteria of myeloid neoplasms (1,7). Demographic characteristics, disease characteristics, comorbidities, and complications of the patients were recorded. Hematological parameters, including hemoglobin (Hb) level, platelet count, and white blood cell count (WBC), were examined at the time of diagnosis Mutation positive (Jak2, Calr) group A, only Jak2 mutation-positive group B, and triple-negative (Jak2, Calr, Mpl negative) group C patients were compared.

Statistical Analysis

Statistical analyses were performed with IBM SPSS (Version 26, Armonk, NY) software. Demographic data were summarized with descriptive statistics. Numerical variables were presented as median (minimum-maximum) and categorical variables were presented as ratios. To compare the groups, Mann Whitney U tests were used for numerical variables and the Chi-square test was used for categorical variables. Comparisons between the patient groups were made using the log-rank test. P value ≤0.05 regarded as statistically significant.

Ethical Approval

All procedures performed in this study were conducted in accordance with the ethical standards of the institutional and/or national research committee and the 1964 Declaration of Helsinki and its subsequent amendments or comparable ethical standards. Approval for this study was given by Ankara Training and Research Hospital Scientific Research and Publication Ethics Committee (Decision number and date: 715/2021-29/07/2021).

RESULTS

76 (80%) of the patients were female (F) and 19 (20%) were male (M). The median age of ET patients was 53 years (18-91). The Jak2V617F mutation was found positive in 42% (n:40) of the patients with ET. Four patients (4%) were Calr mutation-positive, but Mpl mutation was not detected. 51 patients (54%) were triple-negative.

A total of 22 (23%) patients had a thrombotic event at diagnosis and follow-up. Thrombotic events were detected in 27.5% (11/44) of the patients with positive Jak2V617F mutation and in 21.5% (11/51) of the patients with the triple-negative. No thrombotic event was detected in 4 patients with a positive Calr mutation **(Table 1-2).** No statistically significant difference was found for thrombotic events in mutation-positive patients compared with triple-negative patients (p = 0.7) **(Table 4).** No statistically significant difference was found in terms of WBC count, thrombocyte count and spleen size examined at the time of diagnosis **(Table 3).** When compared in terms of Hb, age and gender distribution (M / F), the difference was found statistically significant in those with positive mutation (p= 0.001 *, p= 0.001 *,

Table 1. The frequency of thromboembolism in patients with essential thrombocytosis

Thromboembolism type	Number of patients	Age, median	Comorbid Risks (DM, HT, HL, Obesity, Smoking), median	Jak2 (+)	Triple (-)
Coronary artery disease	11	70	1.8	6	5
Cerebrovascular event	1	76	3	1	0
Deep vein thrombosis	4	55	1.5	1	3
Peripheral vascular disease	0	0	0	0	0
Pulmonary embolism	3	36	0.6	1	2
Portal vein thrombosis	2	52	1	2	0
Other (Retinal vein thrombosis)	1	43	3	0	1

DM; diabetes mellitus, HL; hyperlipidemia, HT; hypertension, Jak2; Janus kinase 2.

p= 0.03*) **(Table 4).** Cardiovascular risk factors were determined in 39 (41%) of 95 patients with ET **(Table 5).**

Table 2. Mutation status of patients with thromboembolism

	Mutation (Yes) (n=44)		Mutation (No) (n=51)
	Jak2 (+) (n=40)	Calr (+) (n=4)	Triple (-) (n=51)
Thromboembolism positive (n=22)	11 (27.5%)	0 (0%)	11 (21.5%)
Thromboembolism negative (n=73)	29 (72.5%)	4 (100%)	40 (78.5%)

Calr; calreticulin, Jak2; Janus kinase 2.

Table 3. Comparison of hematological and clinical features of patients with essential thrombocytosis according to the presence of mutations

Factor	Jak2V617F (+) (n=40)	Calr (+) (n=4)	Triple (-) (n=51)	P value
WBC, x10^9/L, median (min-max)	10.3 (5.8-17.5)	10.9 (8.9-16)	9.5 (4.8-16.7)	0.3
Hb, gr/dl	15 (11-18)	13.5 (13-15)	13 (10-18)	0.001*
Plt, x10^9/L, median (min-max)	627 (471-1242)	758.5 (537-1481)	595 (453-1671)	0.3
Age, median (min-max)	60.5 (24-91)	54.5 (27-72)	44 (18-79)	0.001*
Gender (M / F)	10/30	3/1	6/45	0.01*
Splenomegaly, n (%)	6 (15%)	1 (25%)	4 (7.8%)	0.4
Thromboembolism, n (%)	11 (25%)	0	11 (21.5%)	0.5

Calr; calreticulin, Hb; hemoglobin, Jak2; Janus kinase 2, Plt; platelet**,** WBC; white blood cell *p<0.05

Table 4. Comparison of hematological and clinical features of patients with essential thrombocytosis according to the presence of mutations

Factor	Mutation (+) (A) (n=44)	JAK2 (+) (B) (n=40)	Triple (-) (C) (n=51)	P value	
				A vs C	B vs C
WBC, x10^9/L, median (min-max)	10.4 (5.8-17.5)	10.3 (5.8-17.5)	9.5 (4.8-16.7)	0.1	0.001*
Hb, gr/dl, median (min-max)	15 (11-18)	15 (11-18)	13 (10-18)	0.001*	0.192
Plt, x10^9/L, median (min-max)	630 (471-1481)	627 (471-1242)	595 (453-1671)	0.2	0.001*
Age, median (min-max)	60 (24-91)	60.5 (24-91)	44 (18-79)	0.001*	0.3
Gender (M / F)	13/31	10/30	6/45	0.03*	0.1
Splenomegaly, n (%)	7 (15.9%)	6 (15%)	4 (7.8%)	0.2	0.3
Thromboembolism, n (%)	11 (25%)	11 (27.5%)	11 (21.5%)	0.7	0.5

F; female, Hb; hemoglobin, Jak2; Janus kinase 2, M; male, Plt; platelet**,** WBC; white blood cell *p<0.05

Table 5. Risk factors and risk classification in patients with essential thrombocytosis.

Risk factors and Risk stratification -Age>60 years -Previous thrombosis -Jak2V617F-positive	Cardiovascular risk factors (Yes), n	Cardiovascular risk factors (No), n
Very low No thrombosis history, age≤60 years and Jak2-unmutated	11	29
Low No thrombosis history, age≤60 years and Jak2-mutated	6	11
Intermediate No thrombosis history, age>60 years and Jak2-unmutated	1	4
High Thrombosis history or age>60 years and Jak2-mutated	8	12

Jak2; Janus kinase 2 Cardiovascular risk factors: smoking, hypertension, diabetes mellitus, and hyperlipidemia

DISCUSSION

The presence of Jak2V617F mutation in ET increases the risk of thrombosis, while the presence of Calr mutation reduces the risk of thrombosis (3, 5, 6). However, there are currently very few articles on triple negative ET, which is basically defined as an indolent disease with a low incidence of vascular events. Cattaneo D et al. reported the frequency of thrombus as 5% in 40 triple negative patients with ET (8). In our study, thrombotic events were detected in 27.5% (11/44) of patients with positive Jak2V617F mutations and in 21.5% (11/51) of patients with triple negative mutations, whereas thrombosis was not observed in patients with Calr mutation. However, it was not statistically significant. On the other hand, in this study, it was determined that mutation-positive patients and triple-negative patients had similar thrombosis risk.

Thrombotic complications have been reported in 11-45% of ET cases (4, 5, 6). Furthermore, leukocytosis has been reported to be an independent risk factor for arterial thrombosis in ET (10).

Rumi et al. reported in their study that patients with Jak2V617F were older, had a higher Hb level, higher WBC count, and lower platelet count than those with Calr mutations (3). According to our data, the presence of Jak2V617F mutation, older age, higher-Hb, were consistent with the literature and were found similar in terms of WBC and platelet count.

Rumi et al. reported that ET patients with Calr mutations are characterized by relatively young individuals and a markedly high platelet count, but with a relatively low thrombotic risk (3). In our data, ET patients with Calr mutations were characterized by relatively young individuals and a markedly high platelet count, and no thrombosis was observed in these patients. Mpl mutation was reported in approximately 4% of the ET patients (4). Soyer et al. reported the frequency of Mpl mutation as 2.6% in patients with ET (11). Mpl mutation was not detected in our ET patients. This result might be related to the low number of patients. In our ET patients, the median age at diagnosis, the incidence of thrombosis, and Jak2 mutation positivity were compatible with literature findings (4,9,11,12). While the incidence of thrombosis in patients with ET was 15%, Soyer et al. (11) found it as 23% in our data. The median age at diagnosis of ET has been reported as 55 and 57 years (1, 13). In the current study, the median age at diagnosis for ET was 53. In the ET subgroup, there was a predominance of female patients (80% F vs. 20% M), which was similar to the findings of the International Prognostic Score of thrombosis in World Health Organization- Essential Thrombocythemia (IPSET) study (14).

According to literature, 15-35% of the patients with ET are triple-negative for these mutations (2,3,4). In our study, 54% triple-negative was detected. The reason why the frequency of different genotypic changes is observed in quite different ratio ranges may be patients of different ethnic origins.

In conclusion, the risk of thrombosis in patients with triple-negative ET is similar to patients with Jak2V617F mutation and mutation-positive patients. The most important limitation of the study was its small sample size, due to being a single-centre study. Further larger sample studies are required to investigate this subject and the relationships between the mentioned findings.

Acknowledgement

Competing Interests: The authors declare that they have no competing interests.

Financial Disclosure: There are no financial supports.

Ethical Approval: We received ethical approval for our study from the local Scientific Research and Publication Ethics Committee (E-21-715). It was presented as an oral presentation at the II. Hematology Education and Research Congress.

REFERENCES

1.) Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016; 127: 2391-405.

2.) Cazzola M, Kralovics R. From Janus kinase 2 to calreticulin: the clinically relevant genomic landscape of myeloproliferative neoplasms. Blood. 2014; 123: 3714-19.

3.) Rumi E, Pietra D, Pascutto C, et al. Clinical effect of driver mutations of JAK2, CALR, or MPL in primary myelofibrosis. Associazione Italiana per la Ricerca sul Cancro Gruppo Italiano Malattie Mieloproliferative Investigators. Blood. 2014; 124: 1062-9.

4.) Tefferi A, Barbui T. Polycythemia vera and essential thrombocythemia: 2015 update on diagnosis, risk-stratification, and management. American journal of hematology. 2015; 90:162-73.

5.) Barbui T, Thiele J, Passamonti F, et al. Survival and disease progression in essential thrombocythemia are significantly influenced by accurate morphologic diagnosis: an international study. J Clin Oncol. 2011; 29: 3179-84.

6.)Tefferi A, Wassie EA, Guglielmelli P, et al. Type 1 versus Type 2 calreticulin mutations in essential thrombocythemia: a collaborative study of 1027 patients. American Journal of Hematology. 2014; 89: 121-4.

7.) Tefferi A, Vardiman JW. Classification and diagnosis of myeloproliferative neoplasms: the 2008 World Health Organization criteria and point-of-care diagnostic algorithms. Leukemia. 2008; 22: 14-22.

8.) Cattaneo D, Croci GA, Bucelli C, et al. Triple-Negative Essential Thrombocythemia: Clinical-Pathological and Molecular Features. A Single-Center Cohort Study. Front Oncol. 2021; 11: 637116.

9.) Elliott MA, Tefferi A. Thrombosis and hemorrhage in polycythemia vera and essential thrombocythemia. Br J Haematol. 2005; 128: 275-90.

10.) Palandri F, Polverelli N, Catani L, et al. Impact of leukocytosis on thrombotic risk and survival in 532 patients with essential thrombocythemia: a retrospectivestudy. Ann Hematol. 2011; 90: 933-38.

11.) Soyer N, Haznedaroğlu İC, Cömert M, et al. Multicenter Retrospective Analysis of Turkish Patients with Chronic Myeloproliferative Neoplasms. Turk J Haematol. 2017; 34: 27-33.

12.)Chim CS, Kwong YL, Lie AK, et al. Long-term outcome of 231

patients with essential thrombocythemia: prognostic factors for thrombosis, bleeding, myelofibrosis, and leukemia. Arch Intern Med. 2005; 165: 2651-58.
13.) Tefferi A, Guglielmelli P, Larson DR, et al. Long-term survival and blast transformation in molecularly annotated essential thrombocythemia, polycythemia vera, and myelofibrosis. Blood, The Journal of the American Society of Hematology. 2014; 124: 2507-13.
14.) Passamonti F, Thiele J, Girodon F, et al. A prognostic model to predict survival in 867 World Health Organization–defined essential thrombocythemia at diagnosis: a study by the International Working Group on Myelofibrosis Research and Treatment. Blood. 2012; 120: 1197-201.

Ankara Eğt. Arş. Hast. Derg. (Med. J. Ankara Tr. Res. Hosp.), 2021 ; 54(3) : 429-433
Ankara Training and Research Hospital Scientific Research and Publication Ethics Committee (Decision number and date: 715/2021-29/07/2021).