The Relationship Between the JAK2-V617F Mutation Status and Predictors of Clinical Outcomes in Patients with Essential Thrombocythemia

Esansiyel Trombositemi Hastalarında JAK2-V617F Mutasyonu ile Klinik Sonlanım Belirteçleri Arasındaki Ilişki

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Abstract: The prognostic value of JAK2 - V617F mutation remains unclear in essential thrombocythemia (ET). However, leukocytosis, anemia, thrombocytosis, advanced age, and splenomegaly are all associated with poor clinical outcomes, such as thrombosis, hemorrhage, and short life expectancy. JAK2 mutation may be related to these parameters and may establish prognostic value in this regard. Therefore, we investigated the relationship between JAK2 mutation and these predictors of poor prognosis. This retrospective, cross-sectional study is conducted in archives of a Hematology Clinic in an Education Hospital at 2013. All patients who have been diagnosed with ET and have JAK2 mutation analysis results, are included to the study. Diagnosis of ET was made according to the World Health Organization (WHO) Diagnostic Criteria for ET. Demographic characteristics, complete blood counts, abdominal ultrasounds and JAK2 mutation analyses are collected from patient files for 84 patients. The relationship between JAK2 mutation and age, sex, hemoglobin, leukocyte, thrombocyte counts, and splenomegaly is analyzed by using appropriate statistical analysis. In total, 54.7% of patients with ET tested positive for the JAK2 mutation. Patients with JAK2 mutation had higher leukocyte levels ($p < 0.001$), higher hemoglobin levels ($p = 0.014$), and splenomegaly ($p < 0.001$). There was no relationship between JAK2 mutation and age, sex, thrombocyte counts or anemia. The JAK2 mutation is related to leukocytosis and splenomegaly, which are known to be thrombotic risk factors. Therefore, JAK2 mutation may have a prognostic value and warrants further research with clinical outcomes.
Key Words: essential thrombocythemia, JAK2 - V617F mutation, thrombosis, leukocytosis

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Özet: Esansiyel Trombositoz (ET)'da JAK2- V617F mutasyonunun klinik önemi halen belirsizdir. Bununla beraber, lökositoz, anemi, trombositoz, ileri yaş, splenomegali varlığı tromboz, hemoraji, yaşam süresinde kısalma gibi olumsuz klinik sonlanımlarla ilişkilendirilmektedir. Bu nedenle, çalışmamızda JAK2 mutasyonu ile olumsuz klinik sonlanım gösteren parametrelerin ilişkisini araştırdık. Böylece JAK2 mutasyonunun bu parametrelerle ilişkisinin saptanması prognostik önemi konusunda bilgi verebilir. Bu retrospektif, kesitsel çalışma 2013 yılında Ankara Numune Eğitim Araştırma Hastanesi'nin Hematoloji kliniği arşivlerinde dosya taraması ile gerçekleştirilmiştir. ET tanısı alan ve dosyasında JAK2 mutasyon analizi sonucu bulunan tüm vakalar çalışmaya dahil edilmiştir. ET tanısı Dünya Sağlık Örgütü (DSÖ) ET için Tanı Kriterleri'ne göre konulmuştur. Demografik bilgiler, tam kan sayımı, abdominal ultrason sonuçları, JAK2 mutasyon analizi sonuçları 84 hasta için toplanmıştır. JAK2 mutasyonu ile yaş, cinsiyet, hemoglobin düzeyi, lökosit sayısı, trombosit sayısı ve splenomegali varlığı arasındaki ilişki uygun istatistiksel yöntemler kullanılarak araştırılmıştır.Hastaların yüzde 54,7'sinde JAK2 mutasyonu pozitif saptanmıştır. JAK2 pozitifliği olan hastaların lökosit sayıları daha yüksek ($p <0.001$), hemoglobin düzeyleri daha yüksek ($p = 0.014$) saptanmıştır ve splenomegali bu hastalarda daha sık görülmüştür ($p <0.001$). JAK2 mutasyonu ile yaş, cinsiyet, trombosit sayıları ve anemi durumu arasında istatistiksel anlamlı ilişki saptanmamıştır. JAK2 mutasyonu trombotik risk faktörü olarak bilinen lökositoz ve splenomegali ile ilişkili bulunmuştur. Bu durum JAK2 mutasyonunun prognostik değeri olabileceğini göstermektedir.
Anahtar Kelimeler: esansiyel trombositoz, JAK2 - V617F mutasyonu, tromboz, lökositoz

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1. Introduction

Essential thrombocythemia (ET) is one of the Myeloproliferative neoplasms (MPN) which are a group of diseases in which there is abnormal increase in mature forms of myeloid cells (1). Other MPN such as chronic myeloid leukemia, polycythemia vera (PV), and primary myelofibrosis (PMF) are well defined by clinical and laboratory data; however, ET is a diagnosis of exclusion from other MPN and reactive thrombocytosis (2). The incidence rate of ET is 2.5/100,000 and prevalence rate is 24/100,000 (3). ET is more common in females and the average age of diagnosis is 60 years (4). The 5 and 10 year survival rates are 74%-93% and 61%-84%, respectively (5). Studies have shown that approximately 50% of patients with ET have a mutation in the *JAK2* gene (*JAK2*-V617F) (6). The presence of this mutation proves that the thrombocytosis is myeloproliferative and non-reactive, however, it cannot be used to exclude other MPN (7). Most patients with ET have a normal life expectancy, if there are no complications (8). However, thrombosis and hemorrhage are important complications of ET and cause substantial morbidity and mortality (6).

The relationship between the *JAK2* mutation and clinical outcomes and laboratory parameters of patients with ET is currently unclear. Some studies have found that patients with *JAK2* mutation are at increased risk for thrombosis (9). Another study found that patients with *JAK2* mutation have high leukocyte and hemoglobin levels, and low platelet counts. The same study also suggested a high transformation rate to PV (10).

Several parameters have been identified as risk factors for undesirable clinical outcomes for patients with ET. For example, low hemoglobin levels (<12 g/dl), advanced age (>60 years), high leukocyte counts (>12,000/mm^3), smoking, diabetes, and prior venous thrombosis are shown to be associated with decreased life expectancy. In addition, high platelet counts (>1 million/mm^3) have been suggested as a risk factor for thrombo-hemorrhagic complications (4, 11-13). Low hemoglobin levels (<12 g/dl), advanced age, and high platelet counts (≥1 million/mm^3) are considered as risk factors for leukemic transformation; however, *JAK2* mutation alone, is not associated with transformation to leukemia (14). Leukocytosis has been shown to be an independent risk factor for thrombotic complications (15-18). Another study has shown relationship between splenomegaly and thrombosis (19).

In this study, we hypothesize that the *JAK2* mutation may be associated with some of these undesirable clinical outcomes, such as thrombo-hemorrhagic complications, transformation to leukemia, transformation to PV, and a decreased life expectancy. Therefore, we aimed to assess the relationship between the *JAK2* mutation and the proposed risk factors that are associated with these complications.

2. Patients and Methods

This study was conducted using retrospective scanning of patient files in the archives of a Hematology Clinic in an Education and Research Hospital. The patient files that have a diagnosis of ET between 2005 and 2012 are selected. Diagnosis of ET was made according to the World Health Organization (WHO) Diagnostic Criteria for ET. This resulted in the selection of 84 patients. The *JAK2* mutation was tested in the laboratory of the hospital which the study is based, and same laboratory method was used for all analysis.

Hemoglobin levels, leukocyte and thrombocyte counts, and abdominal ultrasound results were collected from the patient files and hospital patient database. The relationship between *JAK2* mutation and age, sex, hemoglobin levels, leukocyte and platelet counts, and splenomegaly was then investigated. For this assessment, advanced age was defined as ≥ 60 years, high leukocyte count ≥ 12000/mm^3, extremely high platelet count ≥ 1 million/mm^3, and low hemoglobin level as ≤ 12 g/dl. Splenomegaly was

diagnosed where the longitudinal length of the spleen was ≥ 140 mm.

All statistical analyses were conducted using the statistical package SPSS for Windows v.11.5. The normality of the distribution of continuous variables was assessed using Shapiro-Wilk tests. Descriptive statistics are shown as means and (standard deviations) or medians (minima-maxima) for continuous variables, and as the number of cases (*n*) and percentages (%) for categorical variables. Student's *t*-test were used to assess the significance of differences between the group means and Mann-Whitney U test were used to assess the significance of differences between median results for the groups. Pearson's Chi-square test or Fisher's exact test were used to assess the significance of categorical variables. The significance level was set at $p < 0.05$.

3. Results

This present study included 84 patients and 46 (54.7%) of them tested positive for *JAK2* mutation. The 84 patients were assessed for age, gender, leukocyte, hemoglobin, and platelet counts, and splenomegaly. The results are summarized in Table 1.

Table 1.
Clinical and demographic characteristics of 84 patients according to their *JAK2* mutation status

		JAK2 (-) (n = 38)	*JAK2* (+) (n = 46)	p- value	ODDS RATIO (%95 CI)
Age (years), mean (SD)		52.8 (16.8)	56.6 (15.7)	0.289	
Advanced age (years) *n* (%)	<60	23 (60.5)	24 (52.2)	0.443	1,406 (0,589-3,357)
	≥60	15 (39.5)	22 (47.8)		
Gender *n* (%)	Male	17 (44.7)	20 (43.5)	0.908	1,052 (0,443-2,500)
	Female	21 (55.3)	26 (56.5)		
WBC count × 10^3 *n* (min–max)		9.1 (3.6-164)	11.8 (1.2-27)	**< 0.001**	
Patients with leukocytosis, /mm^3 *n* (%)	< 12000	31 (81.6)	23 (50.0)	**0.003**	4,429 (1,624-12,079)
	≥ 12000	7 (18.4)	23 (50.0)		
Hemoglobin level, g/dl Mean (SD)		13.2 (2.3)	14.4 (2.2)	**0.014**	
Patients with anemia, g/dl *n* (%)	< 12	10 (26.3)	6 (13.0)	0.123	2,381 (0,776-7,308)
	≥ 12	28 (73.7)	40 (87.0)		
Thrombocyte count × 10^3 *n* (min-max)		971 (490 - 2811)	856 (457 - 3000)	0.169	
Patients with Thrombocytosis, × 10^3 *n* (%)	<1000	22 (57.9)	28 (60.9)	0.782	0,884 (0,369-2,120)
	≥1000	16 (42.1)	18 (39.1)		
Patients with Splenomegaly, *n* (%)	Negative	30 (90.9)	21 (53.8)	**< 0.001**	
	Positive	3 (9.1)	18 (46.2)		

The mean age and gender was similar in *JAK2* mutation positive and negative patients. The patients positive for *JAK2* mutation had higher median leukocyte counts ($p < 0.001$), and higher median hemoglobin levels ($p = 0.014$)]. However, there was no significant difference in median platelet counts of patients who tested positive and negative for the *JAK2* mutation ($p = 0.169$).

Based on these parameters, the relationship between *JAK2* mutation and postulated risk factors in the literature is investigated.

Significantly more patients with leukocytosis and splenomegaly tested positive for the *JAK2* mutation (23 positives and7 negatives; $p = 0.003$ and 18 positives and 3 negatives; $p < 0.001$, respectively). However, there was no association between advanced age (≥ 60 years), anemia or extremely high platelet counts and *JAK2* mutation status.

4. Discussion

The relationship between *JAK2* mutation and some parameters that are related with complications in ET has been extensively investigated in the literature. In our study, we assessed the relationship between the *JAK2* mutation and age, gender, hemoglobin levels, platelet counts, leukocyte counts, and splenomegaly.

We found that there was no relationship between the JAK2 mutation and gender, which matches the findings of Kittur et al. and Speletas et al., who also found no association between these parameters (20, 21).

Advanced age is a known thrombotic risk factor, and therefore, we assessed the relationship between age and the *JAK2* mutation to indirectly assess if the mutation is related to thrombosis. We found that there was no significant relationship between the *JAK2* mutation and age. However, previous studies by Wolanskyj et al., Campbell et al., and Cheung et al. reported that advanced age was associated with *JAK2* mutation (10, 22, 23).

Several studies have also shown that leukocytosis is a thrombotic risk factor (17, 24), and thus, we analyzed the relationship between the *JAK2* mutation and leukocyte counts. Patients that tested positive for the *JAK2* mutation had significantly higher leukocyte counts and significantly more patients with leukocytosis tested positive than negative for *JAK2* mutation. Similarly, Gangat et al. and Campbell et al. found association between leukocytosis and the *JAK2* mutation (14, 22).

Hemoglobin levels are important in patients with ET because they may be associated with lower life expectancy and transformation to leukemia. Our analysis showed that the *JAK2* mutation was associated with higher hemoglobin levels, supporting the findings of previous studies (10, 20, 22). It is also shown in a study that low hemoglobin levels are associated with leukemic transformation (14). However, in our study there was no relationship between *JAK2* mutation and anemia. There are also studies investigating the relationship between anemia and/or iron deficiency with thrombosis. Potaczek et al. has shown an association between iron deficiency (not anemia) and thrombosis (25). However, our study did not include data for iron deficiency.

Thrombocyte counts are also important in ET because they are related to thrombo-hemorrhagic complications (11). In our study, we found no relationship between the *JAK2* mutation and thrombocyte counts. However, other studies have shown that patients with *JAK2* mutation have lower platelet counts (7, 10).

Splenomegaly can be present in patients with ET and it has been shown to be associated with thrombosis. We found that the incidence of splenomegaly was higher in patients with the *JAK2* mutation, which matches with the previous findings of Lieu et al., Palandri et al., and Vannucchi et al.(26-28).

In conclusion, our study demonstrates that the *JAK2* mutation is associated with several prognostic parameters for ET, including leukocyte counts, hemoglobin levels, and splenomegaly. These associations are very important for furthering our knowledge of this

disorder. The *JAK2* mutation status of patients with ET may alter the likelihood of developing undesirable clinical outcomes.

The main limitation of the present study is the retrospective and observational data collection techniques which restricts making causal assumptions. Also, the indirect investigation of the relationship between *JAK2* mutation and predictors of poor prognosis is a restriction. A prospective study investigating the relationship between *JAK2* mutation and clinical outcomes will be more valuable. However, the findings make a valuable contribution to the current literature on this topic and can be used by future studies which use clinical end-points.

Contribution Statement

MEA conceived the idea of the study. FC, MF, SD, GO contributed to the design of the research. MEA was involved in data collection. FC, MF contributed to the purification of the data. MEA analyzed the data. All authors edited and approved the final version of the manuscript.

- *The study was conducted at Ankara Numune Education and Research Hospital*
- *Part of this study; including data from 2005 to 2008 was accepted as a poster presentation in 35th National Hematology Congress, Turkey in 2009*

REFERENCES

1. Tefferi A. The Philadelphia chromosome negative chronic myeloproliferative disorders: a practical overview. Mayo Clin Proc. 1998;73(12):1177-84.
2. Schafer AI. Thrombocytosis. N Engl J Med. 2004;350(12):1211-9.
3. Ma X, Vanasse G, Cartmel B, Wang Y, Selinger HA. Prevalence of polycythemia vera and essential thrombocythemia. Am J Hematol. 2008;83(5):359-62.
4. .Bellucci S, Janvier M, Tobelem G, Flandrin G, Charpak Y, Berger R, et al. Essential thrombocythemias. Clinical evolutionary and biological data. Cancer. 1986;58(11):2440-7.
5. van Genderen PJ, Mulder PG, Waleboer M, van de Moesdijk D, Michiels JJ. Prevention and treatment of thrombotic complications in essential thrombocythaemia: efficacy and safety of aspirin. Br J Haematol. 1997;97(1):179-84.
6. Schafer AI. Molecular basis of the diagnosis and treatment of polycythemia vera and essential thrombocythemia. Blood. 2006;107(11):4214-22.
7. Gale RE, Allen AJ, Nash MJ, Linch DC. Long-term serial analysis of X-chromosome inactivation patterns and JAK2 V617F mutant levels in patients with essential thrombocythemia show that minor mutant-positive clones can remain stable for many years. Blood. 2007;109(3):1241-3.
8. Beer PA, Erber WN, Campbell PJ, Green AR. How I treat essential thrombocythemia. Blood. 2011;117(5):1472-82.
9. Wong RS, Cheng CK, Chan NP, Cheng SH, Wong WS, Lau KM, et al. JAK2 V617F mutation is associated with increased risk of thrombosis in Chinese patients with essential thrombocythaemia. Br J Haematol. 2008;141(6):902-4.
10. Wolanskyj AP, Lasho TL, Schwager SM, McClure RF, Wadleigh M, Lee SJ, et al. JAK2 mutation in essential thrombocythaemia: clinical associations and long-term prognostic relevance. Br J Haematol. 2005;131(2):208-13.
11. Ruggeri M, Finazzi G, Tosetto A, Riva S, Rodeghiero F, Barbui T. No treatment for low-risk thrombocythaemia: results from a prospective study. Br J Haematol. 1998;103(3):772-7.
12. Fenaux P, Simon M, Caulier MT, Lai JL, Goudemand J, Bauters F. Clinical course of essential thrombocythemia in 147 cases. Cancer. 1990;66(3):549-56.
13. Tefferi A, Fonseca R, Pereira DL, Hoagland HC. A long-term retrospective study of young women with essential thrombocythemia. Mayo Clin Proc. 2001;76(1):22-8.
14. Gangat N, Wolanskyj AP, McClure RF, Li CY, Schwager S, Wu W, et al. Risk stratification for survival and leukemic transformation in essential thrombocythemia: a single institutional study of 605 patients. Leukemia. 2007;21(2):270-6.
15. Wolanskyj AP, Schwager SM, McClure RF, Larson DR, Tefferi A. Essential thrombocythemia beyond the first decade: life expectancy, long-term complication rates, and prognostic factors. Mayo Clin Proc. 2006;81(2):159-66.
16. Carobbio A, Finazzi G, Antonioli E, Vannucchi AM, Barosi G, Ruggeri M, et al. Hydroxyurea in essential thrombocythemia: rate and clinical relevance of responses by European LeukemiaNet criteria. Blood. 2010;116(7):1051-5.
17. Carobbio A, Antonioli E, Guglielmelli P, Vannucchi AM, Delaini F, Guerini V, et al. Leukocytosis and risk stratification assessment in essential thrombocythemia. J Clin Oncol. 2008;26(16):2732-6.
18. Barbui T, Carobbio A, Rambaldi A, Finazzi G. Perspectives on thrombosis in essential thrombocythemia and polycythemia vera: is leukocytosis a causative factor? Blood. 2009;114(4):759-63.
19. Teofili L, Giona F, Torti L, Cenci T, Ricerca BM, Rumi C, et al. Hereditary thrombocytosis caused by MPLSer505Asn is associated with a high thrombotic risk, splenomegaly and progression to bone marrow fibrosis. Haematologica. 2010;95(1):65-70.
20. Kittur J, Knudson RA, Lasho TL, Finke CM, Gangat N, Wolanskyj AP, et al. Clinical correlates of

JAK2V617F allele burden in essential thrombocythemia. Cancer. 2007;109(11):2279-84.
21. Speletas M, Katodritou E, Daiou C, Mandala E, Papadakis E, Kioumi A, et al. Correlations of JAK2-V617F mutation with clinical and laboratory findings in patients with myeloproliferative disorders. Leuk Res. 2007;31(8):1053-62.
22. Campbell PJ, Scott LM, Buck G, Wheatley K, East CL, Marsden JT, et al. Definition of subtypes of essential thrombocythaemia and relation to polycythaemia vera based on JAK2 V617F mutation status: a prospective study. Lancet. 2005;366(9501):1945-53.
23. Cheung B, Radia D, Pantelidis P, Yadegarfar G, Harrison C. The presence of the JAK2 V617F mutation is associated with a higher haemoglobin and increased risk of thrombosis in essential thrombocythaemia. Br J Haematol. 2006;132(2):244-5.
24. Landolfi R, Di Gennaro L, Barbui T, De Stefano V, Finazzi G, Marfisi R, et al. Leukocytosis as a major thrombotic risk factor in patients with polycythemia vera. Blood. 2007;109(6):2446-52.
25. Potaczek DP, Jankowska EA, Wypasek E, Undas A. Iron deficiency: a novel risk factor of recurrence in patients after unprovoked venous thromboembolism. Pol Arch Med Wewn. 2016;126(3):159-65.
26. Lieu CH, Wu HS, Hon YC, Tsai WH, Yang CF, Wang CC, et al. Prevalence of the JAK2-V617F mutation in Taiwanese patients with chronic myeloproliferative disorders. Intern Med J. 2008;38(6):422-6.
27. Palandri F, Ottaviani E, Salmi F, Catani L, Polverelli N, Fiacchini M, et al. JAK2 V617F mutation in essential thrombocythemia: correlation with clinical characteristics, response to therapy and long-term outcome in a cohort of 275 patients. Leuk Lymphoma. 2009;50(2):247-53.
28. Vannucchi AM, Antonioli E, Guglielmelli P, Longo G, Pancrazzi A, Ponziani V, et al. Prospective identification of high-risk polycythemia vera patients based on JAK2(V617F) allele burden. Leukemia. 2007;21(9):1952-9.