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ARAŞTIRMA / RESEARCH

Genetic analysis of BCR-ABL negative chronic myeloproliferative diseases at initial diagnosis and their clinical effects

BCR-ABL negatif kronik myeloproliferatif hastalıkların tanı anındaki genetik analizleri ve bunların klinik etkileri

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Abstract

Purpose: The aim of this study to discuss frequency and clinical significance of JAK2-V617F, Calreticulin (CALR type 1 and type-2) and MPL-W515K/L mutations in patients at initial diagnosis of bcr-abl negative chronic myeloproliferative diseases (CMPD).
Materials and Methods: In this study, the demographic characteristics, subtype, risk status and mutation analysis were investigated between July 2017 and March 2019 in patients diagnosed with bcr-abl negative CMPD.
Results: JAK2 V617F mutation was detected in sum of 27 patients, 18 of them (85,7%) diagnosed with polycythemia vera (PV) and rest of them (N=9, 56,2%) diagnosed with essential thrombocytosis (ET). Calreticulin mutation was positive in 4 (57,1%) patients, who were also JAK2 V617F negative, diagnosed with ET. CALR-type 1 mutation was detected in three patients and CALR-type 2 was in one. MPL-W515K/L was not detected in any of patients diagnosed with ET. Thrombotic event was accompanied 12,6% of patients with PV and 6,25% patients with ET. Splenomegaly was noted in 14 (37,8%) of patients.
Conclusion: Pathogenesis, classification, and risk groups of CMPD have been well characterized with the identification of some genetic mutations in recent years. JAK2 V617F, CALR and MPL are the most common somatic mutations in the pathogenesis of CMPD, which are important in the diagnosis, risk classification and follow-up of the disease and gain importance in personalized medicine.
Keywords:. BCR-ABL1 negative; JAK2 V617F; calreticulin; mpl

Öz

Amaç: Bu çalışmanın amacı, bcr-abl negatif kronik miyeloproliferatif hastalık tanısı alan hastaların tanı anında JAK2 V617F, kalretikulin (CALR tip-1 ve tip-2) ve MPL-W515K / L mutasyonların sıklığını ve bu mutasyonların klinik önemini tartışmaktır.
Gereç ve Yöntem: Bu çalışmada, Temmuz 2017-Mart 2019 tarihleri arasında BCR-ABL negatif kronik miyeloproliferatif hastalık tanısı alan hastaların demografik özellikleri, tanı alt tipi, risk durumu ve mutasyon analizi araştırılmıştır.
Bulgular: JAK2-V617F mutasyonu 18'i (% 85,7) polistemia vera (PV) ve geri kalanı (N = 9, %56,2) esansiyel trombositoz (ET) tanısı alan toplam 27 hastada saptandı. ET tanısı konmuş JAK2 V617F negatif 4 (% 57,1) hastada kalretikulin mutasyonu saptandı. Üç hastada CALR-tip 1 mutasyon ve 1 hastada CALR-tip 2 mutasyon tespit edildi. ET tanısı alan hastaların hiçbirinde MPL-W515K / L saptanmadı. Trombotik olay PV' li hastaların % 12,6'sı ve ET' li hastaların% 6,25'i olarak tespit edildi. Hastaların 14'ünde (% 37,8) splenomegali saptandı.
Sonuç: Kronik myeloproliferatif hastalıkların patogenezi, sınıflandırılması ve risk grupları son yıllarda bazı genetik mutasyonların tanımlanması ile iyi karakterize edilmiştir. JAK2 V617F, CALR ve MPL kronik myeloproliferatif hastalıkların patogenezinde, hastalığın tanı, risk sınıflandırması, takipte önemli olan ve kişiselleştirilmiş tıpta önem kazanan aynı zamanda en sık tanımlanan somatik mutasyonlardır.
Anahtar kelimeler: BCR-ABL1 negatif; JAK2 V617F; kalretikulin; mpl

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INTRODUCTION

Chronic myeloproliferative diseases (CMPD) are clonal stem cell disorders characterized by uncontrolled proliferation of myeloid lineage cells in the bone marrow. Polycythemia vera (PV), essential thrombocytosis (ET), and primary myelofibrosis (PMF) are bcr-abl negative CMPD, namely under the category of CMPD according to the 2008 World Health Organization (WHO) classification of hematopoietic tumors and the 2016 revision. After identifying of the JAK2 V617F mutation, the classification of CMPD has changed and existence of this mutation has been included in the diagnostic criteria of WHO. Thereafter, Myeloproliferative Leukemia Virus (MPL) and calreticulin (CALR) gene mutations were defined in the 2016 revised WHO classification of CMPD, with indicating their roles in the pathogenesis of CMPH[1,2].

The JAK2 V617F mutation occurs in exon 14 of the JAK2 gene and leads to a valine to phenylalanine substitution at codon 617, which consequently increases tyrosine kinase activity, thus resulting in clonal proliferation of one or more myeloid lineages [3]. JAK2 V617F mutation was observed in 90%-95% of patients with PV, in 50%-60% of ET, and in 40%-50% of PMF [4,5].

MPL gene encodes the thrombopoietin receptor, which is the main growth and differentiation factor for megakaryocytes. Exon 10 MPL numerous mutations have been identified such as W515K, W515L, W515S, and 505N. W515K/L is the most frequent mutation seen in CMPD. Mutation leads to thrombopoietin hypersensitivity and uncontrolled megakaryocyte proliferation. MPL mutations were observed in 3-5% of patients with ET and 5% of PMF [6,7].

Calreticulin is found in endoplasmic reticulum, which is involved in calcium regulation, gene activity, cell growth, cell division, cell adhesion, apoptosis regulation, and regulation of immune response. CALR gene contains nine exons. All mutations occur in exon 9 as insertion or deletion, except for a few point mutations. The most common mutations are 52-bp deletion (type 1 mutation) and 5-bp insertion (type 2 mutation). CALR mutations was reported in about 25% of patients with ET and in about 17% of patients with PMF[8-10].

The primary aim of this study is to discuss frequency and clinical effects of JAK2 V617F, MPL, CALR mutations in patients at initial diagnosis of bcr-abl negative CMPD. The secondary aim is to identify the genetic spectrum of Turkish patients and to compare with the literature.

MATERIALS AND METHODS

Thirty-nine patients with bcr-abl negative CMPD, who applied to Trabzon Kanuni Training and Research Hospital, Department of Hematology between July 2017, and March 2019, were investigated at initial diagnosis. Patients with PMF were excluded from outcome analysis due to small number (PMF: 2 subjects). Thirty-seven patients were included in the study.

Procedure

This study was approved by the Ethics Committee of University of Health Sciences Kanuni Training and Research Hospital (protocol number: 2019/03-date: 21.02.2019) and was carried out in accordance with the principles of the Helsinki Declaration. Classification of CMPD is defined according to the 2016 revision of the WHO classification of myeloid neoplasms. Bone marrow biopsy was performed in all patients.

The diagnosis of PV by the WHO requires the presence of all following three major criteria or the presence of the first two major criteria together with the minor criterion. Major criteria; 1. Increased hemoglobin level (>16.5 g/dL in men or >16.0 g/dL in women), hematocrit (>49 percent in men or >48 percent in women), or other evidence of increased red cell volume 2. Bone marrow biopsy showing hypercellularity for age with trilineage growth (panmyelosis) including prominent erythroid, granulocytic, and megakaryocytic proliferation with pleomorphic, mature megakaryocytes (differences in size) 3. JAK2 V617F or JAK2 exon 12 mutation. Minor criterion; serum erythropoietin level below the reference range for normal.

Diagnosis of ET by the WHO criteria require all four of the following major criteria or the first three major criteria plus the minor criterion. Major criteria; 1. Platelet count ≥450 x 109/L (≥450,000/microL) 2. Bone marrow biopsy showing proliferation mainly of the megakaryocyte lineage with increased numbers of enlarged, mature megakaryocytes with hyper lobulated nuclei. No significant increase or left shift in neutrophil granulopoiesis or erythropoiesis and very rarely minor (grade 1) increase in reticulin fibers.

3. WHO criteria for BCR-ABL1 positive chronic myeloid leukemia, polycythemia vera, primary myelofibrosis, myelodysplastic syndrome, or other myeloid neoplasm not met 4. Demonstration of a JAK2, CALR, or MPL mutation. Minor criterion; demonstration of another clonal marker (ASXL1, EZH2, TET2, IDH1/IDH2, SRSF2, or SR3B1 mutation) or no identifiable cause of thrombocytosis.

Risk stratification was defined for PV as high risk; age>60 years old or presence of thrombosis history and low risk; absence of both risk factors. Risk stratification was defined for ET as high risk; presence of thrombosis history or age>60 years old or platelets >1000x 10^9/L and low risk; age<60 years old and no thrombosis history.

Abdominal ultrasonography was performed in all patients and splenomegaly was mentioned when over 120 mm of spleen size.

Mutation analysis

Bone marrow sample specimens were used for all genetic test mentioned below. Common somatic CMPD mutations were examined from DNA. Automated DNA isolation was performed with MagNA Pure. In-house primers and probes used for detection of the JAK2 V617F mutation on the real-time PCR (Roche-LightCycler480). The real time procedure was carried out with the CALR RGQ PCR Kit (Qiagen) for type 1(52-bp deletion) and type 2 mutations (5-bp insertion) and ipsogen MPL W515L/K MutaScreen (Qiagen) on the Rotor-Gene Q instrument according to the manufacturer's instructions.

ET and PMF patients who were negative for JAK2 V617F were further evaluated for CALR and MPL mutations.

Statistical analysis

In the present cross-sectional study, it was aimed to find out the rates and values of the variables. According to the evaluation of the distribution, it is noticed that numerical variables are distributed as non-parametrically. Therefore, numerical variables have been summarized by their median and range, and categorical variables by count and relative frequency (%) of each category. Microsoft office excel was used to calculate variables.

RESULTS

Twenty-one (42.9%) of patients were diagnosed with PV and 16 (57.1%) were ET. Twenty-two 60.7%) of the them were female, 15 (%39.3) were male and the median age was 62 (38-89) years. Demographic, clinical and laboratory features at diagnosis of patients were illustrated in table 1.

All bone marrow biopsies were evaluated as hypercellular and bone marrow fibrosis grade were detected as less than grade 2 in 32 patients. Bone marrow fibrosis grade was detected as grade 2, in rest.

Table 1. Demographic, clinical and laboratory features at diagnosis

Variable	PV*; n=21 (56.75%)	ET**; n=16 (43.25%)	Total; n=37 (100%)
Gender			
Female	10 (47.6%)	12 (75%)	22 (59.5%)
Male	11 (52.4%)	4 (25%)	15 (40.5%)
Median age (minimum-maximum), year	62 (38-89)	53 (40-81)	59 (38-89)
Median hemoglobin (g/dL)	17.0 (12.1-20.4)	13.8 (12.0-16.8)	15.6 (12-20.4)
Median leucocytes ($\times 10^9$/L)	11.9 (5.3-23.8)	10.5 (6.5-21.8)	11.0 (5.3-23.8)
Median platelets ($\times 10^9$/L)	478 (151-2236)	759 (451-1189)	593 (151-2236)
Splenomegaly	9 (42.9%)	5 (31.3%)	14 (37.8%)
Constitutional symptoms	1 (4.7%)	1 (6.2%)	2 (5.4%)
Itching	8 (38%)	0 (0%)	8 (21.6%)
Bleeding	0(%0)	2 (12.5%)	2 (5.4%)
JAK2V617F mutation	18 (85.7%)	9 (56.3%)	27 (73%)
CALR mutation	-	4 (57.1%)	4 (57.1%)
Triple negative	-	3 (42.9%)	3 (42.9%)

*PV; polycythemia vera, **ET; essential thrombocytosis

Splenomegaly was noted in 14 (37.8%) of patients. Portal vein thrombosis was detected in two patients with both splenomegaly and PV at the time of diagnosis. The status of splenomegaly and thrombosis of the patients according to the disease subtypes and mutations was illustrated in table 2.

Table 2. The status of splenomegaly and thrombosis of the patients according to the disease subtypes and mutations

	PV*		**ET****		
	JAK2V617F Positive	**JAK2V617F Negative**	**JAK2V617F Positive**	**CALR Positive**	**Negative Triple**
	18 (85.7%)	3 (14.3%)	9 (56.3%)	4 (25%)	3 (18.7%)
Splenomegaly	9 (50%)	0(0%)	2 (22.2%)	3 (75%)	0(0%)
Artery-Venous Thrombosis	6 (33.3%)	0(0%)	1 (11.1%)	0(0%)	0(0%)

*PV; polycythemia vera**ET; essential thrombocytosis

JAK2 V617F mutation was detected in sum of 27 patients, 18 of them (85.7%) diagnosed with PV and rest of them (N=9, 56.2%) diagnosed with ET. Calreticulin mutation was detected in four (57.1%) patients diagnosed with ET who were negative for JAK2 V617F. CALR-type 1 mutation was detected in three patients and CALR-type 2 mutation was detected in one patient. MPL W515K/L wasn't detected in any of patients diagnosed with ET. Neither of mutations was detected in three of the patients with diagnosed ET, thus they were entitled as triple negatives.

Portal vein thrombosis was present in two patients with PV at the time of diagnosis and in whom JAK2 V617F mutation was positive. Medical history of five patients were remarkable for thromboembolic events (four patients with coronary artery disease and one patient with ischemic cerebrovascular event), and all were JAK2 V617F mutation positive.

Risk status according to the disease subgroups was given in table 3. Twenty-three (62.2%) patients were at high risk at the time of diagnosis and cytoreductive treatment was started. The treatment status of the patients according to risk groups and disease subtypes was shown in Table 4.

Table 3. The risk status according to the disease subgroups and the mutations

Risk status	**PV*; 21 (100%)**		**ET**; 16 (100%)**		
	JAK2V617F positive	JAK2V617F negative	JAK2V617F positive	CALR positive	Triple negative
High risk	16 (88.9%)	1 (33.3%)	3 (33.3%)	2 (50%)	1 (33.3%)
Low risk	2 (11.1%)	2 (66.7%)	6 (66.7%)	2 (50%)	2 (66.7%)

*PV; polycythemia vera **ET; essential thrombocytosis

Table 4. Treatment status of the patients according to risk groups and disease subtypes

	PV*;21 (100%)		**ET**;16 (100%)**	
High risk	HU¥ plus ASA§	5 (29,5%)	HU plus ASA	5 (83,3%)
	HU plus anticoagulant	3 (17,7%)	Anagrelide	1 (10,7%)
	HU plus ASA plus phlebotomy	8 (47%)		
	HU	1 (5,8%)		
Low risk	Phlebotomy	2 (50%)	ASA	10 (100%)
	Phlebotomy plus ASA	2 (50%)		

*PV; polycythemia vera **ET; essential thrombocytosis ¥HU; hydroxyurea §ASA; acetyl salicylic acid

DISCUSSION

JAK2 V617F, CALR and MPL mutations are driver mutations in bcr-abl negative CMPD, associated with clinical effects and outcomes. JAK2 V617F mutation is the most common mutation in bcr-abl negative CMPD and followed by CALR and MPL mutations. CALR and MPL mutations were detected generally in patients with essential thrombocythemia or myelofibrosis without JAK2 mutations.

In this study, we evaluated the mutation status and clinical effect of mutations in patients with bcr-abl negative CMPD at initial diagnosis. JAK2 V617F mutation was detected in patients with PV and ET as 85,7% and 56,2%, respectively. These results were similar with another study which was analysis of Turkish patients with chronic myeloproliferative neoplasms[11]. In the literature, JAK2 V617F mutation is detected in 80-90% of patients with PV, in about 50-60% in patents with ET or PMF.

CALR mutation was detected 57,1% of patients diagnosed with ET, who was negative for JAK2 V617F. MPL mutation was not detected in any patients. In another study from Turkey same results were obtained which evaluated frequency of CALR, JAK2 and MPL mutations in myeloproliferative neoplasm[12]. CALR mutation is the second common mutation in ET without JAK2 V617F which is reported in about 40-50%. MPL mutation is positive 5% in ET without JAK2 V617F[7,13-15]. Our data were compatible with the literature.

Type 1 and type 2 CALR mutations are found in more than 80% of all CALR-mutant patients[8,16]. Although our study included a small number of patients with ET, type 1 and 2 mutations (type 1; 3 patients, type 2; 1 patients) were found in all CALR-mutant patients.

In this study, thromboembolic event was detected in seven patients, six of them were PV and one was ET. Portal vein thrombosis was present in two patients with PV at the time of diagnosis and who were also JAK2 V617F mutation positive. Five patients had a medical history remarkable for thromboembolic event (four patients with coronary artery disease and one patient with ischemic cerebrovascular event), who have JAK2 V617F mutation. Our result was consistent with literature. In the literature, JAK2 V617F mutation represents as a strong independent risk factor for thrombosis. Rumi et al. identified 1235 consecutive patients diagnosed with ET or PV. In their study, patients with JAK2-mutated ET and with PV has higher incidence of thrombosis than CALR-mutated ET[17]. Klampfl T. et al. reported that CALR mutation was associated lower risk of thrombosis and the cumulative incidence at 10 years was 11.0% in CALR mutated group with ET[18]. Another study compared JAK2-mutated ET patients with CALR-mutated ET patients. They detected fewer thrombotic events in CALR mutated patients than JAK2 mutated patients (5.3% vs 20.5%)[13]. Rotunno G et al. reported that CALR mutated patients are less prone to thrombotic events compared with JAK2 and MPL mutated[19]. Reported higher thrombosis risk rate in JAK2-mutated ET patients may be partly attributable to the hyper-viscosity association with higher hematocrit and leukocyte levels[20]. Although our study included few patients, thromboembolic event was not detected any patients with CALR mutations.

Alberto Alvarez-Larrán et al. analyzed the benefit/risk of low dose aspirin in 433 patients with low-risk essential thrombocythemia (271 with a CALR mutation, 162 with a JAK2 V617F). They showed, low-dose aspirin does not reduce the risk of thrombosis and may increase the risk of bleeding in CALR-mutated essential thrombocythemia[21]. Another study evaluated 300 patients with low-risk ET and showed that antiplatelet therapy was effective in reducing the incidence of venous thrombosis in patients with the JAK2 V617F mutation positive and also reducing arterial thrombosis in patients with cardiovascular risk factors[22]. In our study, bleeding was not detected under low dose ASA therapy in CALR mutated patients with ET.

Rumi et al. found that patients with PV had higher frequency of splenomegaly compared with both CALR and JAK2 mutations positive ET patients[17]. Splenomegaly was detected 42,9% of patients with PV and 31,3% of patients with ET, in our study. Subgroup analysis between mutations could not be performed because of small number of patients.

Consistent mutation frequencies and clinical characteristics was observed in patients with bcr-abl negative CMPD in this study. JAK2 V617F, CALR and MPL are the most frequently identified somatic mutations in the pathogenesis of CMPD, which are now important in the diagnosis, risk classification and follow-up of the disease and gain importance in the personalized medicine. Limitations of this study are the small number of patients, the retrospective nature of the study, and an analysis involving a single center.

An additional prospective multicenter study should be performed to allow a more comprehensive genetic analysis and to assess the clinical significance.

Yazar Katkıları: Çalışma konsepti/Tasarımı: AU; Veri toplama: AU, ŞA; Veri analizi ve yorumlama: SU; Yazı taslağı: AU, ŞA, SU; İçeriğin eleştirel incelenmesi: AU, ŞA; Son onay ve sorumluluk: AU, ŞA, SÇ, SU, AHÇ; Teknik ve malzeme desteği: -; Süpervizyon: AU; Fon sağlama (mevcut ise): yok.
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