Check for updates

Clinical and Applied Thrombosis/Hemostasis
18(4) 421-426
© The Author(s) 2012
Reprints and permission:
sagepub.com/journalsPermissions.nav
DOI: 10.1177/1076029611427440
http://cath.sagepub.com

JAK2^{V617F} Mutation Is Not Associated with Thrombosis in Behcet Syndrome

M. Cem Ar, MD, PhD[1], Gülen Hatemi, MD[2], Seda Ekizoğlu, PhD[3], Hülya Bilgen, MD[4], Sevgi Saçlı, MD[5], A. Nur Buyru, PhD[3], Teoman Soysal, MD[6], Birsen Ülkü, MD[6], and Hasan Yazıcı, MD[2]

Abstract

The Janus kinase 2^{V617F} (*JAK2*^{V617F}) mutation is an acquired genetic defect that is considered to enhance thrombosis in Philadelphia chromosome-negative myeloproliferative neoplasms (MPNs). Thrombosis is also a well-defined component of Behcet syndrome (BS). The aim of this study was to determine the frequency of *JAK2*^{V617F} mutation in BS-associated thrombosis. A total of 152 patients with BS (62 with thrombosis and 90 without thrombosis) were enrolled. An additional 186 patients with MPNs and 107 healthy blood donors were included to serve as diseased and healthy controls, respectively. None of the patients with BS and healthy controls carried the *JAK2*^{V617F} mutation, whereas 67% of patients with MPNs were positive for *JAK2*^{V617F}. The frequency of thrombosis in patients with MPNs was not statistically different between carriers and non-carriers of *JAK2*^{V617F} mutation. Our data suggest that *JAK2*^{V617F} is not directly related to thrombosis in MPNs and in other thrombotic entities, such as BS.

Keywords

JAK2, thrombosis, Behcet Syndrome, myeloproliferative neoplasia

Introduction

The Janus kinase 2^{V617F} (*JAK2*^{V617F}) point mutation is a recently identified acquired genetic defect characteristically present in a majority of Philadelphia chromosome-negative myeloproliferative neoplasms (MPNs), including polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF).[1-3] Since MPNs are frequently complicated by arterial and/or venous thrombosis, there has been considerable interest whether *JAK2*^{V617F} per se was associated with increased risk of thrombosis.

Hypothetically, *JAK2*^{V617F} mutation was proposed to enhance thrombosis mainly by creating a hypercoagulable state through rheological changes, activation of platelets, and leukocytes and by inducing hemostatic mechanisms in patients with PV and ET.[4,5] However, the exact mechanism by which this mutation causes thrombosis is not clear. Additional mechanisms, such as increased expression of adhesion molecules on red blood cells, platelet hyperresponsiveness, elevated soluble P-selectin levels, increased levels of circulating platelet–leukocyte aggregates, and disordered hemostasis, that attributed to the malfunctioning *JAK2* gene have been reported.[6-8][Please approve edits made to the sentence "Additional mechanisms that . . . ."]

Whether the presence of the *JAK2*^{V617F} mutation, per se, modifies the thrombotic risk is currently debated. There is some clinical evidence to suggest that the mutation may variably be associated with thrombosis in MPNs, especially in patients with ET.[9] An increased rate of *JAK2*^{V617F} mutation has also been found in patients with unprovoked splanchnic vein thrombosis (SVT).[10-12] This finding encouraged other investigators to search for the thrombogenic potential of the *JAK2*^{V617F} mutation in other diseases. However, no association between *JAK2*^{V617F} and thrombosis has been demonstrated outside MPN, so far.[13,14] We, therefore, thought that it would be interesting to know the relation of *JAK2*^{V617F} mutation to thrombosis in a clinical condition that frequently presents with thrombotic events such as Behcet syndrome (BS).

Thrombosis is a well-defined feature of BS, and thrombotic complications are observed in more than one third of the patients.[15] The majority of vascular events involve veins; arterial thrombosis is less prominent.[16] Thrombotic events most commonly occur within the 5 years of disease onset.[17]

[1] Division of Hematology, Department of Internal Medicine, Istanbul Training and Research Hospital, Istanbul, Turkey
[2] Division of Rheumatology, Department of Internal Medicine, Istanbul University Cerrahpasa Medical Faculty, Istanbul, Turkey
[3] Department of Medical Biology, Istanbul University Cerrahpasa Medical Faculty, Istanbul, Turkey
[4] Blood Bank, Istanbul Bilim University, Istanbul, Turkey
[5] Department of Internal Medicine, Istanbul University Cerrahpasa Medical Faculty, Istanbul, Turkey
[6] Division of Hematology, Department of Internal Medicine, Istanbul University Cerrahpasa Medical Faculty, Istanbul, Turkey

Corresponding Author:
M. Cem Ar, Department of Hematology, Istanbul Training and Research Hospital, Fatih-Istanbul, Turkey
Email: muhcar@superonline.com

However, in up to 30% of patients, vascular involvement has been reported to precede the diagnosis of BS.[18]

Pathogenesis of thrombosis in BS has not been fully elucidated. Microvascular dysfunction, vascular cell injury as reflected by high levels of von Willebrand factor and thrombomodulin, platelet and leukocyte activation, and increased expression of adhesion molecules on endothelial cells are some of the mechanisms contributing to thrombosis in BS.[19-23] Data at hand show that there is no underlying abnormality in the hemostatic systems of the body.[24-26] The role of thrombophilic factors in BS-associated thrombosis has also been investigated by several groups. However, the results were conflicting and mostly inconclusive.[27-34]

The aim of this study was to detect a potential relationship between *JAK2*^{V617F} mutation and BS-associated thrombosis and to compare this with the thrombotic events in MPNs.

Materials and Methods

Patients

In this cross-sectional survey between January 2009 and September 2009, we consecutively enrolled 62 patients with BS with acute or chronic arterial and/or venous thrombosis, 90 patients with BS and without thrombosis, and 107 healthy blood donors. A cohort of 186 patients with Ph(−) MPNs, diagnosed according to the World Health Organization criteria[35,36] and followed at the Hematology Department of Cerrahpasa Medical Faculty, Istanbul University, were also included. Since thrombosis in BS rarely occurs in women,[18] only male patients of older than equal to 18 years of age with BS, who fulfilled the International Study Group criteria[37] for BS, were included in the study. All patients with BS with acute and/or chronic arterial/venous thrombosis were designated as having "BS with thrombosis." Knowing that the majority of BS-related thrombotic events generally occur either before or within the first 5 years of the diagnosis,[17] we defined patients with "BS without thrombosis" as those with a BS diagnosis of more than 5 years and having no history of vascular involvement.

Healthy controls consisted of male blood donors at the Blood Bank of Cerrahpasa Medical Faculty, Istanbul University for blood donation. They were included only if they did not have a history of arterial or venous thrombotic event and did not have clinical signs of thrombosis on examination.

Patients with a diagnosis of Ph(−) MPNs, mainly PV, served as diseased controls. The thrombotic events that these patients had experienced were recovered from patient charts retrospectively.

All patients and controls were inhabitants of Istanbul. Considering its ethnic composition, Istanbul's population adequately represents Turkish population. The study was approved by the local ethics committee and conducted according to the principles of the Declaration of Helsinki. All included patients and healthy volunteers gave written consent before entering the study.

Methods

The *JAK2*^{V617F} mutation was detected by allele-specific polymerase chain reaction (PCR) in all recruited patients. Venous blood (2 mL) was drawn into EDTA-containing sample tubes (Greiner Bio-One GmBH, Kremsmünster, Austria) from patients and healthy controls by peripheral venipuncture. Using standard techniques as described elsewhere,[38] DNA was extracted from blood samples and stored at −70°C. Polymerase chain reaction amplification for the detection of *JAK2*^{V617F} and visualization of the PCR products were carried out using a commercial assay (Seeplex *JAK2* Genotyping Kit; Seegene, Seoul, Korea).

Statistical Analysis

Statistical analysis was done with Statistical Package for the Social Sciences software, release 15.0 (SPSS Inc, Chicago, Illinois). Mann-Whitney *U* test was used for comparison. Odds ratios (ORs) were calculated to determine the role of *JAK2*^{V617F} mutation in thrombotic events in patients with MPN.

Results

Demographic and clinical features of the 152 patients with BS, 186 patients with MPNs, and 107 healthy controls are given in Table 1. Among patients with BS, 62 had a history of documented thrombosis. Almost all of the thrombotic events were of venous origin; the deep vein thrombosis being the most frequently observed type of presentation.

Among the 186 patients with MPNs, who constituted the diseased control group, PV was the most frequent MPN, followed by ET and PMF. In 5 patients, the subgroup of MPNs could not be identified. A total of 42 venous and arterial thrombotic events with almost equal frequencies had been observed in 36 (19.2%) of the 186 patients. Half of the venous thrombosis were located intra-abdominally.

None of the patients with BS, with or without thrombosis, had a *JAK2*^{V617F} mutation. The healthy controls were also negative for the *JAK2*^{V617F} mutation, whereas 67% of patients with MPNs were found to harbor the mutation.

There was no statistically significant difference between patients with *JAK2*^{V617F} (+) and (−) MPN in terms of the frequency of arterial and venous thrombotic events ($P = .098$; Table 2). We noted, however, that 8 of 9 patients with SVT and 7 of 10 patients with ischemic cerebrovascular events (CVEs) were carriers of *JAK2*^{V617F} mutation. Three patients had both arterial and venous thromboses.

Irrespective of the *JAK2*^{V617F} mutational status, patients with arterial thrombosis including cerebral events were significantly older than those without thrombosis ($P < .001$). The frequency of venous thrombotic events, on the contrary, did not seem to be significantly influenced by age ($P = .311$) although the median age was slightly higher in patients with venous thrombosis harboring the *JAK2*^{V617F} mutation (Table 3).

Table 1. Patient Characteristics

Study Groups	BS	MPN	HC
Number of participants	152	186	107
Age, median (range)[a]	30 (18-55)	54 (15-86)	32 (24-45)
Sex, M:F	152:0	102:84	107:0
Pts. with thrombosis, n (%)	62 (40.8)	36 (19.4)	0
Venous thrombosis, n[b]	62	22	
DVT	58	10	
PE	1	2	
SVT	1	3	
PVT		4	
MVT		2	
CVT	2	1	
Arterial thrombosis, n[b]	1	20	
MI	1	5	
PAO		5	
CVA		10	
MPN subgroups			
PV		106	
ET		49	
PMF		26	
Unclassified		5	
JAK2 (+), n (%)	0	125 (67.2)	0

Abbreviations: BS, Behcet syndrome; CVA, cerebrovascular accident (ischemic); CVT, cerebral venous thrombosis; DVT, deep vein thrombosis; ET, essential thrombocythemia; HC, healthy controls; PMF, primary myelofibrosis; MI, myocardial infarction; MPN, myeloproliferative neoplasm; MVT, mesenteric vein thrombosis; PAO, peripheral arterial occlusion; PE, pulmonary embolus; PV, polycythemia vera; PVT, portal vein thrombosis; SVT, splanchnic vein thrombosis; *JAK2*, Janus kinase 2.

[a] Median age of patients in groups BS and HC was not statistically different ($P = .85$).

[b] Total number of thrombotic episodes exceeds the number of patients with thrombosis in study groups BS and MPN since some of the patients had both arterial and venous thrombosis or venous thrombosis at more than 1 site.

Eighteen patients with PV and one patient with ET carried $JAK2^{V617F}$ mutation in homozygous state (17% and 2%, respectively). Homozygosity of $JAK2^{V617F}$ mutation was not found to be associated with increased frequency of thrombosis ($P = .884$) in patients with PV.

Discussion

The current study clearly demonstrates that the $JAK2^{V617F}$ mutation is prevalent in neither BS nor BS-associated thrombosis. Thrombosis is usually observed in about one third of the patients with BS.[15] Endothelial cell damage and activation of leucocytes, platelets, as well as the coagulation system have been accused to lead to thrombosis in BS.[20-26] Thrombotic complications in BS usually involve venous system and present as deep vein thrombosis of the lower extremities and/or superficial thrombophlebitis in most of the cases.[16] Although much more rare than in MPNs, SVT is also observed in patients with BS.[39]

Although thrombosis in BS is mainly attributed to vascular inflammation and endothelial damage, the role of thrombophilia in creating a tendency for thrombosis is also frequently considered. Several studies have been published on the frequency of various thrombophilic factors among patients with BS with thrombosis, with conflicting results.[27-34] Some studies showed an increased risk of thrombosis associated with factors such as increased thrombin activatable fibrinolysis inhibitor levels[25] or activated protein C levels,[32] while others failed to show such associations.[30-31] A recent meta-analysis showed that hyperhomocysteinemia may be associated with thrombosis in patients with BS,[29] whereas another study proposed an association between factor V Leiden (FVL) and prothrombin mutations and thrombosis in BS.[34]

Similar to BS, thrombotic manifestations are observed in about 10% to 30% of patients with myeloproliferative disorders.[4] Thrombosis, in fact, is the most important cause of morbidity and mortality in MPNs, particularly in PV and ET.[4,40] It usually prefers veins more than arteries. Localization of venous thrombosis is unusual with a high frequency of splanchnic vein involvement.[41]

In our cohort of patients with MPNs, 9 of the 22 venous thrombotic events (41% of venous thrombosis and 21% of all thrombotic events) were observed in the splanchnic veins. Of these 9 (7 PV and 2 ET) patients, 8 (88%) were carriers of $JAK2^{V617F}$ mutation. Increased frequency of intra-abdominal vein thrombosis in MPNs can partially be explained by impaired blood flow and pooling of blood in splanchnic area due to splenomegaly. Impaired rheological and hemostatic functions seem to set up the scene for increased thrombotic risk.[5,7,8] Whether the presence of $JAK2^{V617F}$ mutation directly triggers the events leading to thrombosis or induces indirectly a thrombotic background via MPNs is still unclear. Dentali et al[42] has recently reported that about 50% of patients without MPNs presenting with SVT were $JAK2^{V617F}$-positive. More than half of these patients developed signs of MPN, subsequently. This suggests that $JAK2^{V617F}$ positive mutation might have a direct role in the development of SVT through some yet unexplained mechanism. Although in our study, $JAK2^{V617F}$ positivity among patients with SVT was higher than in patients with venous thrombosis at other sites (8 of 9 vs 7 of 13), we could not demonstrate a significant difference ($P = .17$).

Cerebral venous thrombosis (CVT) is another rare but typical clinical manifestation associated with MPNs and BS.[43,44] The frequency of CVT has been found to be 1% and 7.8% in large series of patients with ET and BS, respectively.[44,45] In our study, CVT was detected in 2 patients with BS and 1 patient with ET. They all were $JAK2^{V617F}$ negative.

Ischemic CVEs have also been frequently observed in patients with MPNs.[46] Their association with $JAK2^{V617F}$ mutation is another issue of discussion. In our cohort, 7 of the 10 patients with ischemic CVEs carried the $JAK2^{V617F}$ mutation. The frequency was not significantly different among patients with arterial thrombosis at other sites ($P = .62$). The carriers were mainly male patients with PV (6 PV and 1 ET). We could not explain the occurrence of ischemic CVEs with the presence of $JAK2^{V617F}$ mutation alone since most of these patients were of older age (median 68 years) and had comorbidities predisposing them to ischemic events (hypercholesterolemia, n = 8; arterial hypertension, n = 6; diabetes mellitus, n = 2; and coronary

Table 2. Thrombotic Events in Patients with MPN

	Arterial Thrombosis			Venous Thrombosis			Thrombotic Events (Total)		
	Present	Absent	Odds Ratio	Present	Absent	Odds Ratio	Present	Absent	Odds Ratio
JAK2 (+)	14	111	1.06	15	110	1.79	26	99	1.34
JAK2 (−)	6	55	(95% CI: 0.38-2.95)	7	54	(95% CI: 0.56-5.71)	10	51	(95% CI: 0.60-2.99)
Total	20	166		22	164		36	150	

Abbreviations: MPN, myeloproliferative neoplasm; *JAK2*, Janus kinase 2; CI, confidence interval.

Table 3. Median Age (Years) at the Time of First Thrombotic Event in Patients with MPN

	Arterial Thrombosis		Venous Thrombosis		Thrombotic Events (Total)	
	Present	Absent	Present	Absent	Present	Absent
JAK2 (+)	62	52	56	55	60.5	51
JAK2 (−)	59	50	47	51	57	50

Abbreviation: *JAK2*, Janus kinase 2.

artery disease, n = 2). Interestingly, Zerjavic et al asserted having found a significant association between ischemic stroke and *JAK2*^{V617F} mutation in patients without MPNs.[47]

Arterial thrombosis including CVEs was diagnosed in 20 (11%) of our patients with MPN. Recently, 3 large meta-analyses showed evidence for increased risk of arterial thrombosis in patients with *JAK2*^{V617F}-positive ET (OR, 1.68-2.09).[48-50] In contrast to current literature, we could not demonstrate a significantly elevated risk for arterial thrombotic events in patients with MPNs carrying the *JAK2*^{V617F} mutation. This may result from the relatively low number of patients with ET within the study population. Retrospective design of the study should also be considered as a limitation.

Despite existing controversial results mentioned above, there is some clinical evidence to suggest that the *JAK2*^{V617F} mutation may be variably associated with thrombosis. *JAK2*^{V617F} mutation has been reported by Pardanani et al[51] in 4 of the 210 patients with deep vein thrombosis and/or pulmonary embolism and in 1 of 8 patients with cerebral vein thrombosis. None of the patients harboring *JAK2*^{V617F} mutation met the diagnostic criteria for MPNs. This suggests that *JAK2*^{V617F} mutation may, albeit rarely, be associated with thrombosis with no other clinical associations or may accompany thrombosis-related clinical entities other than MPNs. However, subsequent studies among patients with idiopathic venous thromboembolism (VTE) could not demonstrate any convincing results to designate *JAK2*^{V617F} mutation as an independent risk factor for thrombosis.[52,53] Therefore, screening for *JAK2*^{V617F} mutation in idiopathic thrombotic events is currently not recommended in the absence of findings associated with overt or latent MPN.[54] The significance of *JAK2*^{V617F} mutation in other thrombosis prone clinical entities is yet to be determined.

A number of studies indicated that *JAK2*^{V617F} mutation is absent in healthy control groups as we observed in this study.[55,56] Although speculative, it is interesting to note that acquired mutations like *JAK2*^{V617F}, rarely occur in healthy participants. They seem to be almost always associated with certain clinical conditions. Hereditary mutations, on the other hand, are frequently observed in healthy populations without always causing disease; as it is the case with FVL. The prevalence of FVL in normal Turkish population is about 8%.[57] It has been stated that up to 50% of FVL carriers experience VTE during their lifetime, if they were born to symptomatic families.[58] Accordingly, the presence of FVL does not result in thromboembolic events in more than half of the carriers.

In conclusion, our findings suggest that there is no evidence for *JAK2*^{V617F} mutation occurrence among healthy participants and patients with BS. In fact, no clinical condition other than MPNs has been identified so far to be strongly associated with *JAK2*^{V617F}. The only clinical entity reported to be associated with increased frequency of *JAK2*^{V617F} mutation in the absence of MPNs seems to be the "unprovoked" SVT.[41,59] We found that *JAK2*^{V617F} mutation was frequent in patients with MPN associated SVT and CVEs. But this occurred probably as a result of the relatively higher number of *JAK2*^{V617F}-positive patients in our cohort. In contrast to most of the current literature,[48-50,54] we could not demonstrate a statistically significant difference in the frequency of thrombotic events among patients with MPN with and without *JAK2*^{V617F} mutation. We, therefore, speculate that the thrombogenicity observed in MPNs is multifactorial and does not solely depend on *JAK2*^{V617F} positivity.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship and/or publication of this article: This study

was partially funded by the unrestricted grants of Eczacıbaşı-Baxter Hospital Supply Inc. and Berk İlaç A.Ş.

References

1. James C, Ugo V, Le Couédic JP, et al. A unique clonal *JAK2* mutation leading to constitutive signalling causes polycythaemia vera. *Nature*. 2005;434(7037):1144-1148.
2. Kralovics R, Passamonti F, Buser AS, et al. A gain-of-function mutation of *JAK2* in myeloproliferative disorders. *N Engl J Med*. 2005;352(17):1779-1790.
3. Baxter EJ, Scott LM, Campbell PJ, et al. (Cancer Genome Project). Acquired mutation of the tyrosine kinase *JAK2* in human myeloproliferative disorders. *Lancet*. 2005;365(9464):1054-1061.
4. Elliot MA, Tefferi A. Thrombosis and haemorrhage in polycythaemia vera and essential thrombocythaemia. *Br J Haematol*. 2004;128(3):275-290.
5. Pearson TC. Hemorheologic considerations in the pathogenesis of vascular occlusive events in polycythemia vera. *Semin Thromb Hemost*. 1997;23(5):433-439.
6. Alvarez-Larrán A, Arellano-Rodrigo E, Reverter JC, et al. Increased platelet, leukocyte, and coagulation activation in primary myelofibrosis. *Ann Hematol*. 2008;87(4):269-276.
7. Falanga A, Marchetti M, Vignoli A, Balducci D, Barbui T. Leukocyte-platelet interaction in patients with essential thrombocythemia and polycythemia vera. *Exp Hematol*. 2005;33(5):523-530.
8. Falanga A, Marchetti M, Vignoli A, et al. V617F *JAK-2* mutation in patients with essential thrombocythemia: relation to platelet, granulocyte, and plasma hemostatic and inflammatory molecules. *Exp Hematol*. 2007;35(5):702-711.
9. Wong RS, Cheng CK, Chan NP, et al. *JAK2*V617F mutation is associated with increased risk of thrombosis in Chinese patients with essential thrombocythaemia. *Br J Haematol*. 2008;141(6):902-904.
10. Remacha AF, Estivill C, Sarda MP, et al. The V617F mutation of *JAK2* is very uncommon in patients with thrombosis. *Haematologica*. 2007;92(2):285-286.
11. Bayraktar Y, Harmanci O, Büyükasik Y, et al. *JAK2*V617F mutation in patients with portal vein thrombosis. *Dig Dis Sci*. 2008;53(10):2778-2783.
12. De Stefano V, Fiorini A, Rossi E, et al. Incidence of the *JAK2*V617F mutation among patients with splanchnic or cerebral venous thrombosis and without overt chronic myeloproliferative disorders. *Thromb Haemost*. 2007;5(4):708-714.
13. Karimi O, Crusius JBA, Coucoutsi C, et al. *JAK2* V617F mutation is not involved in thromboembolism in IBD. *Inflamm Bowel Dis*. 2008;14(11):1606-1607.
14. Fouassier M, Girodon F, Cleyrat C, et al. Absence of *JAK2*-V617F in paroxysmal nocturnal haemoglobinuria-associated thrombosis. *Thromb Haemost*. 2009;102(1):180-182.
15. Yazici H, Yurdakul S, Hamuryudan V. Behçet's syndrome. In: Maddison PJ, Isenberg DA, Woo P, Glass DN, eds. *Oxford Textbook of Rheumatology.* 2nd ed. Oxford: Oxford University Press; 1998:1394-1402.
16. Duzgun N, Ates A, Aydintug OT, Demir O, Olmez U. Characteristics of vascular involvement in Behçet's disease. *Scand J Rheumatol*. 2006;35(1):65-68.
17. Kural-Seyahi E, Fresko I, Seyahi N, et al. The long-term mortality and morbidity of Behcet syndrome: a 2-decade outcome survey of 387 patients followed at a dedicated center. *Medicine (Baltimore)* 2003;82(1):60-76.
18. Sarica-Kucukoglu R, Akdag-Kose A, Kayabal IM, et al. Vascular involvement in Behcet's disease: a retrospective analysis of 2319 cases. *Int J Dermatol*. 2006;45(8):919-921.
19. Gül A. Behçet's diease: an update on the pathogenesis. *Clin Exp Rheumatol*. 2001;19(5 suppl 24):S6-S12.
20. Özoran K, Düzgün N, Gürler A, Tutkak H, Tokgöz G. Plasma von Willebrand factor, tissue plasminogen activator, plasminogen activator inhibitor, and antitrombin III levels in Behçet's disease. *Scand J Rheumatol*. 1995;24(6):376-382.
21. Alpagut U, Ugurlucan M, Dayioglu E. Major arterial involvement and review of Behcet's disease. *Ann Vasc Surg*. 2007;21(2):232-239.
22. Haznedaroğlu I, Özcebe OI, Özdemir O, Celik I, Dündar SV, Kirazli S. Impaired haemostatic kinetics and endothelial function in Behçet's disease. *J Inter Med*. 1996;240(4):181-187.
23. Koç Y, Güllü I, Akpek G, et al. Vascular involvement in Behcet's disease. *J Rheumatol*. 1992;19(3):402-410.
24. Ricart JM, Ramón LA, Vayá A, et al. Fibrinolytic inhibitor levels and polymorphisms in Behçet disease and their association with thrombosis. *Br J Haematol*. 2008;141(5):716-719.
25. Dönmez A, Aksu K, Aydin H, et al. The plasma levels of activated thrombin activatable fibrinolysis inhibitor and thrombomodulin in Behçet disease and their association with thrombosis. *Thromb Res*. 2010;126(3):207-210.
26. Espinosa G, Font J, Tàssies D, et al. Vascular involvement in Behçet's disease: relation with thrombophilic factors, coagulation activation, and thrombomodulin. *Am J Med*. 2002;112(1):37-43.
27. Navarro S, Ricart JM, Medina P, et al. Activated protein C levels in Behçet's disease and risk of venous thrombosis. *Br J Haematol*. 2004;126(4):550-556.
28. Lenk N, Özet G, Alli N, Coban O, Erbaşi S. Protein C and protein S activities in Behçet's disease as risk factors of thrombosis. *Int J Dermatol*. 1998;37(2):124-125.
29. La Regina M, Orlandini F, Prisco D, Dentali F. Homocysteine in vascular Behcet's disease: a meta-analysis. *Arterioscler Thromb Vasc Biol*. 2010;30(10):2067-2074.
30. Ricart JM, Vayá A, Todolí J, et al. Thrombophilic risk factors and homocysteine levels in Behçet's disease in eastern Spain and their association with thrombotic events. *Thromb Haemost*. 2006;95(4):618-624.
31. Leiba M, Seligsohn U, Sidi Y, et al. Thrombophilic factors are not the leading cause of thrombosis in Behcet's disease. *Ann Rheum Dis*. 2004;63(11):1445-1449.
32. Gül A, Özbek A, Öztürk C, Inanç M, Koniçe M, Ozçelik T. Coagulation factor V gene mutations increases the riks of venous thrombosis in Behcet's disease. *Br J Rheumatol*. 1996;35(11):1178-1180.
33. Verity DH, Vaughan RW, Madanat W, et al. Factor V Leiden is associated with ocular involvement in Behçet's disease. *Am J Ophthalmol*. 1999;128(3):352-356.
34. Toydemir PB, Elhan AH, Tükün A, et al. Effect of factor V gene G1691A, methylenetetrahydrofolate recuctase gene C677 T and

prothrombin gene G20210A mutations on deep venous thrombogenesis in Behçet's disease. *J Rheumatol*. 2000;27(12):2849-2854.
35. Thiele J, Pierre R, Imbert M, et al. Polycythemia vera, chronic idiopathic myelofibrosis, essential thrombocythemia. In: Jaffe ES, Harris NL, Stein H, Varidman JW, eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues.* 3rd ed. Lyon, France: IARC Press; 2001: 32-44.
36. Thiele J, Kvasnicka HM, Tefferi A, et al. Polycythemia vera, primary myelofibrosis, essential thrombocythemia. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Varidman JW, eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008: 40-50.
37. Wechsler B, Davatchi F, Mizushima Y, et al. (International Study Group for Behçet's Disease). Criteria for diagnosis of Behçet's disease. *Lancet*. 1990;335(8697):1078-1080.
38. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res*. 1988;16(3):1215.
39. Calamia KT, Schirmer M, Melikoglu M. Major vessel involvement in Behcet disease. *Curr Opin Rheumatol*. 2005;17(1):1-8.
40. Barbui T, Firazzi G, Negri M, et al. (Gruppo Italiano Studio Policitemia). Polycythemia vera: the natural history of 1213 patients followed for 20 years. *Ann Intern Med*. 1995;123(9): 656-664.
41. Kiladjian JJ, Cervantes F, Leebeek FW, et al. The impact of *JAK2* and MPL mutations on diagnosis and prognosis of splanchnic vein thrombosis: a report on 241 cases. *Blood*. 2008;111(10): 4922-4929.
42. Dentali F, Squizzato A, Brivio L, et al. *JAK2*V617F mutation for the early diagnosis of Ph-myeloproliferative neoplasms in patients with venous thromboembolism: a meta-analysis. *Blood*. 2009;113(22):5617-5623.
43. Stam J. Thrombosis of the cerebral veins and sinuses. *N Engl J Med*. 2005;352(17):1791-1798.
44. Saadoun D, Wechsler B, Resche-Rigon M, et al. Cerebral venous thrombosis in Behçet's disease. *Arthritis Care Res*. 2009;61(4): 518-526.
45. Bazzan M, Tamponi G, Schinco P, et al. Thrombosis-free survival and life expectancy in 187 consecutive patients with essential thrombocythemia. *Ann Hematol*. 1999;78(12):539-543.
46. Bang S, Lee JS, A JY, et al. Vascular events in Korean patients with myeloproliferative neoplasms and their relationship to *JAK2* mutation. *Thromb Haemost*. 2009;101(3):547-551.
47. Zerjavic K, Zagradisnik B, Stangler Herodez S, Lokar L, Glaser Krasevac M, Kokalj Vokac N. Is the *JAK2* V617F mutation a hallmark for different forms of thrombosis. *Acta Haematol*. 2010; 124(1):49-56.
48. Ziakas PD. Effect of *JAK2* V617F on thrombotic risk in patients with essential thrombocythemia: measuring the uncertain. *Haematologica*. 2008;93(9):1412-1414.
49. Dahabreh IJ, Zoi K, Giannouli S, Zoi C, Loukopoulos D, Voulgarelis M. Is *JAK2* V617F mutation more than a diagnostic index? A meta-analysis of clinical outcomes in essential thrombocythemia. *Leuk Res*. 2008;33(1):67-73.
50. Lussana F, Caberlon S, Pagani C, Kamphuisen PW, Büller HR, Cattaneo M. Association of V617F *JAK2* mutation with the risk of thrombosis among patients with essential thrombocythaemia or idiopathic myelofibrosis: a systematic review. *Thromb Res*. 2009;124(4):409-417.
51. Pardanani A, Lasho TL, Schwager S, et al. *JAK2*V617F prevalence and allele burden in non-splanchnic venous thrombosis in the absence of overt myeloproliferative disorder. *Leukemia*. 2007;21(8):1828-1829.
52. Rossi D, Cresta S, Destro T, et al. *JAK2*V617F in idiopathic venous thromboembolism occurring in the absence of inherited or acquired thrombophilia. *Br J Haematol*. 2007;138(6):813-814.
53. Ugo V, Le Gal G, Lecucq L, Mottier D, Oger E, EDITH Collaborative Study Group. Prevalence of the *JAK2* V617F mutation is low among unselected patients with a first episode of unprovoked venous thromboembolism. *Thromb Haemost*. 2007; 6(1):203-205.
54. Vannucchi AM. *JAK2* mutation and thrombosis in the myeloproliferative neoplasms. *Curr Hematol Malig Rep*. 2010;5(1):22-28.
55. Passamonti F, Rumi E, Pietra D, Lazzarino M, Cazzola M. *JAK2* (V617F) mutation in healthy individuals. *Br J Haematol*. 2007; 136(4):678-679.
56. Xu X, Zhang Q, Luo J, et al. *JAK2* (V617F): prevalence in a large Chinese hospital population. *Blood*. 2007;109(1):339-342.
57. Akar N. Factor V1691GA mutation in a healthy Turkish population. *Turk J Hematol*. 2009;26(1):9-11.
58. Lensen R, Rosendaal F, Vandenbroucke J, Bertina R. Factor V Leiden: the venous thrombotic risk in thrombophilic families. *Br J Haematol*. 2000;110(4):939-945.
59. Regina S, Herault O, D'Alteroche L, Binet C, Gruel Y. *JAK2* V617F is specifically associated with idiopathic splanchnic vein thrombosis. *Thromb Haemost*. 2007;5(4):859-861.