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Diagnostic value of the JAK2 V617F mutation for latent chronic myeloproliferative disorders in patients with budd-chiari syndrome and/or portal vein thrombosis

LIVER

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Original Article

ABSTRACT

Background/Aims: The diagnosis of an underlying myeloproliferative neoplasm (MPN) is often problematic in patients with Budd Chiari syndrome (BCS) or portal vein thrombosis (PVT). This study aimed to assess the diagnostic value of the JAK2 gene V617F gain-of-function mutation for MPN in splanchnic vein thrombosis patients.

Materials and Methods: One hundred eleven patients (80 with PVT, 27 with BCS, and 4 with BCS and PVT) were investigated. The control group included 56 volunteers without any known diseases. LightCycler SNP genotyping was performed to detect the JAK2 V617F mutation in DNA extracted from peripheral blood.

Results: The JAK2 V617F mutation was identified in six of 28 patients (21.4%) with idiopathic PVT or BCS and in eight of 45 patients (17.8%) with PVT or BCS secondary to a known prothrombotic factor, but in only one of 38 patients (2.6%) with PVT and cirrhosis (p=0.049).

Conclusion: The JAK2 V617F mutation is a noninvasive molecular marker for occult MPNs and can be used for the diagnosis of latent MPNs presenting with thrombotic events. Analysis of JAK2 mutation in the patients with idiopathic PVT or BCS showed that 20% had latent MPNs. In addition to this JAK2 mutation, prothrombotic events were observed in a significant number of patients with splanchnic vein thrombosis. JAK2 gene analysis should be included in the research panel for BCS and PVT patients without cirrhosis.

Keywords: JAK2 V617F mutation, Budd Chiari syndrome, portal vein thrombosis, myeloproliferative neoplasms

INTRODUCTION

Portal vein thrombosis (PVT) and Budd-Chiari syndrome (BCS) are relatively rare disorders; however, can be fatal if the underlying etiological factors are not diagnosed and treated. PVT and BCS have similar symptoms (splenomegaly, ascites, pain, and melena, etc.) and etiological factors (factor V Leiden (FVL) mutation, coagulation factor II (F2 (prothrombin) G20210A mutation, protein C and protein S deficiency, and myeloproliferative neoplasms (MPNs)). The diagnosis of etiological factors in both diseases greatly affects prognosis.

Liver cirrhosis is among the most common acquired risk factors for PVT, responsible for approximately 20% of all PVT cases (1). The high prevalence of PVT in patients with cirrhosis (10%-20%) has been attributed to a decrease in portal blood flow and the presence of periportal lymphangitis and fibrosis (2). Moreover, acquired and inherited clotting abnormalities may play a role in the pathogenesis of PVT (1). The F2 G20210A mutation is more common in PVT patients with liver cirrhosis than the C677>T substitution mutation of the methylenetetrahydrofolate reductase (MTHFR) gene (a mutation causing increased plasma levels of homocysteine, a thrombotic factor) or FVL mutation (3-7).

Janus kinase 2 (JAK2) is a cytoplasmic tyrosine kinase that transduces signals triggered by hematopoietic growth factors such as erythropoietin in normal and neoplastic cells. The acquired JAK2 gene mutation on chromosome 9 (JAK2 V617F) is associated with polycythemia vera (PV) and other related MPNs (8). The reported prevalence of the JAK2 mutation has ranged from 65%-97% in PV patients from Europe and North America, 23%-57% in patients with essential thrombocythemia (ET), and 35%-57% in primary myelofibrosis

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(PMF) patients (8-14). The contributions of JAK2 mutation to MPN pathogenesis, disease phenotype, leukemic transformation, and stem cell involvement are unclear at present (15-18).

In this study, we focused on the role of the JAK2 V617F mutation in patients with BCS or PVT, with or without liver cirrhosis. It remains unclear if the JAK2 V617F mutation is associated with an increased risk of thrombosis. A higher rate of thrombotic complications has been reported in some MPN patients with the JAK2 V617F mutation than in those without the mutation; however, this association has not been observed in other studies (19-25). On the other hand, in patients with PVT and/or BCS, factors such as hypersplenism and hemodilution may mask underlying MPNs, which can also occur in patients with both liver cirrhosis and portal hypertension (5).

The present study aimed to determine the prevalence of the JAK2 V617F mutation in patients with BCS and/or PVT. The JAK2 V617F mutation was investigated in 3 groups of patients: PVT or BCS, PVT secondary to cirrhosis, and BCS or PVT secondary to a known thrombotic disorder.

MATERIALS AND METHODS

Patients

The study was conducted at the Department of Gastroenterology, Ege University Hospital, İzmir, Turkey. This study was approved by the local ethics committee and was conducted in accordance with the Declaration of Helsinki and written informed consent was provided by all participants. Doppler ultrasound (US) was performed by two experienced radiologists to determine the patency of the hepatic and portal veins. The study included 111 patients (54 female and 47 male) with a median age of 43.03±14.85 years (range: 18-75 years) diagnosed with BCS or extrahepatic portal vein obstruction (EHPVO) with or without accompanying cirrhosis between January 2005 and January 2009. Transient risk factors for venous thromboembolism (including surgery, pregnancy, puerperium (defined as the 6-week period after delivery), oral contraceptive use, hormone replacement therapy, trauma, malignancy, prolonged immobilization (>10 d), and extensive travel (>8 h) were recorded. Special attention was given to the presence of inflammatory or infectious diseases in anatomical sites of venous drainage (liver, gall bladder, pancreas, and bowels). Patients with malignancy and those who did not provide informed consent were excluded from the study. Thrombophilia screening was performed in all patients as previously described (26-28). The control group included 56 volunteers without any known diseases (28 female and 28 male) with a mean age of 40.71±6.49 years. All those in the control group had patent hepatic and portal veins.

The patients were divided into three subgroups: idiopathic BCS or PVT; PVT secondary to cirrhosis; BCS or PVT due to one or more known etiological thrombotic factors (including MPNs).

Blood samples were obtained from all patients and genomic DNA was extracted from peripheral blood granulocytes using standard procedures (Qiagen, Valencia, California, USA). All samples were stored at -80°C until analysis.

Screening

Screening for prothrombotic factors included measurement of antithrombin (AT III) and protein C (PC) functional activity, free protein S (PS) antigen and fasting homocysteine levels, and MTHFR gene mutations. In addition, screening for FVL and F2 G20210A polymorphisms as well as antiphospholipid antibodies was performed. Deficiency of AT III, PC, or PS was considered inherited only after finding levels below the normal range in a first-degree relative. The JAK2 V617F mutation was detected as previously described (29) using LightCycler SPN genotyping (25) (Roche, Indianapolis, USA) (Table 1, Figure 1).

Statistical analysis

All data were analyzed using SPSS v.13.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Data are expressed as mean±standard deviation (SD) or median as appropriate. Differences in discrete variables between groups were evaluated using the chi-square or Fisher's exact tests according to sample size. The Mann-Whitney U test was used to compare parametric data. Correlation analysis was performed using the parametric Spearman's correlation coefficient (r) when appropriate. The level of statistical significance was set at $p<0.05$.

RESULTS

The participants' clinical and hematological characteristics are summarized in Table 2. There were no significant differences in age or gender ratio between the patient and control groups (p=0.869). In all, 80 of the 111 patients (72.1%) had PVT, 27 (24.3%) had hepatic vein thrombosis, and four (3.6%) had

Table 1. LightCycler primer and probe sequences

LightCycler	
Primers	JAK2-for 5'-ACAACAGTCAAACAACAATTC-3' JAK2-rev 5'-ACACCTAGCTGTGATCC-3'
Probes	JAK2-wt 5'-LCred640-CGTCTCCACAGACACATACTC-C3blocker-3' JAK2-an5'-AAAGGCATTAGAAAGCCTGTAGTTTTACTTACTCT-Fluo-3'

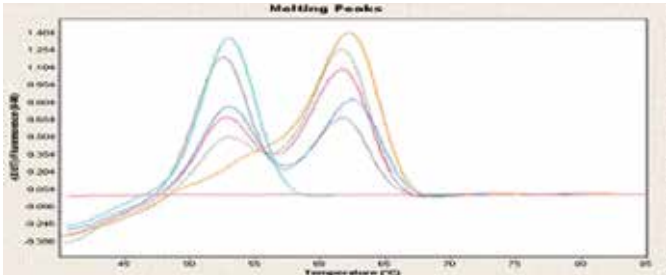

Figure 1. The JAK2 mutation on LightCycler instrument screen
Each color expresses a patient, in the wild type a single peak (60-65C) (yellow) was seen, a single peak (turquoise) was also detected who have homozygous mutation (50-55C) and two peaks were seen in heterozigot mutations.

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Table 2. General characteristics of the study cohort

Characteristics	All patients (n=111)	Idiopathic PVT or/and BCS (n=28)	PVT secondary to cirrhosis (n=38)	PVT or BCS secondary to known etiological factor (n=45)	Controls
Median age (years)	44 (18-77)	44.5 (18-77)	49 (22-75)	34 (18-66)	43 (29-53)
Number of females (%)	54 (48.6)	14 (50)	18 (47.4)	22 (48.9)	28 (50)
Median hemoglobin level (range)	13.25 g L^{-1} (10.1-17 g L^{-1})	13.25 g L^{-1} (10.1-17 g L^{-1})	11.85 g L^{-1} (6.0-16.7 g L^{-1})	12.2 g L^{-1} (6.2-25.4 g L^{-1})	---------
Median WBC count (range)	6.96×10 L^{-1} (2.2-12.2×10 L^{-1})	6.835×10 L^{-1} (2.27-12.25×10 L^{-1})	4.95×10 L^{-1} (0.89-19.17×10 L^{-1})	7.79×10 L^{-1} (1.115-46.73×10 L^{-1})	--------
Median PLT count (range)	154.5×10 L^{-1} (48-538×10 L^{-1})	154.5×10 L^{-1} (48-538×10 L^{-1})	98×10 L^{-1} (25-595×10 L^{-1})	181×10 L^{-1} (30-333.1×10 L^{-1})	---------

PVT; portal vein thrombosis; BCS: budd-chiari syndrome; WBC: white blood cell; PLT: platelet

Table 3. Distribution of JAK2 mutation according to the location of thrombosis and its etiology

	JAK2 V617F (-) (n=96)	JAK2 V617F (+) (n=15)
Location of thrombosis		
PVT	71 (88.8%)	9 (11.13%)
BCS	22 (81.5%)	5 (18.5%)
BCS + PVT	3 (75%)	1 (25%)
Etiology of thrombosis		
Idiopathic PVT/BCS	22 (78.6%)	6 (21.4%)
PVT secondary to cirrhosis	37 (97.4%)	1 (2.6%)
Prothrombotic causes of PVT or BCS	37 (82.2%)	8 (17.8%)

PVT: portal vein thrombosis; BCS: budd-chiari syndrome

Table 4. JAK2 V617F mutation status and biochemical parameters in the patients

	JAK2 V617F (-) (n=96)	JAK2 V617F (+) (n=15)	p
AST	49.90±62.37	75.47±109.45	0.268
ALT	42.44±25.5	59.6±74.4	0.358
Albumin	3.6±3.6	3.8±0.7	0.479
Hg	12.06±3.0	14.03±2.6	0.007
Htc	36.3 ±7.5	43.34±8.6	0.005
WBC	6976.6±5568	15,904±11,340	0.0001
PLT	21,322.92±124,000	340,133±164,104	0.001

both PVT and hepatic vein thrombosis. Among all patients, 28 (25.2%) had idiopathic PVT or BCS, 38 (34.2%) had PVT secondary to cirrhosis, and 45 (40.5%) had either PVT or BCS secondary to one or more prothrombotic factors (except cirrhosis and malignancy).

The JAK2 V617F mutation was detected in 15 of 111 patients (13.5%) with either PVT or BCS, but in none of the control subjects (p=0.0004). There was no significant relationship between the JAK2 V617F mutation and the location of thrombosis (p=0.501; Table 3). The frequency of the JAK2 V617F mutation was significantly higher in patients with idiopathic PVT/BCS (p=0.049).

When all the patients were compared according to the presence of the JAK2 V617F mutation, hemoglobin and hematocrit levels and white blood cell (WBC) and platelet (PLT) counts were significantly higher in those with the mutation (Table 4).

The correlation between secondary causes of hepatic thrombosis/PVT and JAK2 mutation status is shown in Table 5. In total, eight patients were diagnosed with MPN at the time of thrombosis detection, of which four had the JAK2 V617F mutation (p=0.002) (Table 6). Although the association between JAK2 V617F and other prothrombotic genetic events has not been previously reported, the coprevalence of the FVL and JAK2 V617F mutations was significantly higher in the present study (p=0.037).

All patients with PVT or BCS were diagnosed with acute or chronic thrombosis according to radiological findings and the time from the onset of patient complaints to initial hospital admission (classified as acute if <6 weeks). There was no significant relationship between JAK2 mutation status and acute or chronic thrombosis (p=0.821).

Median duration of follow-up was 15 months (range: 1-111 months) in patients with PVT or BCS, 17 months (range: 1-120 months) in those with PVT secondary to cirrhosis, and 10 months (range: 1-177 months) in patients with PVT or BCS secondary to noncirrhotic factors. Median durations of follow-up in the JAK2 mutation-negative and -positive patients were 15 months (range: 1-177 months) and 10 months (range: 1-77 months), respectively (p=0.147). During follow-up, 12 patients died. After a median follow-up of 15 months (range: 1-177 months) in the JAK2 V617F mutation-positive patients and 10 months (range: 1-77 months) in the JAK2 V617F mutation-negative patients (p=0.147), we observed that three of the 15 JAK2

Table 5. Secondary causes of hepatic thrombosis/PVT and JAK2 mutation status

Etiology of thrombosis	JAK2 V617F (-) (n=96)	JAK2 V617F (+) (n=15)	p
Protein C deficiency	4	1	0.664
Protein S deficiency	2	0	0.573
Antithrombin III deficiency	0	0	ND
Hyperhomocysteinemia	3	1	0.494
Antiphospholipid antibody syndrome	3	0	0.573
FVL mutation (heterogeneous/homogenous)	13 /2	6 /0	0.037
F2 G20210A mutation (heterogeneous/homogenous)	5/0	2/0	0.229
MTHFR C677T polymorphism (heterogeneous/homogenous)	38/11	7/0	0.382
MPNs	4	4	0.002
Cirrhosis	36	1	0.018
Abdominal surgery	16	0	0.087
Acute-chronic pancreatitis	4	0	0.421
Pregnancy	1	1	0.128
Behçet's disease	2	0	0.573
FMF	2	0	0.573
Infection (Tbc lymphadenitis)	1	0	0.691
İnferior vena caval web	1	0	0.691
Oral contraceptive	6	1	0.951
Acute thrombosis	23	4	NS
Chronic thrombosis	73	11	NS

FVL: factor V leiden; MPNs: chronic myeloproliferative neoplasms; FMF: familial Mediterranean fever; Tbc: tuberculosis

Table 6. Characteristics of patients according to MPNs and JAK2 status

	JAK2 V617F (+) (n=15)*	JAK2 V617F (-) (n=96)
MPNs (+)		
Overt patients	4	4
Latent patients	6	-
MPNs (-)	2	92

*Three of fifteen JAK2 V617F (+) patients died before the completion of diagnostic procedure for MPN detection.

mutation-positive patients died (20%) compared with nine of the 96 JAK2 mutation-negative patients (9.4%). The mortality rate between the JAK2 gene mutation-positive and -negative patients did not differ significantly (p=0.218). The JAK2 V617F mutation was observed in four patients with acute thrombosis (24.3%) and in 11 patients (75.5%) with chronic thrombosis (p=0.820).

DISCUSSION

Thrombophilic abnormalities (primarily associated with gain-of-function mutations in FVL or with F2 A20210G) and clonal disorders of hematopoiesis, such as Philadelphia chromosome-negative MPN, are etiological factors in a significant proportion of both PVT and BCS cases (30-35). The distinction between these two pathogenic mechanisms may have important clinical implications because anticoagulants are the most rational treatment in cases associated with thrombophilia, whereas cytoreductive therapy is indicated in patients with MPNs. Although the diagnosis of thrombophilia is relatively simple and accurate, diagnosis of MPNs is often problematic in patients with PVT or BCS. At the time of acute thrombosis, as well as during the postthrombotic period, hemodilution, occult bleeding, and hypersplenism due to portal hypertension may mask changes in blood cell counts used for diagnosing MPNs. Moreover, MPNs associated with splanchnic venous thrombosis often present with an atypical phenotype inconsistent with conventional diagnostic criteria (6,36). Overt MPNs are observed in 22.2%-35% of patients with PVT and 23%-31.2% of patients with BCS (37,38); however, the latter proportion increases to 45%-53% when occult MPNs are included as an etiological factor for BCS (39).

In the present study, the JAK2 V617F mutation was detected in 15 (13.5%) of 111 patients, but not in the control group subjects (p=0.004). The reported prevalence of the JAK2 V617 mutation in patients with splanchnic vein thrombosis ranges from 17.2%-36.5% (40-42). Collaizzo et al. (40) reported that the JAK2 V617 mutation was not observed in any of their pediatric patients, which may be why the overall prevalence they reported was relatively low. In the present study, the mutation frequency was lower than in other studies because the number of cirrhotic patients was high, but was within the range previously reported (19.1% vs. 17.2%-36.5%), when the cirrhotic patients were excluded from analysis.

Screening for the JAK2 V617 mutation is a valuable method for diagnosing occult MPNs. In previous studies, the prevalence of the JAK2 V617F mutation has varied widely, from 6%-97% in PV, 23%-57% in ET, and 35%-57% in PMF (11-13,43-45). De Stefano et al. studied 19 MPN patients with thrombosis, of which 18 had the JAK2 V617F mutation (9). In the present study, eight patients were diagnosed with MPN at the time of thrombosis, of which four had the JAK2 V617F mutation.

The prevalence of JAK2 V617F mutation was 21.4% in patients with idiopathic PVT/BCS, 2.6% in PVT secondary to cirrhosis, and 17.6% in PVT/BCS secondary to prothrombotic factors (p=0.49). The prevalence of the mutation in patients with idiopathic splanchnic vein thrombosis was 21.5% in De Stefano et al. and 18% in Regina et al. (42,46). In a study by Patel et al., the JAK2 V617 mutation was confirmed in 58.5% of patients with idiopathic BCS, which is probably high because of the greater prevalence of MPNs in patients with BCS (47). The JAK2 V617

mutation was observed in 40% of BCS and 35% of PVT patients (41). The cause of the low mutation frequency in the present study remains unclear. It is possible that MPN associated with the JAK2 V617 mutation was not a prominent etiological factor for splanchnic thrombosis in the present study population; indeed, eight patients were diagnosed with MPN (PV: n=4; ET: n=4) at the time PVT or BCS was detected, of which four (PV: n=2; ET: n=2) had the JAK2 V617F mutation, which was statistically significant (p=0.002).

In the present study, all MPN patients that had the JAK2 V617F mutation also had a heterozygous FVL mutation, and the association between JAK2 V617F and FVL mutations was statistically significant (P=0.037). As Denninger et al. also reported, the role of multiple factors in the etiology of thrombosis should not be ignored (30); Bununla birlikte D Colaızzo ve ark. çalışmalarında JAK2 gen mutasyonunun hiçbir kalıtsal veya edinsel tromboz nedeni ile istatiksel olarak anlamlı birlikteliği tespit edilmemiştir (34). JAK2 mutasyonu tesit edilen hastalar Hematoloji Bölümü tarafından incelenmiş ve KMPH tanısı alarak tedavileri düzenlenmiştir.however, Colaizzo et al. did not observe a significant relationship between inherited or acquired thrombotic causes and the JAK2 V617 mutation (40). In the present study, a significant relationship was not observed between the JAK2 V617F mutation and protein C deficiency, protein S deficiency, antithrombin III deficiency, F2 G20210A mutation, MTHFR C677T mutation, hyperhomocysteinemia, or any of the other risk factors listed in Table 5.

In our study, five of 27 BCS patients (18.5%), nine of the 80 PVT patients (11.3%), and one of the four BCS + PVT patients (25%) had the JAK2 V617F mutation, but these differences were not statistically significant (p=0.501). Primignani et al. observed the JAK2 V617 mutation in 26 of 73 PVT patients (35.6%) and in eight of 20 BCS patients (40%), which again was not statistically significant (41). De Stefano et al. (42) reported the JAK2 V617 mutation in 33.3% of BCS patients and 41.3% of PVT patients. However, as previously mentioned, the 19 patients diagnosed with MPNs prior to the study may have led to this difference. Consistent with previous reports, patients in the present study with JAK2 V617F mutation had higher hemoglobin (p=0.007) and hematocrit (p=0.005) levels and WBC (p=0.0001) and PLT counts (p=0.001) than JAK2 V617F-negative patients (Table 4) (41,48). Primignani et al. (41) reported that WBC and PLT counts were higher in JAK2-positive patients. In the present study, JAK2 V617F mutation status did not affect duration of hospitalization.

No previous studies have addressed the relationship between JAK2 V617F mutation status and acute or chronic thrombosis. In addition, there are a limited number of studies on PVT secondary to cirrhosis that have examined the contributions of inherited and acquired prothrombotic factors. Amitrano et al. (49) reported that the frequencies of the F2 G20210A and MTHFR C677T gene mutations were significantly higher in patients with PVT secondary to cirrhosis, while Erkan et al. (51) reported that FVL and F2 GA20210 mutation frequencies were higher in PVT secondary to cirrhosis. In contrast, Mangia et al. (52) sirozu ve portal ven trombozu bulunan hastalarda FV Leiden, Protrombin G 20210 ve MTHFR C677T gen mutasyonlarının tromboz riskini arttırmadığını bildirmiştir (5).reported that FVL, F2 G20210, and MTHFR C677T gene mutations did not increase the risk of thrombosis in patients with cirrhosis. Among the 38 patients with PVT secondary to cirrhosis in the present study, five had FVL mutations (all heterozygous), four had the F2 G20210 mutation (three heterozygous and one homozygous), and 21 had the MTHFR C677T mutation (16 heterozygous and five homozygous), while the JAK2 V617F mutation was observed only in one (2.6%) of these 38 patients. Similarly, Colaizzo et al. (53) reported the JAK2 V617F mutation in only five of 91 patients with cirrhosis and PVT.

The present findings indicate that a significant proportion of idiopathic PVT/BCS patients could have occult MPN (41,42,46). The JAK2 V617F mutation is a molecular marker for occult MPNs, and thus can be used for the diagnosis of latent MPNs presenting with thrombotic events. In the present study, screening for the JAK2 V617F mutation in patients with idiopathic PVT/BCS showed that 20% had latent MPNs. Patients that had both the JAK2 V617F mutation and idiopathic thrombosis were referred to an experienced hematologist for consultation.

In conclusion, the JAK2 V617F mutation is an acquired mutation associated with occult MPNs that can be used for the diagnosis of latent MPNs presenting with thrombotic events. Analysis of JAK2 mutations in patients with idiopathic PVT or BCS showed that 20% had latent MPNs. JAK2 gene analysis should be included in the research panel for BCS/PVT patients without cirrhosis.

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