JAK2 Mutation: An Aid in the Diagnosis of Occult Myeloproliferative Neoplasms in Patients with Major Intraabdominal Vein Thrombosis and Normal Blood Counts

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ABSTRACT: **Background:** Janus kinase-2 (*JAK2*) is mutated in a high proportion of patients with polycythemia vera and in a smaller number with essential thrombocythemia and primary myelofibrosis. Mutated *JAK2* is an important diagnostic marker for myeloproliferative neoplasm (MPN) and may also play a major role in the pathogenesis of MPN.

Objectives: To evaluate the prevalence of mutated *JAK2* (*JAK2-V617F*) among patients with major intraabdominal vein thrombosis who had normal blood counts at diagnosis of the initial event.

Methods: The medical records of patients who presented with a major intraabdominal venous thrombosis and normal peripheral blood counts were obtained. *JAK2-V617F* mutation status was determined by real-time polymerase chain reaction.

Results: Twenty-two patients were available for this analysis and 9 (41%) were found to have *JAK2-V617F*. Patients with positive *JAK2-V617F* were younger and had more frequent clinical splenomegaly than those with wild-type *JAK2*.

Conclusions: A high proportion of patients presenting with "idiopathic" major intraabdominal vein thrombosis and normal blood counts carry *JAK2-V617F*. We recommend searching for the mutation in this clinical setting to detect patients with occult MPN.

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KEY WORDS: *JAK2-V617F*, myeloproliferative neoplasm (MPN), abdominal vein thrombosis, normal blood count

JAK2 is an intracytoplamic tyrosine kinase that plays a critical role in signal transduction from multiple hematopoietic factor receptors [1]. In 2005, several groups reported a single somatic mutation in the *JAK2* gene in patients with myeloproliferative neoplasms [2,3]. This mutation, consisting of a substitution of valine to phenylalanine at position 617 in the pseudokinase (JH2) domain of *JAK2* [2-4], is detected in 95% of patients with polycythemia vera and in 50–60% of those with essential thrombocythemia and primary myelofibrosis. The mutation abolishes the self-inhibitory action of the JH2 domain, causing a constitutive activation of JAK and downstream intracellular signal transduction [2]. *JAK2-V617F* has been shown to support endogenous erythroid colony formation independently of erythropoietin and acquires hypersensitivity to erythropoietin and other cytokines [2,4].

MPN are a frequent underlying cause of splanchnic vein thrombosis, and a recently published meta-analysis [5] identified MPN as the underlying cause in 41% of cases of hepatic vein thrombosis and 32% of cases with portal vein thrombosis. However, in 25–65% of these cases MPN may not be apparent at initial presentation and these patients display normal blood counts [6-8]. In the past, the diagnosis of this subset of patients was more problematic and depended on measurement of red blood cell mass, bone marrow examination, spontaneous erythroid colony formation in vitro, and serum erythropoietin measurement. However, in recent years *JAK2-V617F* has become routinely available as a diagnostic tool in the diagnosis of MPN and can easily be utilized to establish a diagnosis in these patients.

Based on our experience presented in this study we suggest that searching for *JAK2-V617F* is of tremendous diagnostic importance in patients presenting with major intraabdominal venous thrombosis who show normal peripheral blood counts.

PATIENTS AND METHODS

We collected clinical and laboratory data from the medical records of patients who presented with a major intraabdominal venous thrombosis and normal peripheral blood counts, who were also evaluated for *JAK2-V617F* mutation. Excluded were patients with thrombosis secondary to malignancy, overt MPN, infection, anatomic malformations, and primary liver disease with cirrhosis. The study was approved by the institutional board review committee.

MPN = myeloproliferative neoplasms

Genomic DNA was isolated using the Wizard Genomic DNA purification Kit (Promega, Madison, WI USA), and a real-time polymerase chain reaction-based allelic discrimination for *JAK2* mutation was performed according to Passamonti et al. [9] using SyBR Green I dye on a LightCycler system (Roche, Roche Applied Science, Mannheim, Germany).

STATISTICAL ANALYSIS

Student's *t*-test was used to assess differences in continuous variables. Data are expressed as mean ± standard deviation. The Fisher exact test was used to examine the categorical variables. Logistic regression was used for multivariate analysis.

RESULTS

Twenty-two patients qualified for assessment according to the inclusion and exclusion criteria, and 9 (41%) of them had the *JAK2-V617F* mutation [Table 1]. In the *JAK2-V617F* group the female:male ratio (3.5:1) was higher than in the wild-type *JAK2* group (1.2:1), but this apparent gender difference was not statistically significant. Patients with *JAK2-V617F* were significantly younger than those with wild-type *JAK2* (mean age 35.7 and 51 years, respectively, $P = 0.022$). Splenomegaly was also significantly more frequently found in patients with *JAK2-V617F* than in those with wild-type *JAK2* (7 and 3 patients, respectively, $P = 0.027$) while there was only a trend (not statistically significant) for other signs of portal hypertension, such as gastroesophageal varices and ascites, in the *JAK2-V617F* group compared to the group with wild-type *JAK2* (8 and 6 patients, respectively $P = 0.074$). There were no statistically significant differences between hemoglobin and hematocrit levels in the two groups (13.2 ± 1.3 vs. 12.2 ± 1.5 g/dl, $P = 0.117$; and 39.3 ± 4.0 vs. 36.6 ± 3.8%, $P = 0.125$, respectively) or in leukocyte and platelet counts (7.50 ± 2.8 vs. 7.7 ± 3.2 x10^9/L, $P = 0.884$; and 263.7 ± 115.3 vs. 259.1 ± 122.4 x10^9/L, $P = 0.93$, respectively).

Details of anatomic sites of thrombosis are given in Table 1. In the *JAK2-V617F* group, thrombosis involving the hepatic veins and/or the portal veins occurred in eight patients, while another patient had thrombosis of the inferior vena cava as well as the iliac and splenic veins. In the wild-type *JAK2* group, hepatic vein thrombosis and/or portal vein thrombosis occurred in 10 patients, while 2 additional patients had isolated mesenteric vein thrombosis and another had an isolated splenic vein thrombosis.

In the *JAK2-V617F* group two patients were heterozygous for factor V Leiden, and one patient was homozygous for prothrombin 20210 mutation. In addition, in the wild-type *JAK2* group, one patient had protein C deficiency, four patients were heterozygous for prothrombin 20210 mutation, one was heterozygous for factor V Leiden, and one was homozygous for this mutation. In the wild-type *JAK2* group there was one patient homozygous for *MTHFR C677T* mutation.

Table 1. Patients' characteristics according to wild-type *JAK2* or *JAK2-V617F*

	Wild-type *JAK-2*	*JAK2-V617F*	*P* value
Patients	13 (59%)	9 (41%)	
Mean age (yr)	51	35.7	0.022
Female/male, ratio	1.2:1	3.5:1	0.380
Splenomegaly Portal hypertension	3 6	7 8	0.027 0.074
Mean Hb level (g/dl)	12.2	13.2	0.117
Mean WBC count (x10^9/L)	7.7	7.5	0.884
Mean platelet count (x10^9/L)	259	263	0.930
Thrombotic site			
Isolated hepatic/Budd-Chiarri syndrome	2	2	
Isolated portal vein thrombosis	4	2	
Isolated splenic vein thrombosis	1	0	
Isolated mesenteric vein thrombosis	2	0	
Combined (more than one site)	4	5	
Thrombophilia			
Protein C deficiency	1	0	
Prothrombin 20210 mutation, heterozygote	4	0	
Prothrombin 20210 mutation, homozygote	0	1	
Factor V Leiden, heterozygote	1	2	
Factor V Leiden, homozygote	1	0	
MTHFR, homozygote	1	0	
Oral contraceptive or hormone replacement therapy	5*	2	

*Additional patient received intravenous immunoglobulin

In the *JAK2-V617F* group two patients used oral contraceptives or hormonal replacement therapy, as compared to five patients in the wild-type *JAK2* group. In the latter group one additional patient was treated with intravenous immunoglobulins prior to the thrombotic event.

Multivariate analysis of clinical parameters, including age, splenomegaly and signs of portal hypertension, revealed that splenomegaly was a predictor for *JAK2-V617F* positivity ($P = 0.036$), while younger age was only marginally significant ($P = 0.06$).

DISCUSSION

Until recently the diagnosis of occult MPN in patients presenting with hepatic or portal vein thrombosis was difficult to establish and was often overlooked. Not uncommonly, patients with occult MPN present with normal blood counts because of blood volume expansion, gastrointestinal bleeding and associated iron deficiency, splenomegaly, or simply because they are in an early stage of their disorder [10].

Splenomegaly, which may frequently develop as a direct result of the hepatic or portal venous thrombosis, makes the clinical diagnosis of occult MPN even more difficult to establish. In recent years, the discovery of the *JAK2-V617F* mutation and its more frequent routine use in the diagnosis of MPN was a major advance. The presence of *JAK2-V617F* strongly correlates with the bone marrow histopathology of MPN and has a high predictive value for the detection of occult MPN [11].

In our study we showed that 41% of patients with normal blood counts who presented with a major intraabdominal venous thrombosis have the *JAK2-V617F* mutation. This is in accordance with the results of a large study in patients with splanchnic vein thrombosis, where the *JAK2-V617F* mutation was found in 39% of the patients and MPN was evident in 40–50% of the cases without any typical peripheral blood findings [12]. This increased prevalence of *JAK2-V617F* is limited not only to patients with hepatic vein thrombosis but is also evident in patients with thrombotic events involving other major intraabdominal veins including the portal and splenic veins and even the inferior vena cava.

In our series, patients with splanchnic vein thrombosis and *JAK2-V617F* were all under the age of 50 (mean 35.7 years) and younger than those with wild-type *JAK2*. Earlier reports had also suggested that young patients with MPN are particularly prone to major intraabdominal vein thrombotic events [11,13,14]. Of further interest is the fact that the *JAK2-V617F* positive patients were predominantly female, a ratio of 3.5: 1, while the female/male ratio in the wild-type *JAK2* subgroup was 1.2:1. Although this difference was not statistically significant, a high female/male ratio was also reported in other studies of patients with MPN and splanchnic vein thrombosis [11,13]. Splenomegaly was also more commonly associated with the *JAK2-V617F* subgroup of patients compared to the non-mutated subgroup, and a trend towards the presence of portal hypertension was also noted in the *JAK2*-mutated patients. Both portal hypertension and occult MPN are possible explanations for the high incidence of splenomegaly in the *JAK2*-mutated patients.

In the *JAK2-V617F* group five patients had other prothrombotic risk factors: two patients were heterozygous for factor V Leiden, one was homozygous for prothrombin 20210 mutation, and two used oral contraceptives or hormone replacement therapy. This observation is in agreement with previous reports of *JAK2-V617F* patients harboring other prothrombotic risk factors [15]. Splanchnic vein thrombosis is a multifactorial disease, and it is indeed important to search for *JAK* mutations in these patients even in the presence of established thrombophilia.

In conclusion, *JAK2-V617F* is present in a significant proportion of patients with major abdominal vein thrombosis who have normal blood counts at the time of diagnosis and at presentation. The detection of this mutation helps to establish the diagnosis in patients with occult MPN who have not been diagnosed after routine clinical or laboratory investigations. Our findings strengthen the premise that searching for the presence of the *JAK2-V617F* mutation should be a part of the routine investigation of patients with major idiopathic abdominal vein thrombosis who have normal blood counts.

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References

1. Ihle JN. The Janus kinase family and signaling through members of the cytokine receptor superfamily. *Proc Soc Exp Biol Med* 1994; 206 (3): 268-72.
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): 1779-90.
3. Baxter EJ, Scott LM, Campbell PJ, et al. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. *Lancet* 2005; 365 (9464): 1054-61.
4. James C, Ugo V, Le Couedic JP, et al. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. *Nature* 2005; 434 (7037): 1144-8.
5. Smalberg JH, Arends LR, Valla DC, Kiladjian JJ, Janssen HL, Leebeek FW. Myeloproliferative neoplasms in Budd-Chiari syndrome and portal vein thrombosis: a meta-analysis. *Blood* 2012; 120 (25): 4921-8.
6. Valla D, Casadevall N, Lacombe C, et al. Primary myeloproliferative disorder and hepatic vein thrombosis. A prospective study of erythroid colony formation in vitro in 20 patients with Budd-Chiari syndrome. *Ann Intern Med* 1985; 103 (3): 329-34.
7. Landolfi R, Di Gennaro L, Barbui T, et al. Leukocytosis as a major thrombotic risk factor in patients with polycythemia vera. *Blood* 2007; 109 (6): 2446-52.
8. Regina S, Herault O, D'Alteroche L, Binet C, Gruel Y. JAK2 V617F is specifically associated with idiopathic splanchnic vein thrombosis. *J Thromb Haemost* 2007; 5 (4): 859-61.
9. Passamonti F, Rumi E, Pietra D, et al. Relation between JAK2 (V617F) mutation status, granulocyte activation, and constitutive mobilization of CD34+ cells into peripheral blood in myeloproliferative disorders. *Blood* 2006; 107 (9): 3676-82.
10. Austin SK, Lambert JR. The JAK2 V617F mutation and thrombosis. *Br J Haematol* 2008; 143 (3): 307-20.
11. Colaizzo D, Amitrano L, Tiscia GL, et al. The JAK2 V617F mutation frequently occurs in patients with portal and mesenteric venous thrombosis. *J Thromb Haemost* 2007; 5 (1): 55-61.
12. 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): 4922-9.
13. Patel RK, Lea NC, Heneghan MA, et al. Prevalence of the activating JAK2 tyrosine kinase mutation V617F in the Budd-Chiari syndrome. *Gastroenterology* 2006; 130 (7): 2031-8.
14. Boissinot M, Lippert E, Girodon F, et al. Latent myeloproliferative disorder revealed by the JAK2-V617F mutation and endogenous megakaryocytic colonies in patients with splanchnic vein thrombosis. *Blood* 2006; 108 (9): 3223-4.
15. Yonal I, Pinarbasi B, Hindilerden F, et al. The clinical significance of JAK2V617F mutation for Philadelphia-negative chronic myeloproliferative neoplasms in patients with splanchnic vein thrombosis. *J Thromb Thrombolysis* 2012; 34 (3): 388-96.