

PREVALENCE OF JANUS KINASE 2 MUTATIONS IN PATIENTS WITH UNUSUAL SITE VENOUS THROMBOSIS

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Abstract We aimed to study patients with splanchnic vein thrombosis (SVT) and cerebral vein thrombosis (CVT) searching for JAK2 mutations. We evaluated 14 patients (median age: 41.5 years) with portal vein thrombosis (PVT) = 7; mesenteric vein thrombosis (MVT) = 3; and CVT = 4. JAK2 V617F was assessed by allele specific PCR of peripheral blood DNA. In addition, DNA was sequenced for other JAK2 mutations. Other inherited and acquired thrombophilia risk factors were evaluated. JAK2 V617F was positive in four out of seven patients with PVT and in one CVT patient. These five patients had a diagnosis of myeloproliferative disorder (MPD) at the moment of the occurrence of thrombosis (n = 2) or later (n = 2). Patients with MVT and CVT were negative for JAK2 V617F, except one patient with CVT and a diagnosis of essential thrombocythemia. No other JAK2 mutations were found in this cohort. Besides MPD, other thrombophilia risk factors were identified in five patients. One patient had MPD as well as thrombophilia risk factor. In this group, 4 out of 7 of the patients with PVT carried the JAK2 V617F mutation with or without overt MPD. However, the investigation of other JAK2 mutations may not be necessary in patients with thrombosis at unusual sites.

Key words: JAK2, myeloproliferative disorders, thrombosis, thrombophilia

Resumen *Prevalencia de las mutaciones del gen quinasa Janus 2 en pacientes con trombosis venosa en sitios poco frecuentes.* Nuestro objetivo fue estudiar pacientes con trombosis de las venas esplánicas (TVE) o trombosis de las venas cerebrales (TVC) en búsqueda de mutaciones del gen quinasa Janus 2 (JAK2). Se estudiaron 14 pacientes (media de edad: 41.5 años) con trombosis de la vena porta (TVP n = 7), trombosis de la vena mesentérica (TVM, n = 3) y TVC (n = 4). La mutación V617F del gen JAK2 fue evaluada por reacción en cadena de la polimerasa (PCR) alelo-específica en muestras de sangre periférica. Además, se realizó secuenciación de ADN en búsqueda de otras mutaciones del gen JAK2 distintas de V617F. También se investigaron factores genéticos y adquiridos para trombofilia. JAK2 V617F fue positiva en 4 de 7 pacientes con TVP y en un paciente con TVC. Estos 5 pacientes con la mutación tuvieron diagnóstico de síndrome mieloproliferativo (SMP) en el momento de la detección de la trombosis (n = 2) o después (n = 3). Un paciente con TVP sufrió el episodio trombótico 18 años después del diagnóstico del SMP y la mutación JAK2 V617F fue negativa. No se encontraron otras mutaciones del gen JAK2 en este grupo de pacientes. Además del diagnóstico de SMP, se identificaron otros factores de riesgo para trombofilia en 4 pacientes. Un paciente tuvo un factor de riesgo para trombofilia además del diagnóstico de SMP. La mutación JAK2 V617F se presentó en 4/7 de los pacientes con TVP con o sin un diagnóstico obvio de SMP. La investigación de otras mutaciones podría no ser necesaria en pacientes con trombosis en sitios poco frecuentes.

Palabras clave: JAK2, síndrome mieloproliferativo, trombosis, trombofilia

Phi negative myeloproliferative disorders (MPD) are common causes of thrombosis outside the lower extremities, such as splanchnic vein thrombosis (SVT; hepatic, portal, mesenteric or splenic) or cerebral vein thrombosis (CVT)¹. In fact, in 28 to 49% of the patients with hepatic vein thrombosis (HVT) and in 14 to 35% of the patients

with extrahepatic portal vein thrombosis (PVT), an underlying MPD can be identified^{2,3}. Conversely, 5 to 10% of the patients with MPD may have SVT thrombosis along the course of the disease and 1% of the patients with essential thrombocythemia (ET) can present with CVT^{4,5}. However, the prevalence of underlying MPD has been variable depending on the diagnostic criteria used². Also, MPD may not be apparent at the moment of the thrombotic event ("latent MPD") but fully develop several years later ("overt MPD")¹.

In 2005, a somatic mutation in the Janus Kinase 2 gene (JAK2), exon 14, was described for most of the patients with PV and for around half of the patients with ET and/

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or IMF. This mutation results in a valine to phenylalanine substitution at position 617 in the autoinhibitory domain of JAK2 tyrosine kinase, leading to the constitutive activation of the protein, which in turn promotes cellular growth independently of exogenous growth factors, and the activation of intracellular activation pathways, such as STAT5. Several techniques based on the polymerase chain reaction (PCR) have been described for mutation detection in clinical practice, being the allele specific PCR the more sensitive one⁶⁻⁸.

Although there is conflicting evidence about the importance of JAK2 V617F mutation in predicting the risk of thrombosis in patients with MPD, two recent meta-analyses have strongly suggested that JAK2 V617F positivity in patients with ET could be associated with increased risk of thrombosis^{9,10}. Interestingly, this association may be stronger in cases of thrombosis at unusual venous sites¹¹. Thus, JAK2 V617F has been proposed as a marker of MPD in patients with SVT, independently of other MPD criteria^{11,12}. Besides JAK2 V617F, other JAK2 mutations have been reported¹³. However, its prevalence in JAK2 V617F negative MPD patients with unusual site venous thrombosis has not been completely addressed.

We aimed to study the occurrence of JAK2 V617F and other JAK2 mutations in a series of patients with splanchnic and cerebral venous thrombosis.

Materials and Methods

We retrospectively screened 14 patients with documented splanchnic and cerebral vein thrombosis, who were referred to our institution for comprehensive thrombophilia testing. Diagnosis of thrombosis occurred between 1985 and 2009. We included patients with or without overt MPD. Laboratory tests searching for hereditary and acquired risk factors for thrombosis were performed in all patients, including genotyping for factor V Leiden and 20210A prothrombin gene allele, measurement of antithrombin, proteins C and S, screening for antiphospholipid antibodies and assay for total homocysteine levels. MPD was diagnosed according to WHO criteria¹⁴. Complete clinical data were obtained, including acquired risk factors. We registered date of thrombosis, diagnosis of MPD, treatment received and recurrence of thrombosis. All patients were managed with anticoagulants after the thrombotic event. Patients were followed up until December 31 2009 or death.

The screening for exon 14 JAK2 V617F mutation was performed with allele-specific PCR on DNA of peripheral blood, as previously published⁶. In patients negative for V617F mutation, we sequenced genomic DNA with primers designed to amplify the coding region exons 12 to 19, and intron/exon boundaries of JAK2 gene. The PCR products were sequenced directly in both directions with automatic sequencer and the sequences were manually analyzed. DNA extraction and PCR studies were performed at the School of Medicine of Universidad Nacional de Córdoba.

The Hospital Privado Institutional Review Board approved the study and all patients gave written informed consent to participate in this study.

Results

We studied 14 patients (median age 41.5 years; range 8 to 70) with SVT or CVT. Median follow-up since diagnosis of the thrombotic event was 48.7 months (range 6.8 to 303.3 months). Clinical characteristics and results of thrombophilia tests are shown in Table 1.

Ten out of 14 patients had splanchnic venous thrombosis and five of them had a diagnosis of MPD: four patients with ET and one patient with primary myelofibrosis (PMF). Four patients had cerebral venous thrombosis and only one of them had a diagnosis of MPD (ET = 1). Two out of six patients had a diagnosis of MPD at the occurrence of the venous thrombotic event (overt MPD), other three patients after the thrombotic event (five years, one year and three months, respectively) and finally, one patient with PMF had the thrombotic event after 18 years of MPD diagnosis. Overall, JAK2 V617F was positive in five out of 14 patients (35.71%). In patients with a diagnosis of MPD, JAK2 V617F was positive in 5 out of 6 (overt MPD = 2; latent MPD = 3); the remaining patient with PMF and JAK2 V617F negative was receiving thalidomide (fourth months) when the thrombosis occurred.

The three patients whose thrombosis developed before MPD diagnosis (latent MPD) had recurrent episodes, even though they were taking oral anticoagulant treatment. Two out of three patients received hydroxyurea when MPD was diagnosed and no further thrombotic events were registered. The other three patients with almost simultaneous diagnosis of thrombosis and MPD (overt MPD) received hydroxyurea from the beginning and no recurrences of thrombosis were registered until end of follow-up. All patients are currently alive.

Four out of 14 patients (28.6%) had one thrombophilia risk factor in the laboratory investigations (Table 1). One of these patients also had JAK2 positive MPD. Transient risk factors for thrombosis were registered in only one patient who had received treatment with thalidomide the four months before the thrombotic event (patient number 7). Also, one patient had a diagnosis of diabetes mellitus prior to the thrombotic event (patient number 11).

Discussion

Our series contributes to confirm that a substantial proportion of patients with SVT have JAK2 V617F mutation with no other JAK2 mutation in the settings of SVT and CVT. In patients with SVT, the presence of portal hypertension, occult blood loss, hypersplenism or hemodilution can mask signs that help to diagnose MPD. Also, some patients may have "latent MPD"¹². Therefore, JAK2 V617F has become a useful tool in the diagnostic approach of patients with SVT. At least 10 series of patients with thrombosis at unusual sites have reported the prevalence of JAK2 V617F

TABLE 1.– Characteristics of patients with splanchnic or cerebral vein thrombosis (n = 14)

Patient	Age/Sex	Site	MPD	Thrombophilia	JAK2 V617F	Upfront Treatment	Thrombosis Recurrence
1	8/F	PVT	No	No	Negative	AC	Yes
2	34/M	PVT	No	APL	Negative	AC	Yes
3	29/F	PVT	ET	HyH	Positive	AC	Yes
4	30/F	PVT	ET	No	Positive	AC	Yes
5	26/M	PVT	ET	No	Positive	AC/HU	No
6	49/F	PVT †	ET	No	Positive	AC/HU	No
7	70/M	PVT †	PMF	No	Negative	AC	No
8	69/M	MVT	No	FVL	Negative	AC	No
9	64/F	MVT	No	No	Negative	AC	No
10	41/M	MVT	No	No	Negative	AC	No
11	27/M	CVT	No	No	Negative	AC	No
12	26/F	CVT	No	No	Negative	AC	No
13	64/F	CVT	No	PCD	Negative	AC	No
14	44/F	CVT	ET	No	Positive	AC	No

*MPD = myeloproliferative disorder; F = female; M = male; PVT = portal venous thrombosis; MVT = mesenteric venous thrombosis; CVT = cerebral vein thrombosis; ET = essential thrombocythemia; PMF = primary myelofibrosis; APL = antiphospholipids; HyH = hyperhomocysteinemia; FVL = factor V Leiden; PCD = Protein C Deficiency; AC = anticoagulation; HU = hydroxyurea.

† These patients had also splenic vein thrombosis.

mutation in this setting^{11, 12, 15-20}. In SVT, the rate of mutation was between 17 and 74%, with most of the series having rates of about 30-40%. Along these series, the precise prevalence of MPD is difficult to assess, since the diagnostic criteria have been variable. For this reason, JAK2 V617F mutation was common in patients with overt MPD at the time of the SVT, ranging from 71-100%, depending on the type of MPD diagnosed (100% for PV and 71% for others)¹². However, this mutation was also observed in patients without MPD at the time of thrombosis (0-32%). In a longer follow-up of these patients some may develop classical features of MPD and in fact, some authors report a diagnosis of MPD as late as 8 years after the thrombotic event. In our series, three JAK2 positive patients had a diagnosis of MPD a long time after the thrombotic event. Interestingly, patients with isolated mesenteric thrombosis in our series were JAK2 negative and did not develop MPD in a mean follow-up of 63.5 months, as previously described^{11, 16}.

Only one patient with CVT had an overt MPD and JAK2 V617F was positive. Literature data about CVT and JAK2 mutations are scarce. The largest series studied 45 patients with CVT and four cases had overt MPD at thrombosis diagnosis with a JAK2 V617F prevalence of 75% (3/4)¹¹.

In our patients with thrombosis at unusual sites, we did not find other JAK2 mutations. Colaizzo et al have described 2 patients with PV presenting with splanchnic venous thrombosis and exon 12 JAK2 mutation¹³. How-

ever, no mutation of JAK2 exon 12 has been reported in other large series of patients with abdominal venous thrombosis, either by direct sequencing or by using allele-specific PCR^{12, 21, 22}. Although Siemiatkowska et al. found three novel mutations in JAK2 exons 12, 19 and 25 in V617F-negative patients with MPD no data about thrombosis were provided²³.

In conclusion, JAK2 V617F is frequently found in patients with splanchnic vein thrombosis and may be considered marker of latent MPD. No other mutations of JAK2 appear to be important in this setting.

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La investigación científica se torna cada vez más una empresa cooperativa; es una tarea social incluso aunque no se la emprenda en equipo. El componente social en la investigación científica es la propiedad de ser públicos los problemas, las técnicas y los resultados (salvo algunos casos aberrantes); y también es público su estudio. El intuicionismo se opone a este carácter social del trabajo científico, puesto que considera a cada pensador como una unidad cerrada y valora más lo inefable y oscuro que lo comunicable y claro.

Mario Bunge

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