Prevalence of the JAK2 V617F mutation associated with splanchnic vein thrombosis.
A 10-year retrospective study

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Dear Sir,
Recent works reported a high prevalence of JAK2 V617F mutation in patients with splanchnic vein thrombosis (SVT) including Budd-Chiari Syndrome (BCS) and portal venous system thrombosis (PVT) (1–6), leading to suspect an underlying myeloproliferative disease (MPD). We have performed a retrospective study on patients with SVT followed-up at Montpellier University Hospital over a period of 10 years (1996–2006). The aim was to determine JAK2 V617F mutation status and the risk for subsequent development of MPD.

We investigated patients with SVT that underwent screening for thrombophilia at least three months after the thrombotic event. Inclusion criteria were the following: adult patients with diagnosis of BCS or PVT, absence of known causes of SVT such as malignancies or cirrhosis, available DNA for molecular testing gathered during thrombophilia screening. Patients who had established criteria for MPD (7, 8) at the time of thrombosis were excluded. Informed consent to study their DNA was obtained from all patients. Collection and use of blood samples was approved by the local Ethics Committees. The JAK2 V617F mutation was detected by single polymorphism genotyping assay using real-time quantitative polymerase chain reaction (PCR) (9) on leukocytes genomic DNA. Proportion of JAK2 V617F allele burden for positive samples was determined by allele-specific semi-quantitative PCR (JAK2 MutaScreen® Kit, Ipsogen, Marseille, France).

Forty-three cases of SVT were analysed (2 BCS, 41 PVT). Our series included 25 females (58.1%) and 18 males (41.9%). Mean age at diagnosis was 47 years (18–78). We detected JAK2 V617F mutation in 9.3% of all patients (n=4). The four patients with mutation were women, 24– to 65-years-old at the time of thrombosis, with diagnosis of PVT. No significant difference was found concerning age at diagnosis of SVT, sex or presence of a splenomegaly. Peripheral blood cells count was in the normal range in most patients of the whole cohort. We found that JAK2 V617F mutation was associated with higher white blood cells (WBC) and platelets count (p<0.05, Table 1) but not with higher haemoglobin levels. A prothrombotic risk factor was present in 32.6% of cases (n=14). The most frequent was factor VIII excess, defined as persistent elevated levels of factor VIII more than 200%, not related to an inflammatory state (11.6%). We also found antiphospholipid antibodies (lupus anticoagulant or antcardiolipin, beta2-glycoprotein I dependant antibodies) (9.3%), factor II 20210A polymorphism (9.3%), and factor V Leiden variant (2.3%). No protein C, S or antithrombin deficiencies were detected. A thrombophilic factor was found in two patients with JAK2 V617F mutation (factor II 20210A polymorphism in one case, antiphospholipid antibodies and factor VIII excess in the other case). Clinical risk factors of thrombosis such as surgery, trauma, immobilisation, plaster cast, pregnancy and post-partum, oral contraception, chronic digestive inflammatory disease, were observed in five patients of the overall cohort (11.6%). Among them, three carried the JAK2 V617F mutation. Thirty-three patients (76.7%) had a personal previous history of thrombosis at the time of SVT. Thirteen patients (30.2%) had a familial history of thrombosis.

The follow-up has been performed for the four patients with the JAK2 V617F mutation. Overt MPD developed in three of...
them. Semi-quantification of JAK2 V617F mutation was done retrospectively on two samples for each patient at different times: one sample of blood collected at SVT diagnosis and one sample at overt MPD diagnosis. Polymorphonuclear cells percentages were similar in the two samples. Patient 1, a 24-year-old woman, had no clinical risk factor of thrombosis. Thrombophilia screening was negative. A rapid increase in platelet count occurred after diagnosis of SVT. She suffered other severe thrombotic events including cerebral vein thrombosis (CVT). Essential thrombocytemia (ET) was diagnosed. Our findings showed a stable mutated allele charge at 12.5–31% between SVT and ET diagnosis. Patient 2, 65 years old, had two biological risk factors for thrombosis: antiphospholipid antibodies and factor VIII excess. Overt ET was diagnosed one year after thrombosis. JAK2 V617F proportion increased from 12.5–31% at SVT diagnosis to 50–78% one year later. For patient 3, a 58-year-old woman, FII 20210A allele was found during screening of thrombophilia in 2006. At the end of our 10-year retrospective study, she did not fit the criteria of MPD, but sustained follow-up showed high platelet count in 2007 and ET diagnosis was made since. The percentage of JAK2-mutated allele also increased when MPD signs developed, from 31–50% to 50–78%. Patient 4 was 54 years old at SVT diagnosis. Mutated JAK2 allele proportion was 12.5–31%. Blood cell count remained in normal range. Unfortunately, no DNA was available to control JAK2 V617F percentage during follow-up.

Thromboses at unusual sites like SVT or CVT are known to be clinical manifestations that can reveal an underlying MPD (10, 11). However, diagnosis of MPD may be difficult. In CVT, the place of a systematic research of JAK2 mutation need to be clarified (2, 12). In SVT classical criteria are hidden or modified by hypersplenism associated to portal hypertension or by possible occult bleeding. Detection of JAK2 V617F mutation represents a new useful tool to confirm diagnosis in these cases (1–5). We report here a retrospective study with long-term follow-up (10 years) on 43 patients that underwent thrombophilia screening after SVT. Prevalence of JAK2 V617F mutation in our study (9.3%) was lower than expected as compared to data recently reported, ranging from 17% to 58%. Of note, if patients with diagnosis of MPD at the time of thrombosis are drawn from the Coiazzo et al. study (4) as we have done, prevalence falls to 11%, similar to our findings. The recruitment of our study based on thrombophilia screening and excluding overt MPD at SVT diagnosis may thus explain this difference. Another explanation may be the cell population studied (total leukocytes versus purified granulocytes) or the different sensitivities of the techniques used (13). In our study, JAK2 V617F was found in patients that had no initial peripheral blood changes. Presence of acquired or inherited thrombophilic factors and association of thrombotic risk factors should not exclude mutation detection. We found the possible coexistence of these abnormalities, as described by other. Follow-up and repeated blood cell counts showed that latent MPD become rapidly overt in three patients among four. Increase of the allele charge of mutated gene was observed in correlation of platelets count increase for two patients. To note, for patient 1, the interval of time between SVT and ET diagnosis was very short. It could explain the absence of increase of the allele burden. The increase observed may be due to the progression of the disease to an overt MPD. Impact of JAK2 V617F mutational load on disease phenotype has recently been shown (14). A high allele burden could be associated with higher risk of thrombosis (15). But to our knowledge, these findings have not been reported. They need to be confirmed with more quantitative methods on a larger cohort.

To conclude, our experience confirms that JAK2 V617F testing on peripheral blood is very simple and rapid, and represents a key element of latent MPD diagnosis in SVT. This work highlights also the potential interest of JAK2 V617F mutation quantification during follow-up of these latent MPD for evolution prediction. Further prospective studies are now needed to clarify evolution of these atypical JAK2 V617F diseases.

References


Table 1: Blood cell counts characteristics associated with the JAK2V617F mutation. Blood cells counts in patient at diagnosis of splenecic vein thrombosis (SVT). Medians are represented with the first and the third quartiles (Q1 and Q3 respectively). P-values were obtained with the Wilcoxon statistical test.

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| Age | Without JAK2V617F (n=39) | With JAK2V617F (n=4) | P-value |
| Median [Q1–Q3] | Median [Q1–Q3] | |
| Haemoglobin (g/dl) | 13.8 [11.9–14.9] | 13.0 [10.8–13.4] | 0.229 |
| Haematocrit (%) | 41 [36.2–44] | 39.4 [33.7–41] | 0.311 |
| Platelet count (x10^11/l) | 199 [126–255] | 319.5 [277–653] | 0.006* |
| WBC count (x10^9/l) | 5.6 [4.1–7.2] | 9.7 [7.2–15.0] | 0.029* |

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