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JAK2 mutation in patients with splanchnic venous thrombosis: A pilot study from India

Sir,

Splanchnic venous thrombosis (SVT) includes thrombosis of the hepatic, portal and mesenteric venous system. SVT may be the presenting feature or a late complication of myeloproliferative neoplasms (MPN). However, at presentation it is difficult to predict which patient will develop overt MPN. Myeloproliferative neoplasms whether overt or latent, represent a major risk factor for the development of thrombosis in the portal, mesenteric, and hepatic areas[1]. More recently, *JAK2 V617F* has been detected in a significant proportion (31-59%) of patients presenting with SVT[2-5]. Many of these *JAK2* (Janus Kinase 2) mutation patients without overt MPNs subsequently develop overt MPNs[6]. No information is available on the presence of the *JAK2 V617F* mutation among patients with SVT from India. Therefore, we analyzed prospectively patients with thrombosis with no signs of overt MPN, for the presence of the *JAK2 V617F* mutation.

Blood samples (10 ml) were obtained randomly from 52 patients [30 males, 22 females; median age 32 yr (range 11-50 yr)] with SVT at the Department of Haematology and Gastroenterology, All India Institute of Medical Sciences (AIIMS), New Delhi, between January 2008 to August 2009. Of these, 32 had portal vein thrombosis (PVT), of whom four had both portal and mesenteric vein thrombosis and 20 had hepatic vein thrombosis (HVT). None of the patients had overt cancer or other disorders, occult or not diagnosed, at the time of SVT occurrence. HVT was diagnosed according to previously published criteria[7]. PVT was diagnosed in the presence of endoluminal material and absence of flow in the portal vein, or cavernous transformation of the vein as shown by duplex-Doppler ultrasound, or contrast enhanced CT scan or magnetic resonance imaging. Patients with hepatocellular carcinoma, liver cirrhosis, previously diagnosed MPN and those diagnosed with an overt MPN at the time of thrombosis were excluded.

Twenty normal healthy individuals (blood donors) were included as control in the study who were staff people. The study protocol was approved by the institute ethics committee, and written informed consent was taken from all patients and controls.

All patients were evaluated for thrombophilia-protein C, protein S, prothrombin gene mutation, factor V Leiden mutation, lupus anticoagulant, antiphospholipid antibodies, and other hypercoagulable states like sepsis, malignancy, pregnancy or oral contraceptives. Peripheral smear was evaluated for leukoerythroblastic picture. Spleen size was determined by ultrasound/ CT scan. Bone marrow biopsy was examined for morphological changes related to myeloproliferative neoplasm. Follow up of clinical and laboratory features was performed to assess for the development of an overt MPN. All patients were followed up for a year. Presence of *JAK2* mutation was assessed by an allele specific (ASO) polymerase chain reaction (PCR) exactly as described by Baxter *et al*[8], in which the mutated allele was specifically amplified together with a fragment common to the mutated and wild type genes (Fig. a). Samples positive for the mutation were subsequently analyzed via PCR amplification and digestion with the restriction endonuclease BsaXI, (New England Biolabs, Hitchin, UK) which allows for an estimation of the ratio between mutated and wild-type alleles (Fig. b). This allows both normal and mutant alleles to be visualized thus distinguishing between homozygous and heterozygous mutations. In all samples scored as homozygous, the mutated allele represented more than 70 per cent of the PCR product. Cases with and without *JAK2V617F* mutation were analysed using Mann-Whitney U (Wilcoxon rank sum test) for WBC, platelet count, and splenomegaly.

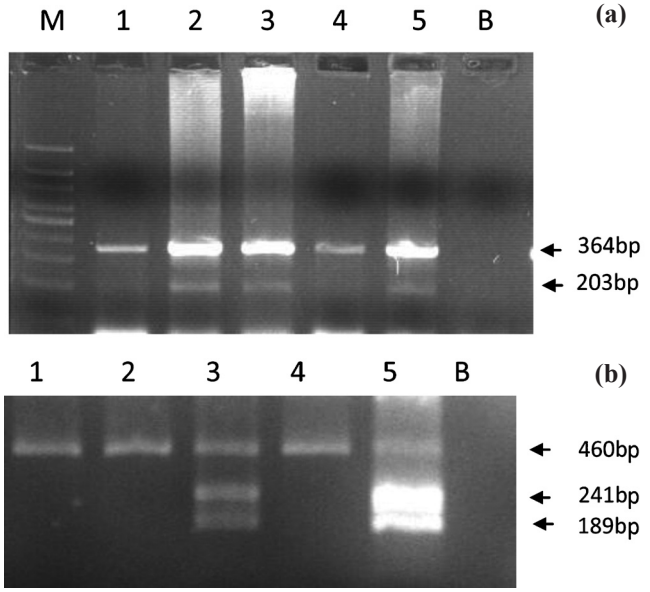

Fig. (a) *JAK2V617F* mutation by Allele specific PCR**.** Lane M, 100-bp ladder; lanes 1-5, samples from patients, lanes 2, 3 and 5 show mutant band (203 bp) carried by the patients. Lanes 1 and 4 show a single band (364bp) which is a wild type band and acts as an internal PCR control, lane B is the negative control. Arrows indicate the product of amplification. **(b)** Restriction enzyme analysis of *JAK2V617F* mutation. Lanes 1-5, samples from patients, lane B is the negative control, lanes 1, 2 and 4 show homozygous mutant band at 460 bp as assessed by BsaXI restriction enzyme. Lanes 3 and 5 show the wild allele which was digested into 241, 189 and 30 bp fragments.

Portal vein thrombosis was observed in 32 (62%) patients and 20 (38%) had HVT. In five SVT patients white blood cell counts were higher than 11 x 10^9/l, four of these were positive for *JAK2* mutation. In all these patients peripheral smear was unremarkable. Bone marrow did not show any morphological evidence of myeloproliferative neoplasm. Reticulin stain was normal. Two patients with portal vein thrombosis had haemoglobin >15 g/dl, both these patients were *JAK2* negative and bone marrow was normal. Red cell mass study was within normal limit. Overall nine patients (17.3%) showed thrombophilic state. Four patients had protein C deficiency, one had protein S deficiency, one patient had factor V Leiden, one had antithrombin III deficiency, and one had antiphospholipid antibody syndrome. One patient had low proglob C but could not be evaluated further. However, a low proglob C by itself has been reported to be an independent risk factor for thrombosis[9]. Among the 20 patients with HVT, four had a thrombophilic state with one each with protein C deficiency, protein S deficiency, antithrombin deficiency and heterozygosity for factor V Leiden. None of these patients had been tested in acute state or while on anticoagulant therapy.

JAK V617F mutation was identified in six patients among 32 with PVT and 4 among the 20 patients with HVT (Table). Patients carrying *JAK2* mutation had significantly (P<0.05) higher median WBC count and median platelet count as compared to JAK2 negative patients. There was no difference in age, haemoglobin and spleen size. Ten JAK2 positive patients were followed up at three monthly intervals with clinical, haemogram and peripheral smear examination. One patient with HVT during follow up developed rising total leukocyte count (20 x10^9/l) and high platelet count (1100 x10^9/l). Bone marrow showed panmyelosis with large megakaryocyte. He was diagnosed with essential thrombocythemia (ET), started on anticoagulation and was symptomatic for a year. Time from diagnosis to progression was 9 months. None of the JAK 2 negative patients developed MPN over the study period. Only one patient positive for JAK2 progressed to MPN. Though, *JAK2V617F* mutation has been described in SVT; there are varied results about its prevalence. It has been found to occur in 34-36 per cent in SVT patients, 33-40 per cent in HVT patients and 17-41 per cent PVT patients[4,5]. We observed presence of *JAK2V617F* mutation in 19 per cent of patients with SVT (18% with PVT and 20% with HVT) which is lower than that reported by others[4]. Inherited thrombophilic risk factors that contribute to the multifactorial aetiology of venous thrombosis, was observed in nine patients (17.31%); this is lower than that reported by others[2,4,5]. Two of

Table. General characteristics and JAK 2 mutation status of the patients

Characteristics	JAK2 positive (n=10)	JAK 2 negative (n=42)
Median age (yr)	32.5 (11-49)	30 (15-55)
Sex (M/F)	6/4	24/18
Median spleen size (cm)	15.7	15.3
Median haemoglobin level (g/dl)	13.1 (9.4-14.6)	12.1 (5.8-16)
Median WBC count (x10^9/l)	8.3 (3.8-11.8)*	5.3 (1.4-12.1)
Median platelet count (x10^9/l)	254 (80-441)*	146 (23-490)

WBC, white blood cell; Hb, haemoglobin, n, number of patients; P value refers to the comparison of JAK2 V617F positive vs. negative subjects. Values in parentheses denote range
*P<0.05 compared with JAK2 negative

the thrombophilic patients had *JAK2* mutation; both were HVT. None of the controls were positive for the JAK2 mutation.

One *JAK2* mutation positive HVT patient developed ET after nine months of follow up. In earlier studies, progression to overt MPN has been reported at a median of 38 months[3] to 4 yr[4]. Colaizzo *et al*[2] studied 99 patients with portal and mesenteric vein thrombosis, of whom seven *JAK2* patients had an overt MPN at diagnosis. Three of the 10 *JAK2* positive patients without an overt MPN at diagnosis developed an overt MPN at a median of 41 months[2].

In the present preliminary study, none of the patients had an overt MPN at diagnosis. However, one *JAK2* positive patient developed ET while none of the *JAK2* negative patients progressed to MPN. The low occurrence of progression to MPN may be due to short follow up period in our study.

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