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ORIGINAL ARTICLE

Incidence of the *JAK2* V617F mutation among patients with splanchnic or cerebral venous thrombosis and without overt chronic myeloproliferative disorders

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Summary. *Background:* Thrombosis of splanchnic or cerebral veins is a typical manifestation of polycythemia vera (PV) or essential thrombocythemia (ET). The recently identified Janus kinase 2 (*JAK2*) V617F somatic mutation is closely related to chronic myeloproliferative disorders (CMD). *Objective:* To assess the incidence of the *JAK2* V617F mutation among patients with splanchnic or cerebral venous thrombosis with or without overt CMD. *Patients and methods:* We searched for the mutation in 139 adult patients (> 18 years old) with thrombosis of hepatic veins (HVT, $n = 15$), or extrahepatic portal vein (PVT) and/or mesenteric vein (MVT) ($n = 79$), or cerebral veins (CVT, $n = 45$). Only 19 patients fulfilled criteria for diagnosis of PV ($n = 8$) or ET ($n = 11$) at the time of thrombosis: four had HVT, 11 PVT and/or MVT, and four CVT. *Results:* The *JAK2* V617F mutation was found in 94.7% [95% CI 75.3–99.0] of the patients with overt CMD at the time of thrombosis, in 21.5% (95% CI 13.8–31.7) of the patients with abdominal venous thrombosis and without overt CMD, and in 4.8% (95% CI 1.3–16.1) of the patients with CVT and without overt CMD. Among the patients without overt CMD or thrombophilia and with unprovoked thrombosis, 29.4% (95% CI 16.8–46.1) with splanchnic venous thrombosis and 42.8% (95% CI 24.4–63.4) with PVT had the *JAK2* V617F mutation. *Conclusions:* A substantial proportion of patients with splanchnic venous thrombosis and a small, but significant, number of patients with CVT can be recognized as carriers of the *JAK2* V617F mutation in the absence of overt signs of CMD. The clinical significance of such findings deserves further investigation.

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Introduction

Thrombosis is a major cause of morbidity and mortality in patients with *Bcr/Abl*-negative chronic myeloproliferative disorders (CMD) [1–3]. Thrombosis of major abdominal veins has been reported in 5–10% of the patients with polycythemia vera (PV) and essential thrombocythemia (ET) [4,5]. Conversely, overt CMD can be diagnosed in 28–49% of the patients with hepatic vein thrombosis (HVT) and in 14–35% of the patients with extrahepatic portal vein thrombosis (PVT) [6–12]. Cerebral venous thrombosis (CVT) is another rare but typical clinical manifestation associated with PV or ET [13]. The incidence of CVT has previously been measured as 1% in a large series of patients with ET [5].

CMD (whether at early stages, or at latent stages that do not fulfill the diagnostic criteria) can be recognized in an additional, and also substantial, proportion of patients with HVT or PVT. This is performed usually on the basis of the growth of erythroid colonies in the absence of exogenous erythropoietin, referred to as spontaneous endogenous erythroid colonies (EEC) [14,15]. In some patient series, this approach has doubled the overall incidence of CMD diagnosis [6,7]. However, EEC assay demands specialist facilities, which are neither widely available nor fully standardized. This, combined with the possibility of false-positives in assays in both non-clonal causes of erythrocytosis and in healthy controls, has raised some doubts on the use of EEC as the only diagnosis of latent CMD [16].

Recently, a somatic mutation of the Janus kinase 2 (*JAK2*) gene was identified in patients with CMD. The V617F substitution results in a constitutive activation of the tyrosine kinase [17–21]. This mutation has been detected in high frequency in patients with PV (65–97%) or ET (23–57%) [17,19,20,22–27], and correlates with the ability to form EEC [28]. Although the mutation is absent in healthy controls

[17,19–22], it has been found in 0.9% of a large Chinese hospital population of 3935 individuals [29]. It is uncertain whether the *JAK2* V617F mutation is associated with an increased risk of thrombosis, because a higher rate of thrombotic complications has been reported in some series of CMD *JAK2* V617F carrier patients when compared with non-carrier CMD patients [17,19,25,27], although not in all patient series [23,24,26].

The close relationship of the *JAK2* V617F mutation with CMD [17–28] has led to its consideration as a major diagnostic criterion for PV and ET [30]. It can be expected that the detection of the *JAK2* V617F mutation could simplify the diagnosis in a significant proportion of patients with HVT or PVT. Recently, the *JAK2* V617F mutation has been reported in a high proportion of patients with Budd–Chiari syndrome [31], PVT and mesenteric venous thrombosis (MVT) [32,33]. No data are available on the presence of the *JAK2* V617F mutation among patients with CVT. To address this, we have analyzed patients with thrombosis at unusual sites, and with no signs of overt CMD, for the presence of the *JAK2* V617F mutation.

Patients and methods

Patients

From November 1994 to August 2006, 1568 unrelated patients with previous venous thromboembolism (VTE) were referred to our thrombosis center for thrombophilia screening. Preliminary evaluation ruled out patients with overt cancer. Transient risk factors at the times of all episodes of VTE [i.e. surgery, pregnancy and puerperium (defined as the 6-week period after delivery), oral contraceptive intake, hormone replacement therapy, trauma, leg cast, prolonged bed immobilization ($>$ 10 days) or long travel ($>$ 8 h)] were recorded. In the case of abdominal thrombosis or CVT, special attention was paid to the presence of inflammatory or infectious diseases in anatomical sites of venous drainage (liver, cholecystis, pancreas, bowels, brain, mastoid and neck). In the absence of transient risk factors, VTE was labeled as spontaneous.

This study was approved by the institutional review board. All patients gave informed consent and underwent laboratory screening for thrombophilia. Blood samples were taken from all patients; genomic DNA was extracted from peripheral blood granulocytes using standard procedures and was archived. All patients (or their parents in the case of children) gave informed consent for future investigations on archived DNA aimed to obtain novel insight on the putative cause of thrombosis.

We first selected patients with diagnosis of splanchnic venous thrombosis (involving hepatic, portal, mesenteric, or splenic veins) or diagnosis of CVT. Of these, DNA samples were available in 158 of 190 patients.

Nineteen patients had thrombosis prior to 18 years of age (two HVT, three PVT, and 14 CVT). Although these individuals were analyzed for comparative purposes, they were excluded from the final analysis, which was limited to adult patients.

Out of the 139 adult patients, 19 (13.7%) had overt CMD at the time of thrombosis: four had HVT, seven had PVT [including one also with superior MVT, three with splenic vein thrombosis (SVT), and one with both MVT and SVT], one MVT, three SVT, and four CVT. Diagnosis of PV (eight patients) or ET (11 patients) was made according to updated criteria of the Polycythemia Vera Study Group [34,35]. The rate of overt CMD was 15.9% among patients with splanchnic venous thrombosis and 8.9% among patients with CVT.

Of the remaining 120 adult individuals, 11 had HVT (including one combined with PVT), 50 had PVT (combined with MVT in 14 cases, with SVT in two, with both MVT and SVT in 14, and associated with thrombosis of the superior mesenteric artery in one), 16 MVT, two SVT, and 41 CVT.

None of the patients with PVT had liver cirrhosis. All thrombotic events were objectively proven by means of computed tomography or magnetic resonance imaging. Eleven of the 47 patients with MVT underwent laparotomy for resection of infarcted bowel.

Methods

Screening for thrombophilia included measurement of antithrombin (AT) and protein C (PC) functional activities, free protein S (PS) antigen, and fasting homocysteine levels. The identification of FV Leiden and prothrombin (PT) G20210A polymorphisms, as well as the detection of antiphospholipid antibodies (i.e. lupus anticoagulant and anticardiolipin antibodies) were also performed as previously described [36,37]. Deficiency of AT, PC, or PS was considered inherited only after finding levels both below the normal range and in a first-degree relative.

The *JAK2* V617F mutation was detected by allele-specific polymerase chain reaction (PCR) essentially according to Baxter *et al.* [17]. For sequencing analysis we amplified genomic DNA by PCR according to the procedure of Wolanskyj *et al.* [23]. Samples were analyzed on an ABI PRISM 3100 Avant DNA analyzer (Applied Biosystems, Foster City, CA, USA) and the presence of mutation G to T positioning the V617 position in exon 12 identified.

All the assays were performed blinded to the diagnosis and clinical history of the patients. We also carried out a retrospective revision of the laboratory data available at the referral in all the patients who, at the time of thrombosis, were considered to be without overt CMD.

Statistical methods

Differences between groups were estimated by Fisher's exact test (statistical significance for $P < 0.05$).

Results

The clinical characteristics of the investigated patient groups are shown in Table 1.

Table 1 Clinical characteristics of the investigated patients and results of the laboratory investigation for thrombophilia

	Sex (M/F)	Age (years) median (range)	Transient risk factor				Thrombophilia					
			None	OC	Pregnancy or puerperium	Other transient risk factors	AT, PC or PS deficiency	Factor V Leiden	PT G20210A	HyO	LAC/ ACA	Multiple defects
Patients with thrombosis and overt CMD												
HVT ($n = 4$)	0/4	27 (22–28)	4	0	0	0	0	1	0	0	0	0
PVT ($n = 8$)	4/4	38 (21–63)	6	1	1	0	1	0	0	1	1	0
SVT ($n = 3$)	2/1	34 (33–55)	3	0	0	0	1	0	0	0	0	0
Total ($n = 15$)	6/9	33 (21–63)	13	1	1	0	2	1	0	1	1	0
CVT ($n = 4$)	1/3	35 (25–80)	3	0	1	0	0	1	0	0	0	0
Patients with thrombosis but without overt CMD												
HVT ($n = 11$)	4/7	27 (18–67)	7	2	0	2	1	2	0	0	0	0
PVT ($n = 50$)	23/27	46 (18–76)	33	7	2	8	5	3	6	1	3	1
MVT ($n = 16$)	11/5	45 (24–79)	11	1	0	4	2	2	1	1	0	1
SVT ($n = 2$)	1/1	35,54	1	0	0	1	0	0	0	0	0	0
Total ($n = 79$)	39/40	44 (18–79)	52	10	2	15	8	7	7	2	3	2
CVT ($n = 41$)	8/33	35 (19–80)	13	15	8	5	3	4	4	0	3	2

ACA, anticardiolipin antibodies; AT, antithrombin; CMD, chronic myeloproliferative disorders; CVT, cerebral vein thrombosis; HVT, hepatic vein thrombosis; HyO, hyperhomocysteinemia; LAC, lupus anticoagulant; MVT, mesenteric vein thrombosis; OC, oral contraceptive; PC, protein C; PS, protein S; PT, prothrombin; PVT, portal vein thrombosis; SVT, splenic vein thrombosis.

First manifestation of thrombosis at unusual sites was found in 18 of the 19 patients with overt CMD (94.7%), and in 106 patients without CMD (88.3%, $P = 0.69$). In all other cases, thrombosis at unusual sites was secondary to deep venous thrombosis of the legs ($n = 9$), superficial venous thrombosis of the legs ($n = 4$), or a deep venous thrombosis of the arms ($n = 2$). Thrombosis was unprovoked in 16 patients with overt CMD (84.2%) and in 65 patients without CMD (54.1%, $P = 0.02$).

Screening for thrombophilia

Thrombophilia was diagnosed in 45 patients without CMD [37.5%, 95% confidence interval (95% CI) 29.3–46.4]. In patients with splanchnic venous thrombosis, 36.7% (95% CI 26.9–47.7) had thrombophilia: 10.1% had inherited deficiency of AT, PC, or PS; 8.9% had FV Leiden; 8.9% PT G20210A; 2.5% mild hyperhomocysteinemia; 3.8% antiphospholipid antibodies; and 2.5% had multiple abnormalities. In the patients with CVT but without CMD, 39% (95% CI 25.6–54.2) had thrombophilia: 7.3% had inherited deficiency of AT, PC, or PS; 9.7% FV Leiden; 9.7% PT G20210A; 7.3% antiphospholipid antibodies; and 4.9% had multiple abnormalities. Finally, thrombophilia was diagnosed in six patients with overt CMD (31.5%, 95% CI 15.3–53.9) (Table 1).

Among the patients without overt CMD, 34 with splanchnic venous thrombosis (43.0%) and eight with CVT (19.5%, $P = 0.01$) had unprovoked thrombosis and no known thrombophilic abnormality.

Screening for the JAK2 V617F mutation

None of the 19 pediatric patients with thrombosis carried the *JAK2* V617F mutation.

The distribution of the *JAK2* V617F mutation in the adult patients is shown in Table 2. Of these, the *JAK2* V617F mutation was found in 37 individuals: 32 patients among the 94 with splanchnic venous thrombosis (34.0%, 95% CI 25.2–44.0) and five among the 45 patients with CVT (11.1%, 95% CI 4.8–23.5) ($P = 0.004$). The incidence of the mutation was 33.3% (95% CI 15.1–58.2) among the 15 HVT patients, and 41.3% (95% CI 29.6–54.2) among the 58 PVT patients.

JAK2 V617F was identified in 94.7% (95% CI 75.3–99.0) of the 19 patients with overt CMD at the time of thrombosis (Table 2). Among the 120 patients without overt CMD, the mutation was present in 21.5% (95% CI 13.8–31.7) of the patients with splanchnic venous thrombosis, and in 4.8% (95% CI 1.3–16.1) of those with CVT. In this group not fulfilling the diagnostic criteria for CMD, the mutation was found in 16 patients with PVT (32.0%, 95% CI 20.7–45.8), in one with HVT (9.0%, 95% CI 1.6–37.7), and in none with isolated MVT (95% CI 0.0–0.2) or SVT (95% CI 0.0–0.6) (Table 2). The incidence of the mutation among the patients with PVT was not significantly different from that found among the patients with HVT ($P = 0.15$), but was significantly higher than that found in patients with MVT ($P = 0.007$). When the analysis was restricted to the patients without diagnosis of CMD and with unexplained thrombosis (i.e. occurred in the absence of thrombophilia or a provoking circumstance), the *JAK2* V617F mutation was detected in 10 of 34 patients with thrombosis of splanchnic veins (29.4%, 95% CI 16.8–46.1) and in nine of 21 patients with PVT (42.8% 95% CI 24.4–63.4) (Table 2).

Twenty-seven out of the 37 samples containing the *JAK2* V617F substitution were sequenced successfully, revealing 24 heterozygous and three homozygous patients. Because of the paucity of DNA, we performed allele-specific PCR on the remaining samples, which was more sensitive than sequencing [17]. The heterozygous patients had overt CMD in 14 cases (five PV and nine ET); of the remaining 10 patients without

Table 2 Results of the laboratory investigation for the presence of the *JAK2* V617F mutation

	Patients (*n*) with *JAK2* V617F mutation (%)	Patients (*n*) with thrombophilia or transient risk factor (*JAK2* V617F mutation)	Unexplained thrombosis (*JAK2* V617F mutation)
Patients with thrombosis and overt CMD			
HVT ($n = 4$)	4 (100.0)	1 (1)	3 (3)
PVT ($n = 8$)	8 (100.0)	4 (4)	4 (4)
SVT ($n = 3$)	3 (100.0)	1 (1)	2 (2)
Total ($n = 15$)	15 (100.0)	6 (6)	9 (9)
CVT ($n = 4$)	3 (75.0)	2 (2)	2 (1)
Patients with thrombosis but without overt CMD			
HVT ($n = 11$)	1 (9.0)	6 (0)	5 (1)
PVT ($n = 50$)	16 (32.0)	29 (7)	21 (9)
MVT ($n = 16$)	0	9 (0)	7 (0)
SVT ($n = 2$)	0	1 (0)	1 (0)
Total ($n = 79$)	17 (21.5)	45 (7)	34 (10)
CVT ($n = 41$)	2 (4.8)	33 (2)	8 (0)

CMD, chronic myeloproliferative disorders; CVT, cerebral vein thrombosis; HVT, hepatic vein thrombosis; MVT, mesenteric vein thrombosis; PVT, portal vein thrombosis; SVT, splenic vein thrombosis.

signs of overt CMD, one had CVT and nine PVT (one with combined HVT, two with MVT, one with SVT, and four with both MVT and SVT). Out of the three homozygous patients, one had PVT as a first manifestation of overt PV at 50 years of age, when she was tested. The second patient, with PC deficiency, suffered from PVT (combined with both MVT and SVT) at 34 years of age during intake of oral contraceptives; 6 years later she had a CVT following oral anticoagulation. ET was diagnosed 8 years after the first event. The third patient was a woman who suffered from unprovoked PVT associated with thrombosis of the superior mesenteric artery at the age of 58 years. She had a recurrent unprovoked DVT of the left leg and of the inferior cava vein 7 years later, when we obtained the DNA sample that was tested. She died from hepatic failure aged 73 years, without signs of overt CMD.

Retrospective analysis of the laboratory data of the patients without diagnosis of CMD at the time of thrombosis

To evaluate the possibility of under-diagnosis of CMD at the time of thrombosis, we carried out a retrospective analysis of the laboratory data of the patients who were considered to be without overt CMD. Among the patients with CVT, none had a palpable splenomegaly. Furthermore, none had hemoglobin (Hb) levels > 18 g dL^{-1} (men) or > 16 g dL^{-1} (women), or hematocrit values > 0.52 (men) or > 0.47 (women), which are both common criteria to suspect PV [38,39]. Finally, none had a sustained leukocyte count $> 12 \times 10^9$ L^{-1} and/or a sustained platelet count $> 500 \times 10^9$ L^{-1} in the absence of known causes [39].

In patients with splanchnic venous thrombosis and portal hypertension, the criteria adopted to suspect PV or ET may be altered because of the presence of hypersplenism and increased plasma volume, which can mask erythrocytosis or thrombocytosis [11,14,15,39]. None of the patients without diagnosis of overt CMD underwent measurement of red blood cell mass at the time of referral to the center. Consequently, we adopted the criteria previously reported by Primignani *et al.* [10] for suspicion of CMD in patients with PVT and enlarged spleen volume. We considered possible under-diagnosis of CMD in those patients with Hb > 15 g dL^{-1} (men) or > 13 g dL^{-1} (women), or with leukocyte count $> 10 \times 10^9$ L^{-1}, or with platelet count $> 300 \times 10^9$ L^{-1} in the absence of known causes. Among the 17 patients with splanchnic venous thrombosis and with the *JAK2* V617F mutation, none had a leukocyte count $> 10 \times 10^9$ L^{-1}. One male individual had Hb 16.4 g dL^{-1} and a platelet count of 319×10^9 L^{-1}. However, he was a heavy smoker (60 cigarettes per day) and the Hb level was considered appropriate. Among the 62 patients without mutation, two male patients had Hb > 15 g dL^{-1}, two female patients had Hb > 13 g dL^{-1}, one had a leukocyte count $> 10 \times 10^9$ L^{-1}, and three had a platelet count $> 300 \times 10^9$ L^{-1}. Three patients were considered not evaluable because of previous splenectomy, and were thus not included in further analysis. Therefore, among the patients with abdominal venous thrombosis, one out of 17 (5.9%) with the *JAK2* V617F mutation, and eight out of 59 (13.5%) in those without ($P = 0.67$), gave laboratory values that might suggest CMD. A second retrospective analysis was also carried out according to Chait *et al.* [11], who identified a platelet count $> 200 \times 10^9$ L^{-1} in the presence of splenomegaly as strongly suggesting CMD in patients with splanchnic venous thrombosis. A platelet count $> 200 \times 10^9$ L^{-1} was present in eight patients with the *JAK2* V617F mutation (47%), and in 26 without (44.1%, $P = 1.0$). This gave no significant difference even when adopting such a stringent criterion of suspicion.

We obtained follow-up information on 12 of the 19 patients with the *JAK2* V617F mutation but who lacked overt CMD. The median follow-up was 4 years (range 2–9 years). In one patient, overt ET developed 8 years after thrombosis. One patient died 8 years after blood drawing without signs of overt CMD. In two patients, platelets were 411×10^9 L^{-1} and 517×10^9 L^{-1} (4 and 9 years after PVT, respectively). In another patient, the platelet count was 511×10^9 L^{-1} 5 years

after CVT. No other patient developed signs inducing suspicion of CMD.

Discussion

Although thrombosis in splanchnic or cerebral veins is regarded as a typical manifestation of CMD [1,4,5,13], no systematic data are available concerning the incidence of CMD as an underlying cause of CVT. However, overt CMD can be recognized in about one-third of the patients with splanchnic venous thrombosis [6–12]. In patients with HVT or PVT, portal hypertension and consequent hypersplenism and hemodilution can alter or mask the signs usually employed as conventional criteria for diagnosis of CMD [11,39]. Therefore, a special effort to diagnose CMD is required [6,7,11,12,14,15]. The recent identification of the *JAK2* V617F mutation is viewed as a promising tool to simplify the diagnostic approach [17–28,30].

We systematically evaluated the archived samples of the patients with splanchnic or cerebral venous thrombosis that were consecutively referred to our thrombosis center. A thrombophilic abnormality was diagnosed in 37% of the patients, consistent with previous estimates [7,8,10,40].

An overt CMD was identified at the time of thrombosis in 15.9% of the patients with splanchnic venous thrombosis, and in 8.9% of the patients with CVT. The *JAK2* V617F mutation was detectable in almost all of these patients. To date, it is uncertain whether this mutation is associated with an increased risk for thrombosis [17,19,23–27]. In CMD patients with a history of thrombosis, the incidence of the *JAK2* V617F mutation in previous studies has ranged from 50% to 79.3%, with a pooled incidence of 61.0% (210 of 344 reported CMD patients with thrombosis) [17,19,23–27]. This is significantly different ($P = 0.002$) to the 94.7% incidence of the *JAK2* V617F mutation detected in our series of patients with thrombosis at unusual sites and with overt CMD. At this time, we have no explanation for the discrepancy in incidence of the mutation. Our data suggest a greater association of *JAK2* V617F with a more pronounced risk for thrombosis at unusual sites, indicating that further investigations in larger samples are warranted.

We demonstrated the presence of the *JAK2* V617F mutation in 34.0% of all the patients with splanchnic venous thrombosis and in 21.5% of those without diagnosis of CMD at the time of thrombosis. The mutation was found in 33.3% of the HVT patients, with incidence lower than that reported by Patel *et al.* [31] but similar to that reported by Primignani *et al.* [32], who found the mutation in 40% of the HVT patients. Yet, all HVT patients with overt CMD had the mutation, which fits with the 90.9% incidence reported by Patel *et al.* [31] in patients who subsequently developed CMD.

We detected the *JAK2* V617F mutation in 41.3% of all the patients with PVT, in agreement with Primignani *et al.* [32] who reported the mutation in 35.6% of these patients. In our study, the analysis was restricted to PVT patients without overt CMD, without thrombophilia, and without a provoking circumstance in their clinical history. This confirmed a incidence of the *JAK2* V617F mutation as high as 42.8%. Colaizzo *et al.* [33] reported a incidence of the *JAK2* V617F mutation in 17.2% of patients with PVT and/or MVT, i.e. in 77.8% of the patients with overt CMD at the time of thrombosis, and in 11.1% of the patients without overt CMD. The incidence of the *JAK2* V617F mutation among patients with no known inherited or acquired cause of thrombosis was 21.2%, with a frequency lower than that found in the present study. A possible contribution to this discrepancy could be the inclusion in their estimate of an unreported number of pediatric patients, none of whom carried the *JAK2* V617F mutation.

The identification of the *JAK2* V617F mutation appears to be a useful tool in the diagnosis of patients with apparent idiopathic PVT, and may possibly overcome the difficulties for diagnosis of CMD in this setting. The association of thrombosis with the *JAK2* V617F mutation in the absence of factors such as erythrocytosis or thrombocytosis could potentially be mediated by a possible *JAK2*-mediated effect that enhances platelet and leukocyte activation [41].

There was low incidence of the *JAK2* V617F mutation (4.8%) in CVT patients without diagnosis of CMD. This may, in part, be due to a higher incidence of transient risk factors among the patients with CVT in our study. However, in the only two *JAK2* V617F patients an additional risk factor was present. Therefore, a site-linked thrombotic risk could only be suggested in the patients with HVT or PVT. This hypothesis is reinforced by the observation that the mutation was not found in our patients with isolated MVT, in line with the report of Colaizzo *et al.* [33].

Our study has several limitations. Firstly, it should be taken into consideration that a portion of the patients with splanchnic venous thrombosis may have been simply under-diagnosed because of the difficulties in applying conventional diagnosis criteria for CMD. However, retrospective analysis of laboratory values at the time of thrombosis showed that the incidence of the *JAK2* V617F mutation was equally distributed among the patients who could have been potentially under-diagnosed with CMD, and those who were not. Second, our study was retrospective and only some of the patients had a follow-up. Of these, only one of the *JAK2* V617F patients developed an overt CMD during the follow-up period, although one-third of the patients developed increased platelet counts during the intervening years. However, whereas the follow-up in our series did not exceed 9 years, a thrombotic event has been reported to precede diagnosis in some cases by up to 15 years in patients with CMD [2,42]. Third, none of our patients without diagnosis of overt CMD underwent measurement of red cell mass at the time of thrombosis, and almost none had a bone marrow biopsy, which is regarded as an important source of information for diagnosis of CMD [11,12]. Nevertheless, the close relationship of the *JAK2* V617F mutation with CMD and the notion that the mutation can be absent in a significant portion of patients with CMD (ET) renders it possible that

our study may have underestimated (rather than overestimated) the incidence of CMD in patients with splanchnic thrombosis or CVT.

In conclusion, a substantial proportion of patients with splanchnic venous thrombosis (in particular PVT) and a small proportion of patients with CVT can be recognized as carriers of the *JAK2* V617F mutation in the absence of overt signs of CMD. This suggests that screening for the mutation might appear critical for accurate diagnoses. The clinical significance of such findings deserves further investigation.

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Disclosure of Conflict of Interests

The authors state that they have no conflicts of interest.

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