Clinical utility of screening for CALR gene exon 9 mutations in patients with splanchnic venous thrombosis

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Dear Sirs,

Current evidences indicate that screening for the V617F JAK2 mutation is relevant in patients with splanchnic venous thrombosis (SVT), a positive finding being a strong predictor of a subsequent diagnosis of a myeloproliferative neoplasm (MPN), but not of a specific subtype (1–5).

Very recently, in patients with Essential Thrombocythemia (ET) or Myelofibrosis (MF), but not Polycythemia Vera (PV), somatic mutations in the exon 9 of CALR, the gene encoding calreticulin, an endoplasmic reticulum protein, which controls calcium homeostasis, were discovered in 15 to 25% of patients with ET and MF (6, 7). At variance with this, the occurrence of somatic CALR gene mutations seems, at the best, rare in patients with PV (6–8). Noteworthy, JAK2 V617F and CALR exon 9 mutations were found to be mutually exclusive. Like investigation for the JAK2 V617F mutation, assessment of CALR mutations in peripheral blood leukocytes has been suggested as a powerful tool for the diagnosis of ET and MF.

Notwithstanding the mechanisms by which CALR gene exon 9 mutations affects abnormal proliferation of megakaryocytes are largely unknown at present, a lower thrombotic incidence has been reported for CALR-positive patients as compared with that observed in JAK2-positive ones (9, 10).

To further address this issue, we have investigated the prevalence of CALR gene exon 9 mutations and the clinical utility of screening for CALR mutations in the diagnosis of underlying MPN in patients with SVT. A cohort of 155 non-cirrhotic patients (79 men and 76 women) with a documented extrahepatic portal vein thrombotic occlusion was enrolled between January 1997 and June 2014, at the Gastroenterology Unit of the “A. Cardarelli” Hospital, Naples. All subjects with Budd-Chiari syndrome, liver cirrhosis, hepatocellular carcinoma, and abdominal or extra-abdominal malignancies were excluded from the study. After approval of the local Ethics Committees, the study was carried out according to the Principles of the Declaration of Helsinki, informed consent was obtained from all the subjects.

Twenty-three patients (11.6%) were not studied because of refusal of the consent (n=3), DNA samples were not available (n=15), or for technical problems (n=5). Thus, we analysed 132 (85.2%) patients (68 men and 64 women; median age: 53 years; range, 24 to 86 years).

From 2006 to 2008, the V617F JAK2 mutation was investigated as previously reported (5). Then, all mutations were genotyped by real-time quantitative PCR on an ABI Prism 7700 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) using TaqMan single-nucleotide polymorphism (SNP) genotyping assays (Applied Biosystems). From 2007, MPL exon 10 gene mutations were investigated by sequencing analysis. Mutations in exon 9 of CALR gene were assessed by direct bidirectional sequencing analysis.

Demographic characteristics and the prevalence of circumstantial and thrombophilic risk factors both in patients and in controls are shown in Table 1. A history of a MPN was found in 45 patients (29.0%); PV in 13, ET in nine, and MF 23. Overall, 39 MPN patients (86.7%), 11 with PV, 7 with ET, and 21 with MF, carried the JAK2 V617F mutation; all were heterozygotes. These results confirm data which emerged over the last decade, showing MPN as the leading systemic cause of non-malignant and non-cirrhotic SVT (11).

The presence of the JAK2 V617F mutation at the occurrence of the venous thrombotic event was recorded in 11 out of 13 PV patients (84.6%), four men and nine women, seven of nine ET patients (77.8%), five men and four women, and 21 of 23 MF patients, 10 men and 13 women (91.3%). These results further confirm, even in the absence of known MPN, the predicting role of the presence of JAK2 V617F mutation, a gain-of-function mutation strongly associated with the development of MPN (14). Indeed, the prevalence is very high, up to one third, in patients with objectively diagnosed SVT, as compared to that of about 1% in patients with VTE in other sites (12–14). In keeping with this, SVT may represent the first clinical manifestation of MPN, and of a MPN diagnosed during the follow-up period (1).

Mutations in exon 9 of CALR gene were not found in any patients. In addition, no patients displayed a MPL gene exon 10 mutation. At variance with this, in a setting of 195 patients (81 men and 114 women median age: 61 years; range, 17 to 88 years), with a clinical suspicion of ET consecutively investigated for somatic gene mutations, 85 patients (42.6%) resulted positive for JAK2 V617F and 33 (16.8%) for mutations within the exon 9 of the CALR gene. As previously reported, two mutations, del p.L367fs*46 and ins p.K385fs*47 were the most often represented, being found in 22 (66.7%) and 8 (24.2%) patients, respectively. In the remaining ET patients, three different rare mutations were found (del p.K375 fs*49, delins p.384fs*49 complex, and delins p.384fs*46 complex). CALR-mutated MPN appears to be substantially different from those carrying the JAK2 V617F mutation in terms of clinical outcomes. Indeed,

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Received: December 18, 2014
Accepted after minor revision: January 16, 2015
Epub ahead of print: March 12, 2015
http://dx.doi.org/10.1160/TH14-12-1055
Thromb Haemost 2015; 113: 1381–1382

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the prevalence of exon 9 of CALR gene mutations in SVT patients was significantly lower of that recorded in patients with a clinical suspicion of ET. These observations are in close agreement with the findings of recent studies, showing a lower rate of thrombosis in CALR-mutated patients both in sporadic and in familial MPN (6, 10).

Present data, in keeping with current evidence, indicate that screening for somatic gene mutations, i.e. JAK2 V617F, exon 10 MPL, and exon 9 CALR genes is of great importance for MPN diagnosis. In patients presenting with SVT, the molecular status is associated with a different clinical prognosis, search for JAK2 mutation having a key role in the management of patients with SVT. In contrast, CALR gene exon 9 mutations are much less frequent and, to provide that are mutually exclusive, should be investigated only in SVT patients lacking the JAK2 mutation. In agreement with other studies (15–18), in patients with SVT we suggest a sequential approach for the investigation of MPN first providing JAK2 V617F screening and then the investigation for CALR gene exon 9 mutations. Indeed, this approach represents an affordable, time saving, way to manage MPN diagnosis in SVT patients.

Author contributions
D.C. performed experiments, collected and analysed data; L.A. and M.A.G. recruited participants, collected and interpreted the data, and contributed to and revised the manuscript; G.L. T. and G.F. performed experiments and made the table; G.D. revised the manuscript, and contributed to the analysis of the results; R.S. performed experiments and revised the manuscript; E.G. designed the study, interpreted the data, and revised the draft and final manuscript; M.M. designed and performed the research, collected, analysed, and interpreted the data, and wrote the draft and final manuscript.

Conflicts of interest
None declared.

References

Table 1: Clinical presentation and laboratory characteristics of SVT patients.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Age, years median (range)</th>
<th>Men, n (%)</th>
<th>Family history of thrombosis (%)</th>
<th>Previous thrombosis (%)</th>
<th>Arterial (%)</th>
<th>Venous (%)</th>
<th>Arterial + Venous (%)</th>
<th>Clinical onset (%)</th>
<th>Vein(s) occluded (%)</th>
<th>Circumstantial risk factors (%)</th>
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<td>47.0 (15–80)</td>
<td>68 (51.5)</td>
<td>13 (9.9)</td>
<td>21 (15.9)</td>
<td>4 (19.0)</td>
<td>16 (76.2)*</td>
<td>1 (4.8)</td>
<td>24 (18.2)</td>
<td>40 (30.3)</td>
<td>Local 9 (6.8)</td>
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<td>MPN (%) 47 (35.6)</td>
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<td>JAK2 V617F mutation (%) 42 (31.8)</td>
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<td>Thrombophilic risk factors (%) 26 (19.7)</td>
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<td>Antiphospholipid antibodies 13 (9)</td>
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Seven patients carried a thrombophilic risk factor; ** A patient carried both FV Leiden (homozygote) and FIL A20210 allele.