Venous thrombosis in oral contraceptive users and the presence of the JAK2 V617F mutation

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Dear Sir,

Oral contraceptive (OC) use is known to be associated with increased risk of venous thromboembolism (VTE). OCs induce changes in coagulation and fibrinolysis, although in healthy women haemostatic variables remain within normal ranges (1, 2).

The absolute risk of VTE in healthy women using OCs is low (20–40 per 100,000 pill years) (4). The presence of acquired or inherited causes of thrombophilia increases this risk (5); with the exception of antithrombin deficiency, the reported odds ratios for thrombophilic women developing VTE during OCs vary substantially in individual studies (5), ranging between 6 to 15 (5).

Thromboses in unusual sites, as cerebral vein thrombosis (CVT) or portal and mesenteric venous thrombosis (PMVT) are less frequent than VTE in OCs users (6, 7). PMVT is a rare but severe cause of intestinal ischemia (8). OC may be an associated risk factor for the development of MTV (9) and its use accounts for 9% to 18% of the episodes of MTV in young women (8, 10, 11). PMVT is classified as either primary or secondary. Currently, an etiologic factor can be identified in about three quarters of patients.

Myeloproliferative disorders (MPD) are an intrinsic factor for the development of thrombosis in the portal, mesenteric, or hepatic areas (13). Recently, several groups identified a recurrent activating tyrosine kinase mutation, Val617Phe, in the JH2 pseudo-kinase domain of Janus Kinase-2 (JAK2) gene in patients with sporadic MPD. This mutation was found in most patients with MPD (14–16) and is an acquired somatic event in sporadic MPD leading to a constitutive activation of the JAK-STAT signal transduction pathway (17).

In a setting of patients with portal and mesenteric thromboses, we recently showed that determination of JAK2 V617F mutation may contribute to the search for genetic determinants of these thrombotic events (18).

Thus, we have investigated whether this mutation occurs in women with OCs VTE, CVT or PMVT.

From January 1998 to December 2006, 440 patients with venous thrombosis were referred for a work-up to two Thrombosis Centres of Southern Italy, Cardarelli Hospital (Naples) and IRCCS “Casa Sollievo della Sofferenza”, S. Giovanni R (FG) at least three months after the thrombotic episode. Among them, 55 women with VTE (median age: 31 years, range 16–44; median observation time: 3 years, range 1–10), seven with CVT (median age: 34 years, range 18–38; median observation time: 2 years, range 1–8), and nine with a PMVT (median age: 35, range 23–44; median observation time: 5 years, range 2–11) during OC were recorded. A complete clinical summary with emphasis on personal and family history for thromboembolic disease was obtained from all subjects by a specially trained staff. Venous thromboses were diagnosed by Doppler ultrasonography, spiral computed tomography, or magnetic resonance imaging as required during the routine diagnostic work-up.

MPD was diagnosed according to established criteria (19). One hundred thirty-two apparently healthy women (median age 44.0 years, range 19–46) randomly selected from a Southern Italian general population of employees of the “Casa Sollievo della Sofferenza” Hospital, S. Giovanni Rotondo, without a history of VTE served as controls. The two groups were comparable for social status and age.

After approval of the Institutional Review Board, the study was carried out according to the Principles of the Declaration of Helsinki; informed consent was obtained from all the subjects.

Blood samples were collected into vacuum plastic tubes containing 3.8% trisodium citrate and centrifuged at 2,000 g for 15 min to obtain platelet-poor plasma. After having filtered a portion for the assessment of lupus anticoagulant, samples were frozen and stored in small aliquots at −70°C until tested. DNA was extracted from peripheral blood leukocytes according to standard protocols (17). Acquired and inherited thrombophilias and homocysteine plasma levels were investigated in all patients, as reported elsewhere (18). Amplifications of regions of JAK2 gene containing the V617F was performed as previously described (18).

Among the 55 women with previous VTE, six (10.9%) carried the FV Leiden mutation (all heterozygotes), 13 (23.6%) the FII A20210 allele (all heterozygotes), two (3.6%) showed an antithrombin deficiency, three (5.5%) a moderate hyperhomocysteinemia, and three (5.5%) antiphospholipid antibodies.

In the group of patients with CVT (n = 7), one (1.8%) showed an antithrombin deficiency and two (3.6%) a moderate hyperhomocysteinemia.

Among the nine women with previous PMVT, one showed the FV Leiden mutation, one protein C deficiency and three (33.3%) a moderate hyperhomocysteinemia (one of them was the woman with protein C deficiency).
As far as the JAK2 V617F mutation is concerned, none of the 55 women with VTE or of the seven with CVT showed it, whereas two (22.2%) of the nine PMTV (Table), both without known thrombophilias, carried it. None of them was homozygous for the JAK2 V617F mutation in circulating blood cells. These patients, aged 34 and 38 years, used OCs since two and five years, respectively.

None in the control group (Fisher exact test p=0.004 vs. PMVT) was found to carry the mutant allele.

These two patients were followed for three and six years, respectively.

Interestingly, DNA sample of one of them (patient [Pt] 7), who suffered at the age of 38 years from a PMVT (December 2000), at that time did not show the JAK2 V617F mutation; on the contrary in 2006, when she developed a MPD, the mutation was identified in a new sample. At the time of diagnosis blood cell values were: Hb: 11.4 g/dl , Htc: 34.4% , WBC: 8.6 x10^9/l, Plt: 189 x10^9/l, while in 2006 were: Hb: 17.2 g/dl , Htc: 47.6%, WBC: 6.8 x10^9/l, Plt: 241 x10^9/l. Diagnosis was made according to the WHO criteria (19).

Pt6 showed the following cell values: Hb: 13.3 g/dl , Htc: 35.6%, WBC: 6.7 x10^9/l, Plt: 253 x10^9/l. As far as the spleen size is concerned, it was reported slightly enlarged at CT scan in Pt7 since December 2000, while it was reported normal in Pt6.

Since their introduction in 1960, OCs have been described to be associated also with PMVT; the first case was described by Reed and Coon in 1963 (20) and more recently, a review (9) reported 27 other cases in the English literature. Thus, this is a rare but recognized complication of OCs.

Although the extensive work-up carried out in all women developing OC vein thromboses, nevertheless it is not always possible to identify a “thrombophilic” risk factor, that could explain why only some women exposed to OCs develop a thrombosis. Also in this setting of patients, we were not able to identify in all patients another “trigger” in addition to OCs. Recent data from our (18) and other groups (21, 22) show that JAK2 mutation might be a new candidate risk factor for the occurrence of vein thrombosis.

In the present study, we do not find the JAK2 mutation in women with a previous OC-related VTE or CVT. On the other hand, in about 22% of women with OC-related PMVT, we describe this mutation; more interestingly, none of these patients carried another known cause of thrombophilia, suggesting that, in addition to OCs, JAK2 might represent a “trigger” for PMTV in young women without other well established causes of thrombophilia.

Another interesting issue is the relationship between PMVT in OC users and the following development of MPD. In one of our patients the mutation was observed at the diagnosis; in the other one, the first DNA sample obtained at the time of the MTV did not show the presence of the JAK2 mutation. In the same patient, six years later, we are able to demonstrate the presence of the mutation in the same time of the diagnosis of a MPD.

Our hypothesis is that an OC-related PMTV might be the first sign of a MPD. If other prospective evaluations will confirm our finding, this could allow us to carefully follow patients with previous OC-related PMVT and early diagnose and treat those who will develop a MPD.

<table>
<thead>
<tr>
<th>JAK2 G1849T mutation</th>
<th>Age at the event</th>
<th>OC type</th>
<th>Duration of OC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pt1 *</td>
<td>Absent</td>
<td>32</td>
<td>EE+ gestodene Third generation</td>
</tr>
<tr>
<td>Pt2</td>
<td>Absent</td>
<td>45</td>
<td>EE+ gestodene Third generation</td>
</tr>
<tr>
<td>Pt3</td>
<td>Absent</td>
<td>43 (died)</td>
<td>EE+ gestodene Third generation</td>
</tr>
<tr>
<td>Pt4 ^</td>
<td>Absent</td>
<td>27 (died)</td>
<td>EE+ gestodene Third generation</td>
</tr>
<tr>
<td>Pt5</td>
<td>Absent</td>
<td>23</td>
<td>EE+ cyproterone</td>
</tr>
<tr>
<td>Pt6</td>
<td>Present</td>
<td>34</td>
<td>EE+ gestodene Third generation</td>
</tr>
<tr>
<td>Pt8</td>
<td>Absent</td>
<td>26</td>
<td>EE+ drospirenone Fourth generation</td>
</tr>
<tr>
<td>Pt9</td>
<td>Absent</td>
<td>44</td>
<td>EE+ desogestrel Third generation</td>
</tr>
</tbody>
</table>

*FVL Leiden heterozygous. ^Protein C deficiency and mild hyperhomocisteinemia. EE: ethinyl estradiol.
References