Thrombophilia Is Significantly Associated With Severe Preeclampsia
Results of a Large-Scale, Case-Controlled Study

Giorgio Mello, Elena Parretti, Luca Marozio, Cristina Pizzi, Andrea Lojacono, Tiziana Frusca, Fabio Facchinetti, Chiara Benedetto

Abstract—The role of thrombophilia in the pathogenesis of preeclampsia is controversial. The aim of this case-controlled study was to determine whether thrombophilia increases the risk of preeclampsia or interferes with its clinical course. A total of 808 white patients who developed preeclampsia (cases) and 808 women with previous uneventful pregnancies (controls) matched for age and parity were evaluated for inherited and acquired thrombophilia (factor V Leiden; factor II G20210A; methylenetetrahydrofolate reductase C677T; protein S, protein C, and antithrombin III deficiency; antiphospholipid antibodies; lupus anticoagulant; and hyperhomocysteinemia). Odds ratios (ORs) with 95% confidence intervals (CIs) for risk of being carriers of thrombophilia in cases compared with controls and for risk of maternal life-threatening complications and adverse perinatal outcomes in preeclamptic patients with or without thrombophilia were calculated. Women with severe preeclampsia (406 cases) had a higher risk (OR, 4.9; 95% CI, 3.5 to 6.9) of being carriers of either an inherited or acquired thrombophilic factor, except for protein S, protein C, and antithrombin deficiency. In women with mild preeclampsia (402 cases), only prothrombin and homozygous methylenetetrahydrofolate reductase gene mutations were significantly more prevalent than in the controls. Thrombophilic patients with severe preeclampsia are at increased risk of acute renal failure (OR, 1.8; 95% CI, 1.5 to 2.2), disseminated intravascular coagulation (OR, 2.7; 95% CI, 1.1 to 6.4), abruptio placentae (OR, 2.6; 95% CI, 1.2 to 6.0) and perinatal mortality (OR, 1.7; 95% CI, 1.5 to 2.2) compared with nonthrombophilic preeclamptic patients. Our study demonstrates a significant association between maternal thrombophilia and severe preeclampsia in white women. Thrombophilia also augments the risk of life-threatening maternal complications and adverse perinatal outcomes in preeclamptic patients. (Hypertension. 2005;46:1270-1274.)

Key Word: preeclampsia ■ thrombophilia ■ maternal outcome ■ perinatal outcome

Preeclampsia (PE) greatly contributes to maternal and fetal morbidity and mortality. Its cause is unknown, but its association with impaired placentation and activation of coagulative cascade has been demonstrated.1,2 In the past few years, attention has been focused on the role that inherited or acquired thrombophilia may play in the pathogenesis of PE. New causes of thrombophilia are still being identified, and a panel of inherited and acquired thrombophilic factors has been assessed and thoroughly investigated in patients with PE.3,4 Unfortunately, the results of previous studies are controversial, and a systematic review of the association between maternal thrombophilia and PE has not led to definite conclusions.5 About 1300 cases have been reported in 16 studies. However, these included patients with very mild to the most severe form of PE and from different ethnic groups in which the prevalence of thrombophilic mutations is known to vary.6 Therefore, because of the size of their samples or the heterogeneity of their inclusion criteria, they cannot be used to evaluate the real impact of thrombophilia on the onset and course of PE.

The aim of our multicenter case-controlled study was to definitely assess in a large white population whether severe and mild PE or specific acute complications of PE are associated with thrombophilia.

Subjects and Methods

In this multicenter case-control study performed between March 2000 and July 2003, the cases were 808 white women who had singleton pregnancies complicated by PE and agreed to participate. They were consecutively selected from those referred for postpartum visit or preconceptional counseling to the high-risk pregnancy units of the university hospitals of Brescia, Florence, Modena, and Turin, which are tertiary care hospitals. The controls were 808 white women who delivered at the same hospitals after uneventful singleton pregnancies, identified at the postpartum
visit; for each PE patient included, a control matched for age and parity was enrolled. The study adhered to the principles of the Declaration of Helsinki and was approved by the local ethics committees and, written informed consent was obtained from each woman and her partner.

PE was diagnosed from the presence of blood pressure ≥140/90 mm Hg and proteinuria ≥0.3 g in a 24-hour urine specimen that occurred after 20 weeks of gestation, according to the definition of the American College of Obstetricians and Gynecologists. It was classed as severe if ≥1 of the following criteria were present: blood pressure ≥160/110 mm Hg; 24-hour proteinuria ≥5 g; oliguria <500 mL in 24 hours; cerebral or visual disturbances; pulmonary edema; epigastric or right upper-quadrant pain; impaired liver function; and thrombocytopenia.

Eclampsia was defined as the presence of new-onset seizures in a PE patient.

Abruptio placentae was diagnosed clinically. Only cases with grade 2 or 3 abortion (abruption associated with vaginal bleeding or concealed hemorrhage, uterine tenderness, and fetal distress) were included.

Fetal growth restriction (FGR) was defined as birth weight below the fifth centile for gestational age according to Italian birth weight distribution in the absence of congenital malformations or chromosomal aberrations, recent cytomegalovirus infection, or drug or alcohol abuse.

The HELLP (Hemolysis, Elevated Liver enzymes, and Low Platelet) syndrome was diagnosed in accordance with the following laboratory findings: hemolysis (lactic dehydrogenase >600 IU/L, serum bilirubin >1.2 mg/dL, or the presence of schistocytes in the peripheral blood); increased serum aspartate aminotransferase concentrations (>70 IU/L); and thrombocytopenia (platelet count <100,000/mm²).

Disseminated intravascular coagulation (DIC) was defined as platelet count <100,000/mm³, plasma fibrinogen <1 g/L, and fibrin degradation products >40 mg/dL.

Acute renal failure was diagnosed in the presence of oliguria or anuria in association with a creatinine clearance ≤20 mL/min or serum creatinine ≥2 mg/dL. Pulmonary edema and pleural effusion were diagnosed on the basis of clinical findings and chest radiography.

Chronic hypertension and concomitant diabetes or renal and cardiovascular diseases were regarded as exclusion criteria for cases and controls because they may affect the outcome of pregnancy or increase the incidence and the severity of PE and thus mask the pathogenetic role of thrombophilia. The absence of chronic hypertension was confirmed by normal blood pressure at enrollment. Subjects with previous thromboembolic diseases were also excluded because most of them were thrombophilic, and this might represent a selection bias. Ninety-eight patients with severe PE and 65 patients with mild PE were excluded, mainly because of chronic hypertension (95%). Moreover, only whites were included.

On enrollment, 4 to 12 months from the last pregnancy (index pregnancy), blood was drawn from all the women for DNA analysis for factor V Leiden, factor II G20210A, and methylenetetrahydrofolate reductase (MTHFR) C677T, and for the assessment of protein S, protein C, or antithrombin deficiencies, antiphospholipid antibodies, and combined thrombophilic defects were examined. The prevalences of the thrombophilic factors in severe and mild PE were compared with 17.2% in the matched controls. In contrast, in women with mild PE (Table 2), the prevalence of thromboembolic defects (16.7%) was similar to that in the matched controls (14.9%). Women with severe PE had a higher risk than controls of being carriers of either an inherited or an acquired thrombophilic factor, except for protein S, protein C, and antithrombin deficiencies, the prevalence of which is, in any case, very low. In the mild PE cases, heterozygous prothrombin gene and homozygous MTHFR gene mutations alone were significantly more prevalent than in the controls.

The risk of carrying combined thrombophilic defects was 17× higher in patients with severe PE (Table 1). Homozygosity for factor V Leiden was detected only in 3 women with severe PE.

Patients with a positive first-degree family history of PE displayed a 5.8-fold (95% CI, 3.3 to 10.3; P = 0.000001) risk of being thrombophilic. Similarly, a positive family history of thromboembolism (deep venous thrombosis or pulmonary embolism) was associated with an 8.6-fold risk of thrombophilia (95% CI, 4.0 to 18.6; P = 0.000001). Independent variables such as maternal age, maternal age >35 years, prepregnancy BMI, prepregnancy BMI >25, smoking, parity ≥3, family history of PE, family history of thromboembolism, factor V Leiden+/− or factor V Leiden++/−, F2 G20210A+/−, MTHFR++/−, hyperhomocysteinemia, protein S, protein C, or antithrombin deficiencies, antiphospholipid antibodies, and combined thrombophilic defects were exam-
TABLE 2. Prevalence of Inherited or Acquired Thrombophilia in Severe PE

<table>
<thead>
<tr>
<th>Thrombophilic Defect</th>
<th>Patients With Severe PE (n=406)</th>
<th>Controls (n=406)</th>
<th>Odds Ratio (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor V Leiden (^{-/-}) or (^{+/-})</td>
<td>68 (16.7%)</td>
<td>15 (3.7%)</td>
<td>5.2 (2.9–9.8)</td>
<td>0.000001</td>
</tr>
<tr>
<td>Factor II G20210A (^{-/-})</td>
<td>44 (10.8%)</td>
<td>8 (2.0%)</td>
<td>6.0 (2.7–14.1)</td>
<td>0.000001</td>
</tr>
<tr>
<td>MTHFR (^{-/-})</td>
<td>49 (12.1%)</td>
<td>3 (0.7%)</td>
<td>17.1 (5.9–55.9)</td>
<td>0.000001</td>
</tr>
<tr>
<td>Hyperhomocysteinemia</td>
<td>49 (12.1%)</td>
<td>21 (5.2%)</td>
<td>2.5 (1.4–4.4)</td>
<td>0.00073</td>
</tr>
<tr>
<td>PS, PC, or AT deficiencies</td>
<td>3 (0.7%)</td>
<td>0 (0.0%)</td>
<td>1.2 (0.6–2.5)</td>
<td>0.95</td>
</tr>
<tr>
<td>Antiphospholipid antibodies</td>
<td>52 (12.8%)</td>
<td>15 (3.7%)</td>
<td>3.8 (2.1–7.2)</td>
<td>0.000044</td>
</tr>
<tr>
<td>Combined defects</td>
<td>59 (14.5%)</td>
<td>4 (1.0%)</td>
<td>17.1 (5.9–55.9)</td>
<td>0.000001</td>
</tr>
<tr>
<td>Any defect</td>
<td>206 (50.7%)</td>
<td>70 (17.2%)</td>
<td>4.9 (3.5–6.9)</td>
<td>0.000001</td>
</tr>
</tbody>
</table>

PS indicates protein S; PC, protein C; AT, antithrombin. χ² test, 2-way, and Fisher’s exact test were used when needed.

TABLE 1. Prevalence of Inherited or Acquired Thrombophilia in Severe PE

<table>
<thead>
<tr>
<th>Thrombophilic Defect</th>
<th>Patients With Severe PE (n=406)</th>
<th>Controls (n=406)</th>
<th>Odds Ratio (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor V Leiden (^{-/-}) or (^{+/-})</td>
<td>13 (3.2%)</td>
<td>13 (3.2%)</td>
<td>1.0 (0.42–2.32)</td>
<td>0.84</td>
</tr>
<tr>
<td>Factor II G20210A (^{-/-})</td>
<td>16 (4.0%)</td>
<td>5 (1.2%)</td>
<td>3.3 (1.1–10.3)</td>
<td>0.027</td>
</tr>
<tr>
<td>MTHFR (^{-/-})</td>
<td>30 (7.5%)</td>
<td>12 (3.0%)</td>
<td>2.6 (2.3–5.5)</td>
<td>0.007</td>
</tr>
<tr>
<td>Hyperhomocysteinemia</td>
<td>11 (2.7%)</td>
<td>19 (4.7%)</td>
<td>0.6 (0.3–1.2)</td>
<td>0.19</td>
</tr>
<tr>
<td>PS, PC, or AT deficiencies</td>
<td>1 (0.2%)</td>
<td>1 (0.2%)</td>
<td>1.0 (0.2–4.0)</td>
<td>0.95</td>
</tr>
<tr>
<td>Antiphospholipid antibodies</td>
<td>13 (3.2%)</td>
<td>21 (5.2%)</td>
<td>0.6 (0.3–1.3)</td>
<td>0.21</td>
</tr>
<tr>
<td>Combined defects</td>
<td>16 (4.0%)</td>
<td>9 (2.2%)</td>
<td>1.8 (0.7–4.5)</td>
<td>0.22</td>
</tr>
<tr>
<td>Any defect</td>
<td>67 (16.7%)</td>
<td>60 (14.9%)</td>
<td>1.1 (0.8–1.7)</td>
<td>0.56</td>
</tr>
</tbody>
</table>

PS indicates protein S; PC, protein C; AT, antithrombin. χ² test, 2-way, and Fisher’s exact test were used when needed.

Discussion

Over the past few years, research has focused on whether factors known to predispose to thrombosis may cause or contribute to the development of PE, but the existence of an association between maternal thrombophilia and PE has been asserted and denied.15–32 Our findings in a large white population provide a clear demonstration of the strong association of severe PE with thrombophilia. Diagnosis of PE and assessment of its severity were in strict accordance with the American College of Obstetricians and Gynecologists criteria.7 All 4 units participating in the study used the same inclusion criteria and shared the same clinical approach to the management of severe early-onset PE. The subjects enrolled in the study had no previous thromboembolic disorders, chronic hypertension, or diabetes, and thus constituted a homogenous population for statistical analysis according to the severity of PE. In the 406 women with severe PE, the prevalence of each thrombophilia factor tested, except for inherited deficiency of protein S, protein C, or antithrombin III, was significantly higher than in controls. Overall, 50.7% of the severe PE cases had an inherited (37.9%) or acquired thrombophilia (12.8%) compared with 17.2% (13.5% inherited and 3.7% acquired) in the control group, whereas in the 402 mild PE cases, only the prothrombin and the MTHFR gene mutations were higher than in controls.

These data may explain why some studies that included forms of hypertensive disorders of indifferent severity in pregnancy have led to negative results.25,31 Pooling mild and severe PE, gestational hypertension, and chronic hypertension is likely to result in inaccurate determination of the exact impact of thrombophilic defects and perhaps a selection bias toward healthier subjects. Our study clearly indicates that only severe PE is associated with a very high prevalence of thrombophilia, in which the prevalence of inherited and acquired thrombophilia in mild PE is similar to that observed.
in the control group. As pointed out by Dekker and Sibai, this finding highlights the need to draw a distinction between true pathological conditions, such as severe early-onset PE with maternal and fetal life-threatening complications and mild hypertension and proteinuria at term, both of which meet the criteria for the diagnosis of PE but are probably different diseases in terms of etiology and pathophysiology.

We studied only white women (98% Italian), and our results are solely significant for similar populations. Ethnic differences in the prevalence of thrombophilic mutations may influence the association of thrombophilia with PE. Livingston et al's prospective case-controlled study of patients with severe PE did not reveal a higher prevalence of thrombophilia compared with the controls. However, ≈57% of their subjects were blacks, who are known to have a high incidence of PE and a very low prevalence of inherited thrombophilia. In addition, the limited size of studies of the association of thrombophilia with PE reduces their statistical significance and may result in its overestimation or underestimation. The size of our sample is one of the strengths of our study. Even so, it was insufficient to detect significant differences in the prevalence of inherited deficiency of protein C, protein S, and antithrombin III, all rather infrequent.

Our data are in agreement with those reported by Kupferminc et al, although they found an overall higher prevalence of inherited thrombophilia, except for prothrombin mutation, both in severely PE patients (64.7%) and in controls (18.0%). The differences may be attributable to the size and the ethnicity of the populations studied.

This is probably the first investigation of maternal and perinatal outcomes in preeclamptic subject carriers or non-carriers of thrombophilia. Its results show that inherited or acquired thrombophilia contribute to the severity of PE. Severe PE patients with thrombophilia are at higher risk of acute life-threatening complications than those without thrombophilia (Table 4), and their incidence of acute renal failure, DIC, and placental abruption is statistically significantly higher. Moreover, early-onset PE (before 28 weeks of gestation) is more frequent in thrombophilic women.

Overall, thrombophilia exacerbates perinatal outcomes in patients with severe PE, and their risks of delivery before 28 weeks of gestation, FGR, and perinatal mortality are significantly higher (Table 5). This may be because of the greater severity of PE and a direct effect of thrombophilia on the fetus and placenta. The role of fetal thrombophilia in these subjects must also be investigated.

In conclusion, our results demonstrate a statistically highly significant association between severe PE and maternal thrombophilia in white women. Thrombophilia also augments the risk of life-threatening maternal complications and adverse perinatal outcomes. These findings should be taken into account when planning preconceptional counseling and dur-

### Table 4. Incidence of Early-Onset and Acute Complications of Severe PE

<table>
<thead>
<tr>
<th>Acute Complications</th>
<th>Severe PE With Thrombophilia (n=206)</th>
<th>Severe PE Without Thrombophilia (n=200)</th>
<th>Odds Ratios (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE onset &lt;28th week</td>
<td>118 (57.3%)</td>
<td>38 (19%)</td>
<td>5.7 (3.6–9.2)</td>
<td>0.000001</td>
</tr>
<tr>
<td>HELLP syndrome</td>
<td>43 (20.9%)</td>
<td>49 (24.5%)</td>
<td>0.8 (0.5–1.3)</td>
<td>0.45</td>
</tr>
<tr>
<td>Abruptio placenta</td>
<td>25 (12.1%)</td>
<td>10 (5.0%)</td>
<td>2.6 (1.2–6.0)</td>
<td>0.017</td>
</tr>
<tr>
<td>DIC</td>
<td>23 (11.2%)</td>
<td>9 (4.5%)</td>
<td>2.7 (1.1–6.4)</td>
<td>0.021</td>
</tr>
<tr>
<td>Acute renal failure</td>
<td>19 (9.2%)</td>
<td>3 (1.5%)</td>
<td>1.8 (1.5–2.2)</td>
<td>0.00064</td>
</tr>
<tr>
<td>Pulmonary edema</td>
<td>8 (3.9%)</td>
<td>4 (2.0%)</td>
<td>1.3 (0.8–2.0)</td>
<td>0.38</td>
</tr>
<tr>
<td>Eclampsia</td>
<td>7 (3.4%)</td>
<td>6 (3.0%)</td>
<td>1.1 (0.3–3.9)</td>
<td>0.95</td>
</tr>
</tbody>
</table>

χ² test, 2-way, and Fisher's exact test were used when needed.

### Table 5. Incidence of Perinatal Complications in Severe PE

<table>
<thead>
<tr>
<th>Perinatal Outcomes</th>
<th>Severe PE With Thrombophilia (n=206)</th>
<th>Severe PE Without Thrombophilia (n=200)</th>
<th>Odds Ratio (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age at delivery</td>
<td>31.8±7.1</td>
<td>35.9±6.3</td>
<td>—</td>
<td>0.002</td>
</tr>
<tr>
<td>(weeks; mean±SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delivery &lt;28th week (n, %)</td>
<td>117 (56.8%)</td>
<td>35 (17.5%)</td>
<td>6.2 (3.8–10.1)</td>
<td>0.000001</td>
</tr>
<tr>
<td>FGR (n, %)</td>
<td>125 (60.6%)</td>
<td>61 (30.5%)</td>
<td>3.5 (2.3–5.4)</td>
<td>0.000001</td>
</tr>
<tr>
<td>Placental weight (mean±SD)</td>
<td>304±195</td>
<td>488±243</td>
<td>—</td>
<td>0.0002</td>
</tr>
<tr>
<td>Birth weight (mean±SD)</td>
<td>1107±547</td>
<td>1893±811</td>
<td>—</td>
<td>0.00044</td>
</tr>
<tr>
<td>Birth weight distribution (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25th centile</td>
<td>3.0</td>
<td>13.5</td>
<td>—</td>
<td>0.01</td>
</tr>
<tr>
<td>Median</td>
<td>11.5</td>
<td>31.0</td>
<td>—</td>
<td>0.001</td>
</tr>
<tr>
<td>75th centile</td>
<td>28.0</td>
<td>58.0</td>
<td>—</td>
<td>0.001</td>
</tr>
<tr>
<td>Perinatal mortality (n, %)</td>
<td>19 (9.2%)</td>
<td>3 (1.5%)</td>
<td>1.7 (1.5–2.2)</td>
<td>0.00064</td>
</tr>
</tbody>
</table>

χ² test, 2-way, Mann–Whitney U test, and Student t test were used when appropriate.
ing the clinical management of such patients. Randomized trials of antithrombotic intervention to prevent the recurrence of PE in thrombophilic women are needed.

Perspectives

The results of our study provide a clear demonstration of the strong association of thrombophilia with severe PE. Thrombophilia also augments the risk of life-threatening maternal complications and adverse perinatal outcomes in preeclamptic patients. Further prospective studies are needed to assess whether inherited or acquired thrombophilia increases the risk of development and recurrence of PE. At this regard, few observational studies suggest that the administration of prophylactic doses of low–molecular weight heparin from the beginning of pregnancy may reduce the recurrence rate of PE in thrombophilic women. These observations highlight the need for randomized trials of antithrombotic intervention to prevent the recurrence of PE in thrombophilic women and to assess the cost-effectiveness of such an intervention. If the use of antithrombotic therapy will be proven to be effective in reducing maternal and perinatal morbidity and mortality, acceptable, and cost effective, then a screening program reducing maternal and perinatal morbidity and mortality, use of antithrombotic therapy will be proven to be effective in thrombophilic women. These observations highlight the need for randomized trials of antithrombotic intervention to prevent the recurrence of PE in thrombophilic women and to assess the cost-effectiveness of such an intervention. If the use of antithrombotic therapy will be proven to be effective in reducing maternal and perinatal morbidity and mortality, acceptable, and cost effective, then a screening program should be planned to identify women with thrombophilia and a past history of severe PE.

References

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