Low-molecular-weight heparin versus low-dose aspirin in women with one fetal loss and a constitutional thrombophilic disorder

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The prospective evaluation of the effect of thromboprophylaxis in women with one unexplained pregnancy loss from the 10th week of amenorrhea was performed. A total of 160 patients with heterozygous factor V Leiden mutation, prothrombin G20210A mutation, or protein S deficiency were given 5 mg folic acid daily before conception, to be continued during pregnancy, and low-dose aspirin 100 mg daily or low-molecular-weight heparin enoxaparin 40 mg was taken from the 8th week. Twenty-three of the 80 patients treated with low-dose aspirin and 69 of the 80 patients treated with enoxaparin had a healthy live birth (odds ratio [OR], 15.5; 95% confidence interval [CI], 7-34, \( P < .0001 \)). Enoxaparin was superior to low-dose aspirin in each subgroup defined according to the underlying constitutional thrombophilic disorder. An associated protein Z deficiency and/or positive antiprotein Z antibodies were associated with poorer outcomes. The neonate weight was higher in the women successfully treated with enoxaparin, and neonates small for gestational age were more frequent in patients treated with low-dose aspirin. No significant side effects of the treatments could be evidenced in patients or newborns. As there is no argument to prove that low-dose aspirin may have been deleterious, these results support enoxaparin use during such at-risk pregnancies. (Blood. 2004;103:3695-3699)

Introduction

The use of serial ultrasonography studies during early pregnancy have shown that the arterial signals in the yolk circulation disappear and the umbilicalplacental circulation increases between 8 and 10 weeks of gestation, indicating that the placenta replaces the yolk sac as an essential source of blood supply to the embryo at that time.\(^1\) Thus, it can be deduced that during the switch and at least from the beginning of the 11th week of gestation the maintenance of the permeability of the maternal placental intervillous space becomes a crucial necessity for the viability of the fetus. This pathophysiologic perception has been reinforced by a demonstration, in the late 1990s, mainly by means of a series of case-control studies performed after the first one published by Sanson et al.,\(^2\) that thrombophilic disorders in the mother are associated with an increased risk of fetal loss, before or after (stillbirths) 22 weeks of gestation. This finding has led to a recent meta-analysis showing that factor V Leiden mutation, activated protein C resistance, prothrombin G20210A mutation (factor II G20210A mutation), and protein S deficiency are likely to be associated with a significant risk of fetal loss,\(^3\) giving legitimacy to secondary prevention trials using antithrombotic agents, mainly low-molecular-weight heparin (LMWH).

Concerning antithrombotic prophylaxis in women with thrombophilia and pregnancy complications, 2 distinct opinions are currently developed. The first one,\(^4\) based on the results of noncontrolled published studies in which outcomes were compared with the patients’ previous history of pregnancy loss,\(^5-8\) favors the use of LMWH during the next possible pregnancy. The second one,\(^9\) because of the absence of controlled studies, does not support the use of LMWH.

It is, however, very difficult to propose placebo to women with such a potentially harmful, at least in its psychological dimension, medical antecedent. Most patients, because of moral suffering but also because of abundant data currently available, (ie, on the Web), concerning the use of LMWH during at-risk pregnancies, do not accept it.

We thus performed, in women with a single antecedent of unexplained fetal loss, a prospective trial comparing 2 antithrombotic therapies: low-molecular-weight heparin enoxaparin and low-dose aspirin.

Patients and methods

Patient inclusion criteria

This therapeutic trial took place in our Mediterranean Abnormal Pregnancy Study Program, which has led to the previously published Nîmes Obstetricians and Haematologists (NOHA) studies on hemostasis-related risk factors for pregnancy losses.\(^10-15\) Patients were selected from those who had been referred to our laboratory by practitioners and obstetricians of the
Southern French Region Languedoc-Roussillon because of at least one antecedent of pregnancy loss from the 10th week of amenorrhea.

Medical history with specific attention to obstetric history (pregnancies; childbirth; treatments; infectious disease during pregnancy, including HIV, erythroblastosis fetalis Rh-negative disease, immune thrombocytopenic purpura [ITP], and fetomaternal alloimmune thrombocytopenia [FAT]; gravidic hypertension and its complications; trauma; obstetric complications; diabetes mellitus; morphologic malformation in the dead fetus) was taken into consideration by investigators who were unaware of the laboratory results. Any positive pathology mentioned here was an exclusion criterion.

We looked for presumptive etiologic factors: hysterosalpingogram, karyotype in both parents, glucose tolerance test, toxoplasmosis serology, thyroid function, serum prolactin levels, normal luteal phase of at least 12 days and plasma progesterone above 25 ng/mL, absence of antinuclear factor, or antiphospholipid/antiprotein antibodies (lupus anticoagulant, anticardiolipin, anti–β2-glycoprotein I, anti–annexin V, anti-phenyldialdentic-
anolamine, immunoglobulin G [IgG], and IgM, by the methods previously described in our laboratory12,13), absence of antithrombin or protein C deficiency,13 fasting plasma total homocysteine lower than 15 μM/L. All these data were obtained between 6 and 12 months after fetal loss. All women finally included in the study were negative for the various tests or assessments mentioned here.

Exclusion criteria were any presumptive etiologic factor, as described earlier; any antecedent of venous or arterial thrombosis; any pregnancy loss before the beginning of the 10th week of amenorrhea; any lethal fetal defect; fetal hemorrhage; pregnancy-induced hypertension with its complications; any infectious disease during pregnancy; known erythroblastosis fetalis, ITP, or FAT; trauma during pregnancy; diabetes mellitus; tobacco consumption at least equal to 10 cigarettes a days.

Finally, the ultimate inclusion criteria were one single unexplained pregnancy loss from the 10th week of amenorrhea with no unexplained pregnancy losses before the beginning of the 10th week of amenorrhea and no explained pregnancy losses associated with a factor V Leiden mutation, a factor II G20210A mutation (all heterozygous), or a protein S deficiency (performed as previously described11; functional activity in a procoagulant assay and free protein S antigen all lower than 55% of normal values). Protein Z plasma concentrations and antiprotein Z antibodies, IgG, and IgM were systematically assayed.14 Protein Z was considered to be deficient in the case of concentrations lower than 1 mg/L,13 antiprotein Z IgG was considered positive if higher or equal to 7.1 arbitrary units (AU) in 2 consecutive evaluations, and antiprotein Z IgM was considered positive if higher or equal to 5.3 AU.14 Thus, patients had one principal thrombophilic disorder among the 2 Leiden mutations and protein S deficiency and may also have protein Z deficiency or/and positive antiprotein Z antibodies. We included the 184 consecutive patients meeting our criteria.

Treatment regimens

The study was approved by our local hospital ethics committee. All patients were fully informed of the aim of the trial and of the proposed treatment regimens, and, before de

| All women | 1 (1-2) | 15 (10-29) | 96 | 50 | 14 | 26 (18-36) | 74 | 64 | 22 | 24.2 (21-32) | 108 | 44 | 8 |
| AIFVIL | 1 (1-2) | 15 (10-29) | 48 | 15 | 9 | 25 (18-35) | 31 | 31 | 10 | 24.8 (21.4-31) | 54 | 14 | 4 |
| AIFIL | 1 (1-2) | 15 (10-27) | 32 | 26 | 2 | 27 (20-34) | 28 | 22 | 10 | 23.7 (21.1-32) | 34 | 22 | 4 |
| AIPS | 1 (1-2) | 16 (11-23) | 16 | 9 | 3 | 26 (19-36) | 15 | 11 | 2 | 24.5 (21-29.5) | 20 | 8 | 0 |

BMI indicates body mass index; AIFVIL, all patients carrying the heterozygous factor V Leiden mutation; AIFIL, all patients carrying the heterozygous factor II G20210A mutation; AIPS, all patients carrying a protein S deficiency.
deficiency, or positive protein Z antibodies, was more frequently present in the case of treatment failures (respectively, \( P = .020 \) and \( P = .019 \)), as was the complex protein Z deficiency positive antiprotein Z antibodies (\( P = .004 \); 15 of the 20 cases led to pregnancy failure, 9 being treated with aspirin, 6 with enoxaparin). Table 4 gives the results of the multiparametric logistic regression model, adjusted by the type of treatment, type of principal thrombophilic disorder, protein Z status, and antiprotein Z status. The reference being a patient with a factor V Leiden mutation but no protein Z deficiency nor positive antiprotein Z antibodies treated with low-dose aspirin during pregnancy. Low molecular weight heparin use was associated with a dramatic increase in the chance of giving birth to a living child, protein Z deficiency or antiprotein Z antibodies were independently associated with a significant decrease of this chance, and factor II G20210A mutation and protein S deficiency indicated a nonsignificant trend for a lower chance of good pregnancy outcome.

Aspirin was associated with 57 pregnancy losses and enoxaparin with 11. In patients taking aspirin, losses occurred between the 11th and the 18th week of amenorrhea (median, 15; lower and upper quartiles, 13 and 16). In patients taking enoxaparin, losses occurred later on: from the 17th to the 24th week (during weeks 23 and 24 in 2 patients).

Seventy-six (83%) of the 92 successful pregnancies ended at term after 37 weeks of gestation. None severe preeclampsia developed in 7 cases, 4 women treated by enoxaparin and 3 with low-dose aspirin, with no pejorative secondary consequence for the women or their neonate. Of the 92 neonates, 65 were delivered vaginally and 29 (32%) by cesarean section.

The neonate weight was higher in the 69 women successfully treated with enoxaparin (median, 3043 g; interquartile range, 373 g; range, 2310-3787 g) than in the 23 women treated with low-dose aspirin (median, 2742 g; interquartile range, 522 g; range 2010-3268 g) (\( P = .0005 \)). Neonates’ weights were not, for each of the treatments, correlated to the intensity of tobacco consumption before pregnancy nor to the residual tobacco consumption during pregnancy. Neonates small for gestational age, defined as having a weight lower or equal to the 10th percentile corresponding to the gestational age at birth, were delivered by 7 of the 71 successful mothers treated with enoxaparin (10%) and in 7 of the 23 successful mothers treated by aspirin (30%; \( P = .04 \), Fisher exact test). None of these small-for-gestational-age neonates had, finally, any significant sequela.

We have not observed any case of heparin-induced thrombocytopenia, abnormal skin reactions, or clinical manifestation of spontaneous bone pain among the women treated with enoxaparin. No case was seen of digestive intolerance to low-dose aspirin either. There were no hemorrhages, except slight bruising at the injection sites for enoxaparin and for both treatments in case of local domestic trauma.

### Discussion

The present study included women with one pregnancy loss from the 10th week of amenorrhea and carrying a factor V Leiden mutation, or a factor II G20210A mutation, or a protein S deficiency. The participants also took 5 mg folic acid per day. The

### Table 3. Effect of the two treatments on pregnancy outcome

<table>
<thead>
<tr>
<th>N</th>
<th>Live births</th>
<th>( P^* )</th>
<th>OR</th>
<th>95% CI</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>All women</td>
<td></td>
<td>&lt; .0001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>80</td>
<td>23 (29%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enoxaparin</td>
<td>69 (86%)</td>
<td>15.5</td>
<td>7.34</td>
<td>&lt; .0001</td>
<td></td>
</tr>
<tr>
<td>AIIFVL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>36</td>
<td>12 (33%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enoxaparin</td>
<td>34 (94%)</td>
<td>34</td>
<td>7.166</td>
<td>&lt; .0001</td>
<td></td>
</tr>
<tr>
<td>AIIFIL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>30</td>
<td>10 (33%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enoxaparin</td>
<td>24 (80%)</td>
<td>8</td>
<td>2.526</td>
<td>.0005</td>
<td></td>
</tr>
<tr>
<td>AIIPS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>14</td>
<td>01 (7%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enoxaparin</td>
<td>11 (79%)</td>
<td>48</td>
<td>4.256</td>
<td>.0016</td>
<td></td>
</tr>
</tbody>
</table>

\*OR indicates crude odds ratio for giving birth to a live healthy baby after treatment with low-molecular-weight heparin enoxaparin, low-dose aspirin being the treatment of reference; CI, confidence interval; AIIFVL, all patients carrying the heterozygous factor V Leiden mutation; AIIFIL, all patients carrying the heterozygous factor II G20210A mutation; AIIPS, all patients carrying a protein S deficiency.

### Table 4. Multiparametric logistic regression model on a normal live birth after treated pregnancy

<table>
<thead>
<tr>
<th>Adjusted OR</th>
<th>95% CI</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enoxaparin use</td>
<td>32</td>
<td>11.89</td>
</tr>
<tr>
<td>Factor II G20210A mutation</td>
<td>0.44</td>
<td>0.17-1.15</td>
</tr>
<tr>
<td>Protein S deficiency</td>
<td>0.29</td>
<td>0.08-1.02</td>
</tr>
<tr>
<td>Positive protein Z deficiency</td>
<td>0.22</td>
<td>0.08-0.58</td>
</tr>
<tr>
<td>Positive antiprotein Z antibodies</td>
<td>0.20</td>
<td>0.08-0.51</td>
</tr>
</tbody>
</table>

study shows that treating the next pregnancy with the low molecular weight enoxaparin from the 8th week is associated with a greater number of live births and with more normal weight neonates than using a low-dose aspirin treatment. There were no consistent clinical complications.

This is the first study in which the outcome of antithrombotic-treated, constitutional thrombophilia-associated pregnancies in women with a clearly defined obstetric history is not compared with the patients’ previous history of pregnancy loss but in which 2 antithrombotic treatments are prospectively compared. One may argue that, in such cases, a placebo-controlled trial should have been done first.9 We agree to this theoretical argument which was tried out, but failed, because very few women having suffered fetal loss adhered to placebo trial. However, we are not in a classical situation in which we only try to prevent a special subtype of thrombosis recurrence. Here, we try to prevent death recurrence by treating women who in their special “future-mother context” always, in case of failure, lose a part of their own life. We thus thought that comparing 2 antithrombotic treatments was a humane ethical option.

This study was not a blind test study. Patients and physicians were aware of the treatment being taken. It would have been necessary for blind tests to have access to 2 placebo formulations, one for oral aspirin and one for subcutaneous low-molecular-weight heparin. Producing them, for such potentially long treatments, is of significant cost. This trial was performed without any financial support from pharmaceutical industries. It was difficult to imagine that the 2 laboratories, the one producing aspirin and the other producing the LMWH, would accept to collaborate in the same trial, potentially leading to only one of them supporting the trial. This would have opened the door to the masked criticism of credibility generally associated to studies sponsored by the industry. So, in absence of sufficient institutional funding, we chose not to perform a double-placebo–controlled trial, and we think that our results are likely to be independent from industrial influences.

Our patients had the 3 constitutional thrombophilic disorders that have been validated by the available meta-analysis of the published studies,3 and mainly the 2 that are the most frequently diagnosed, namely the factor V and factor II mutations. We did not stratify the obtained results by the level of fasting total homocysteine, because all patients were taking folic acid from at least 1 month before conception, to eliminate this potential cofactor of vasculopathyplacental complications.17 However, patients were stratified according to the presence or absence of protein Z deficiency and/or antiprotein Z antibodies that we had previously described to be associated with poor pregnancy outcome.13,14 Protein Z deficiency has been described to increase the severity of the prothrombotic phenotype of factor V Leiden in mice18 and in patients,19 and it was thus necessary to take into account these potential cofactors. Finally, our results show that protein Z deficiency and positive antiprotein Z antibodies are independent risk factors for a poor outcome of treated pregnancies, particularly in patients treated with aspirin.

The spontaneous prognosis of pregnancy in nonthrombotic women with factor V or factor II mutations or with protein S deficiency and a single unexplained fetal loss from the 10th week is basically still unknown. However, Rai et al20 recently reported the prospective outcome of untreated pregnancies in 25 women heterozygous for the factor V Leiden mutation. In 16 women with 3 or more miscarriages at less than 12 weeks gestation, the spontaneous live birth rate was 6 of 16, but in 9 women with fetal loss after 12 weeks gestation the rate was 1 of 9. Our patients receiving low-dose aspirin had a good outcome in roughly one third of the cases. Because 86% of our patients had experienced fetal loss after 12 weeks, it is thus not impossible that low-dose aspirin may have a positive significant clinical effect, by itself or in association with folic acid. A recent study showed that exposure to aspirin during pregnancy increases miscarriages.21 The risk was however limited to the prenatal use of aspirin and treatments. Our patients did not begin treatment before the sixth week after the extrapolated date of conception.

In pregnancies with a good outcome, low birth weight has been consistently shown to be associated with coronary heart disease which appears to be, from an epidemiologic point of view, a developmental disorder that originates through 2 widespread biological phenomena, developmental plasticity in utero and compensatory growth during infancy.22 Treating mothers having the lowest rate of neonates with a small weight for gestational age may thus be associated to the lowest incidence of cardiovascular diseases in future adults. If this relationship was also validated after therapeutic interventions, this would be another reason to prefer low-molecular-weight heparin to low-dose aspirin in our patients.

In conclusion, enoxaparin given from the eighth week of amenorrhea to prevent pregnancy loss in nonthrombotic women carrying the factor V Leiden mutation, or the factor II G20210A mutation, or protein S deficiency and having a single antecedent of unexplained fetal loss from the 10th week of amenorrhea seems to be a safe, much more effective treatment than low-dose aspirin.

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References


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