We describe the case of a 39-year-old woman who suffered two iliofemoral venous thromboses, a cerebral ischemic infarct and recurrent fetal loss. Initial studies showed high levels of antiphospholipid antibodies (APAs) and a moderate thrombocytopenia. After her second miscarriage, laboratory diagnosis revealed that the woman was heterozygous for the factor V Leiden mutation and had a functional protein S deficiency as well as anti-protein S and anti-β2-glycoprotein I antibodies. The impairment of the protein C pathway at various points could well explain the recurrent thromboses in the patient and supports the role of a disturbed protein C system in the pathophysiology of thrombosis in patients with APAs.
ing of dizziness, right motor deficiency and loss of consciousness. Over the following days she developed a complete flaccid right hemiplegia, hyporeflexia with right Babinski’s sign, right hemihypotonia and mixed aphasia, and was diagnosed by a CAT scan as having right Sylvian ischemic infarction. Since neither the ECG nor the EEG showed alterations, a potential embolic etiology was ruled out. Laboratory analysis at that point showed an APTT of 61.2 sec (normal range, 26-36 sec). The diluted Russell viper venom test gave a ratio of 1.75 (normal index <1.2), and the correction with a mixture of platelet phospholipids was 40% (normal range <10%), which indicated the presence of LA. ACAs were GPL 160 U (normal level <15 U), MPL 29 U (normal level <13 U). The patient had a moderate thrombocytopenia (103,000 platelets/µL). Underlying systemic lupus erythematosus disease was ruled out. These laboratory and clinical findings led to the diagnosis of antiphospholipid syndrome. Initially, the patient was anticoagulated with therapeutic doses of sodium heparin (1200 IU/hr) and then with long-term acenocoumarol to maintain the INR between 3.0 and 4.0. Her clinical progress was partially satisfactory. She regained movement in the right arm and leg and began to utter some phonemes.

The woman was again brought to our Department in August, 1996 for a new study of thrombophilia because she had another spontaneous abortion. The only noteworthy aspect of the medical history in previous reports.27,28

The study done in our laboratory in 1996, after the patient’s second miscarriage, confirmed that the thrombocytopenia and APAs persisted (Table 1). Fur-

Table 1. Laboratory data of the patient.

<table>
<thead>
<tr>
<th></th>
<th>Platelets (x 1,000/µL)</th>
<th>LAs</th>
<th>ACAs</th>
<th>anti-β2-GPI</th>
<th>APC-R</th>
<th>FV Leiden</th>
<th>Protein S (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>Free</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Functional</td>
<td></td>
</tr>
<tr>
<td>Patient</td>
<td>113</td>
<td>Positive</td>
<td>&gt;140</td>
<td>36</td>
<td>26</td>
<td>1.39</td>
<td>1.84</td>
</tr>
<tr>
<td>Control</td>
<td>150-300</td>
<td>Negative</td>
<td>&lt;15</td>
<td>&lt;10</td>
<td>&lt;20</td>
<td>2.7±0.2</td>
<td>2.9±0.4</td>
</tr>
</tbody>
</table>

LAs: Lupus anticoagulants; ACAs: anticardiolipin antibodies; β2-GPI: β2-glycoprotein-I; APC-R: activated protein C resistance; RVF: APC-R ratio evaluated by diluting the patient’s plasma 1/5 with factor V-depleted plasma; RFL: APC-R ratio evaluated by diluting the patient’s plasma 1/1 with a suspension of platelet phospholipids.

APA and recurrent thrombosis and abortion
thermore, abnormal levels of anti-β²GPI were detected (26 U; normal levels, <20 U). Additional studies showed the presence of APC-R (APTT + APC/APTT ratio below the cut-off point of 2.3), as evaluated with a modified test using a previous 1/5 dilution of the patient’s plasma with factor V-depleted plasma.²¹,²² When the APAs were neutralized with a suspension of platelet phospholipids the ratio remained below the cut-off point, suggesting the presence of a molecular abnormality. PCR analysis showed that the patient was heterozygous for the factor V Leiden mutation. Functional protein S was decreased. Antibodies to protein S were detected by immunoblotting. The blots showed a single band at about 70 kDa (Figure 1). The patient was screened for antiphosphatidylethanolamine and was negative. The circulating activated protein C/total protein C ratio (0.681) was within normal limits (0.61-1.3, n=35), suggesting normal protein C activation. All other hemostatic parameters studied were found to be normal. It is not uncommon to find extensive venous thrombosis, arterial thrombosis or recurrent abortions in patients with APAs. What makes the present case unusual is the co-existence of all these clinical manifestations in the same patient. This can be explained by the coincidence in this patient of several laboratory abnormalities: presence of LAs, ACAs, anti-β²GPI antibodies, anti-protein S antibodies and the factor V Leiden mutation.

The presence of anti-β²GPI autoantibodies in patients with ACAs and LAs has been associated with an increase in thrombotic risk.²⁶ Although Horbach et al.³⁰ have recently speculated that anti-β²GPI antibodies are only relevant in assays to detect APAs, but have no role in vivo thrombosis. Similarly, acquired protein S deficiency could also contribute to the development of thrombosis.²⁵ Co-occurrence of APAs, anti-protein S and anti-β²GPI antibodies has been reported previously.¹²,³⁷ Mörange et al. described a similar case of a 26-year-old woman with venous thrombosis and the combination of APAs, anti-β²GPI and anti-protein S autoantibodies, factor V Leiden mutation and systemic lupus erythematosus.³¹ However, their patient did not develop arterial thrombosis or suffer recurrent abortions.

APAs are seen as a heterogeneous population of antibodies that, depending of their specificity, can interfere with different anticoagulant reactions. In 7 patients with a history of thrombotic complications, Oosting et al. showed the presence of IgGs which inhibited APC activity.³² Phospholipids are known to participate in the activation and function of protein C. Specifically, phosphatidylethanolamine appears to play an important role both in protein C activation and in the anticoagulant function of activated protein C.³³ In vitro studies have provided some clues to explain the thrombotic tendency observable in patients with APAs. Plasma or immunoglobulin fractions were used to show that both thrombomodulin-dependent activation of protein C³⁶,³⁷ and activated protein C anticoagulant function³⁸,³⁹ were inhibited by APAs. Studies by Berard et al. showed a high frequency of patients with clinical symptoms suggesting the occurrence of an antiphospholipid syndrome in patients whose only APA was antiphosphatidylethanolamine. They also showed a strong association between antiphosphatidylethanolamine and thrombosis, suggesting that inhibition of activated protein C was the pathogenic mechanism. Galli et al. recently studied the differential effects of anti-β²GPI and anti-prothrombin antibodies on the anticoagulant activity of activated protein C. They found that anti-β²GPI, but not anti-prothrombin antibodies, hampered the inactivation of factor Va by endogenous activated protein C. They also found that 20 of the 24 APA patients with anti-β²GPI had reduced inactivation of factor Va whereas only six of the 18 patients with anti-prothrombin antibodies showed abnormal factor Va inactivation. Moreover, an abnormal rate of factor Va inactivation was found in 73%
of APA patients with venous thrombosis versus 56% of patients without venous thrombosis. The authors conclude that anti-β2-GPI antibodies are associated with thromboembolic events that occur in patients with APAs and suggest that acquired APC-R resulting from the impairment of the anticoagulant activity of the protein C system may represent one of the possible pathogenetic mechanisms responsible for the increased thrombotic risk in some APA patients. Our patient did not have antiphosphatidyl ethanolamine antibodies, which is consistent with the finding of a normal activated protein C/total protein C ratio since the presence of these antibodies would induce a decrease in protein C activation and hence a reduced APC/protein C ratio. However, the relatively low APC level and the presence of anti-β2-GPI could hamper the anticoagulant function of activated protein C.

The presence of antibodies to protein S in the patient’s plasma strongly suggest that the reduced functional protein S level is due to inhibition of protein S activity by specific anti-protein S antibodies. However, functional assays for protein S can give falsely low levels in patients with APC-R. Since we did not demonstrate directly that the patient’s anti-protein S IgG inhibits protein S activity, the presence of a true functional protein S deficiency in the patient remains uncertain.

The co-presence of factor V Leiden mutation and protein S antibodies in our patient may, therefore, have contributed to the severe clinical manifestations since the patient’s protein C system is impaired at different levels: reduced anticoagulant function of activated protein C as a consequence of decreased protein S function due to the presence of anti-protein S antibodies, and poor anticoagulant response of the activated protein C due to the presence of the factor V Leiden mutation and anti-β2-GPI.

Contributions and Acknowledgments

YM and AV were responsible for the clinical care of the patient and commented on the draft of the manuscript. MR and FE performed protein S and activated protein C studies. SG and AE performed genetic studies and commented on the draft. PV was responsible for all other laboratory analyses. FE wrote the manuscript and JA revised it and gave final approval. The authors thank Drs. E. Balada and J. Ordí for the evaluation of antiphosphatidyl ethanolamine antibodies.

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Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

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