Serum markers thrombophilia in pregnant women with Systemic Lupus Erythematosus

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Abstract

Objectives: to determine the frequency of serum markers for hereditary and acquired thrombophilia and their association with pregnancy in women with Systemic Lupus Erythematosus (SLE).

Methods: a case-control study was conducted among 25 pregnant women with SLE (study group) and 32 pregnant women without known disease and with at least one previous pregnancy (control group). The presence of antiphospholipid antibodies and hereditary thrombophilia were examined in both groups. We used the χ² Test with Yates correction or Fisher’s Exact Test to verify the associations and calculate the relative risk.

Results: thrombophilia was present in 72.0% of pregnant women with SLE and in 6.0% of patients in the control group. A significant association was found between the presence of SLE and serum markers for hereditary thrombophilia / antiphospholipid antibodies (p<0.05). The relative risks for antiphospholipid antibodies were 13.20 (ICR95%= 1.81 - 96.46) in pregnant women with SLE, 7.26 (CI95%= 1.77 - 29.86) for the presence of serum markers of hereditary thrombophilia and 7.92 (CI95%= 2.62 - 3.94) for the presence of hereditary thrombophilia and/or antiphospholipid antibodies.

Conclusions: the identification of markers for hereditary and/or acquired thrombophilia in pregnant women with lupus may be clinically useful to determine which patients have a higher risk of obstetric complications.

Key words Systemic lupus erythematosus, Thrombophilia, Antiphospholipid syndrome, Pregnancy complications, hematologic
Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease induced by multiple factors, characterized by the production of autoantibodies, which lead to the damage of multiple organs and changes in immunological patterns. It is a serious disease with significant mortality and morbidity, and approximately 90% of affected patients are women of childbearing age.

The disease has a variety of signs and symptoms that are often unspecific, thereby making its diagnosis difficult. The diagnosis of SLE is made when at least four of the 17 criteria defined by the American College of Rheumatology for SLE are present (Table 1).

As the manifestations of a normal pregnancy may sometimes be confused with lupus activity, it is important to understand the changes that occur during pregnancy. During normal pregnancy, changes occur in clotting factors, which result in protein S deficiency, increased fibrinogen levels, and changes in factor II and factor VII. These changes increase the risk of thromboembolism by five-fold. Thrombocytopenia is usually mild and does not cause bleeding problems.

Thrombophilias are mainly related to the presence of antiphospholipid antibodies that are present in one-third of patients with SLE (currently a diagnostic criteria according to the American College of Rheumatology), and their presence is associated with poor pregnancy outcomes. These antibodies

| Table 1 |
|Clinical and Immunologic Criteria for SLE.

<table>
<thead>
<tr>
<th>Clinical Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Acute cutaneous lupus</td>
</tr>
<tr>
<td>a) including lupus malar rash (do not count if malar discoid)</td>
</tr>
<tr>
<td>i) bullous lupus</td>
</tr>
<tr>
<td>ii) toxic epidermal necrolysis variant of SLE</td>
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<td>iii) maculopapular lupus rash</td>
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<td>iv) photosensitive lupus rash</td>
</tr>
<tr>
<td>(1) in the absence of dermatomyositis</td>
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<tr>
<td>b) or subacute cutaneous lupus</td>
</tr>
<tr>
<td>i) nonindurated psoriaform and/or annular polycyclic lesions that resolve without scarring, although occasionally with postinflammatory dyspigmentation or telangiectasias)</td>
</tr>
<tr>
<td>2) Chronic cutaneous lupus</td>
</tr>
<tr>
<td>a) including classical discoid rash</td>
</tr>
<tr>
<td>i) localized (above the neck)</td>
</tr>
<tr>
<td>ii) generalized (above and below the neck)</td>
</tr>
<tr>
<td>b) hypertrophic (verrucous) lupus</td>
</tr>
<tr>
<td>c) lupus panniculitis (profundus)</td>
</tr>
<tr>
<td>d) mucosal lupus</td>
</tr>
<tr>
<td>e) lupus erythematosus tumidus</td>
</tr>
<tr>
<td>f) chillblains lupus</td>
</tr>
<tr>
<td>g) discoid lupus/lichen planus overlap</td>
</tr>
<tr>
<td>3) Oral ulcers: palate</td>
</tr>
<tr>
<td>a) buccal</td>
</tr>
<tr>
<td>b) tongue</td>
</tr>
<tr>
<td>c) or nasal ulcers</td>
</tr>
<tr>
<td>d) in the absence of other causes, such as vasculitis, Behcets, infection (herpes), inflammatory bowel disease, reactive arthritis, and acidic foods</td>
</tr>
</tbody>
</table>

Criteria are cumulative and need not be present concurrently. Adapted from Petri et al.
## Table 1

### Clinical and Immunologic Criteria for SLE.

#### Clinical Criteria

4) Nonscarring alopecia (diffuse thinning or hair fragility with visible broken hairs)
   a) in the absence of other causes such as alopecia areata, drugs, iron deficiency and androgenic alopecia

5) Synovitis involving two or more joints, characterized by swelling or effusion OR tenderness in 2 or more joints and thirty minutes or more of morning stiffness.

6) Serositis
   a) typical pleurisy for more than 1 day
      i) or pleural effusions
      ii) or pleural rub
   b) typical pericardial pain (pain with recumbency improved by sitting forward) for more than 1 day
      i) or pericardial effusion
      ii) or pericardial rub
      iii) or pericarditis by EKG
         1) in the absence of other causes, such as infection, uremia, and Dressler's pericarditis

7) Renal
   a) Urine protein/creatinine (or 24 hr urine protein) representing 500 mg of protein/24 hr or
   b) Red blood cell casts

8) Neurologic
   a) seizures
   b) psychosis
   c) mononeuritis multiplex
      i) in the absence of other known causes such as primary vasculitis
   d) myelitis
   e) peripheral or cranial neuropathy
      i) in the absence of other known causes such as primary vasculitis, infection, and diabetes mellitus
   f) acute confusional state
      i) in the absence of other causes, including toxic-metabolic, uremia, drugs

9) Hemolytic anemia

10) Leukopenia (< 4,000/mm$^3$ at least once)
    i) in the absence of other known causes such as Felty’s, drugs, and portal hypertension
    b) Lymphopenia (< 1,000/mm$^3$ at least once)
    i) in the absence of other known causes such as corticosteroids, drugs and infection

11) Thrombocytopenia (<100,000/mm$^3$) at least once
    a) in the absence of other known causes such as drugs, portal hypertension, and TTP

#### Immunological Criteria

1) ANA above laboratory reference range
2) Anti-dsDNA above laboratory reference range, except ELISA: twice above laboratory reference range
3) Ant–Sm
4) Antiphospholipid antibody; any of the following
   a) lupus anticoagulant
   b) false-positive RPR
   c) medium or high titer anticardiolipin (IgA, IgG or IgM
   d) anti-$\beta_2$ glycoprotein I (IgA, IgG or IgM)
5) Low complement
   a) low C3
   b) low C4
   c) low CH50
6) Direct Coombs test in the absence of hemolytic anemia

Criteria are cumulative and need not be present concurrently. Adapted from Petri et al.$^3$

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Thrombophilia and Systemic Lupus Erythematosus
promote arterial and venous thrombosis, thrombocyto-
topenia and pregnancy loss. In despite of these
information, previous reports regarding hereditary
thrombophilia serum markers are addressed in
nonpregnant women with SLE.
Large numbers of complications can occur in
pregnant women with SLE. Possible adverse events
include reactivation of the disease, thrombosis and
miscarriage in women with antiphospholipid anti-
8 bodies, neonatal lupus, hypertension and toxicity of
drugs used to treat SLE. The high disease activity
during pregnancy results in an increased frequency
of preterm births and a decreased likelihood of live-
birth, with almost 25% of pregnancies resulting in
fetal death. It is recommended that the immuno-
suppressive drugs needed to control SLE continue to
be administered, particularly if the patient has major
organ involvement, such as lupus nephritis. Corticosteroids are relatively safe for use during
pregnancy, therefore it may contribute in increasing
maternal hypertension and gestational diabetes.
Prednisone is the choice drug option in cases of
active SLE disease upon the maternal organism, for
it does not cross the placental barrier. By the other
hand, dexamethasone should enter fetal circulation,
so that it becomes the best choice in treatment of
congenital heart block.

The objective of the present study was to deter-
mine the frequency of serum markers for hereditary
and acquired thrombophilia and antiphospholipid
antibodies and to examine their association with
pregnancy in women with SLE.

Methods

We carried out a prospective, observational, cross-
sectional controlled study in pregnant women from
the Service of Gynecology and Obstetrics at the
Hospital University Maria Aparecida Pedrossian
(HUMAP) from June 2007 to June 2012. These were
two distinct groups of patients, who were classified
according to the following criteria:

Control Group (CG) - consisted of pregnant
women without known disease who had at least one
previous pregnancy, whose previous pregnancies
were all low-risk and resulted in term deliveries and
who had no history of miscarriage. Patients with a
prior history of serum markers of hereditary throm-
bophilias and antiphospholipid antibodies were
excluded from this group.

Study Group (SG) – consisted of pregnant
women with a diagnosis of Systemic Lupus
Erythematosus (SLE) prior to pregnancy based on
clinical and laboratory criteria (Table 1) who also
might not have been diagnosed with hereditary
thrombophilia or antiphospholipid antibodies prior
to the current pregnancy.

Pregnant women whose SLE was in the active
phase, who were less than 18 years of age and those
whose diagnosis of SLE was made in the current
pregnancy were excluded from the study.

The selection of patients was conducted prospec-
tively and at random according to the order in which
cases meeting the inclusion criteria of the outpatient
high-risk pregnancy (SG) group and the low-risk
(CG) group presented during the study period.

The calculation of the simple random sampling
minimum number of pregnant women with a prior
history of SLE to be included in SG took the
following factors into account: the total population
of primary care patients at the Clinic of High-Risk
Pregnancy HUMAP (1,000 pregnant patients/year);
the prevalence of antiphospholipid antibodies in this
group of patients, whose values are well established
in the literature and are approximately 40%,
the prevalence of thrombophilia in the general popu-
lation, which is approximately 5%, and test power of
85% and a sampling error of 5% using a two-tailed
test. With these parameters, the minimum number of
pregnant women required in both groups was 24.

All patients were screened for the presence of
antiphospholipid antibodies by serum IgM and IgG
anticardiolipin, lupus anticoagulant and anti-β2
glycoprotein I (anti-β2,GPI). In despite of the litera-
ture recommendation of repeating the dosage within
12 weeks interval, it was not performed due to
specific characteristics of our routine service.

Pregnant women in whose blood sample the
antiphospholipid antibodies were identified, were
considered as antiphospholipid serum markers
carriers, hence they were not classified as
Antiphospholipid Antibody Syndrome (APS) disease
carriers. The presence of hereditary thrombophilia
was identified through the measurement of protein C
and S coagulation, antithrombin, homocysteine and
research Q506 mutation of factor V (factor V
Leiden). Among pregnant women in the study group,
the measurements were performed at the first
prenatal consultation of the current pregnancy, this
visit was prior to 20 weeks gestation. Among preg-
nant women in the control group who met the inclu-
sion criteria, samples were collected in the imme-
diate postpartum period (up to six hours postpartum)
to avoid sample loose. The screening for acquired
inherited thrombophilia is routinely performed at
the Clinic of High-Risk Pregnancy at our institution.
The serum levels of factor VIII, prothrombin
G20210A mutation, and enzyme methylenetetrahy-
The parametric variables (age and parity) are expressed as the mean ± standard deviation and were compared using the Student t test. The nonparametric variables (presence/absence of hereditary thrombophilia and antiphospholipid antibodies) were evaluated in double-entry contingency tables using the χ² test with Yates correction or Fisher’s Exact Test. Significant associations were those with p value <0.05. Relative risks were calculated and are presented with 95% confidence intervals (CI).

The patients included in the study signed an informed consent form (ICF). The study and its ICF were approved by the Ethics Committee on Human Research of the Federal University of Mato Grosso do Sul - UFMS, Protocol 884, as of May 18, 2007.

Results

Of the 57 pregnant women studied, 25 (43.9%) were in the study group and 32 (56.1%) were in the control group. The mean age in the SG was 29.4 years, and the average number of pregnancies was 2.1±1.4. In the CG, the mean age was 30.3 ±4.4 years, and the mean number of pregnancies was 1.2±0.4. No difference between the groups was found for maternal age or parity.

Positive screening for hereditary thrombophilia and/or antiphospholipid antibodies was found in 72.0% (18/25) of pregnant women in the SG. Among the CG patients, the presence of thrombophilia and/or antiphospholipid antibodies was found in 9.4% (3/32) of the sample (Table 2).

Screening for antiphospholipid antibodies in pregnant women with lupus demonstrated positive results in ten women (40%), out of these, six (24%) patients were identified with isolated antibodies and 4 (16%) patients had antibodies associated with other thrombophilia serum markers. In the pregnant women from the CG the presence of antiphospholipid antibodies was detected in only one patient (3.1%) (Table 2).

There was an association (p<0.05) between the presence of SLE in pregnant women and the occurrence of serum markers for hereditary thrombophilia.
Table 2

Association between the presence of SLE and the occurrence of serum markers of thrombophilias in pregnant women.

<table>
<thead>
<tr>
<th>Study group 1</th>
<th>Serum markers of antiphospholipid antibodies*</th>
<th>Serum markers of hereditary thrombophilia</th>
<th>Serum markers of hereditary thrombophilia and/or antiphospholipid antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td>Total</td>
</tr>
<tr>
<td>n</td>
<td>%</td>
<td>n  %</td>
<td>n  %</td>
</tr>
<tr>
<td>Study group 1</td>
<td>10</td>
<td>40.0</td>
<td>15 60.0</td>
</tr>
<tr>
<td>Control group 2</td>
<td>1</td>
<td>3.1</td>
<td>31 96.9</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>19.3</td>
<td>46 80.7</td>
</tr>
<tr>
<td>RR (IC95%)</td>
<td>13.20 (1.81 - 96.46)</td>
<td>7.26 (1.77 - 29.86)</td>
<td>7.92 (2.62 - 23.94)</td>
</tr>
<tr>
<td>P</td>
<td>0.001</td>
<td>0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Fisher's Exact Test; 1pregnant women with SLE; 2pregnant women with at least one successful pregnancy.
Table 3

<table>
<thead>
<tr>
<th>Hereditary thrombophilia and / or antiphospholipid antibodies</th>
<th>Study Group 1</th>
<th>Control Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolated antiphospholipid antibodies</td>
<td>24.0% (6/25)</td>
<td>3.1% (1/32)</td>
</tr>
<tr>
<td>Antibody IgM anticardiolipin</td>
<td>4.0% (1/25)</td>
<td>3.1% (1/32)</td>
</tr>
<tr>
<td>Lupus anticoagulant</td>
<td>20.0% (5/25)</td>
<td>- (0/32)</td>
</tr>
<tr>
<td>Isolated hereditary thrombophilia</td>
<td>24.0% (6/25)</td>
<td>6.2% (2/32)</td>
</tr>
<tr>
<td>Protein C Deficiency</td>
<td>4.0% (1/25)</td>
<td>- (0/32)</td>
</tr>
<tr>
<td>Protein S Deficiency</td>
<td>4.0% (1/25)</td>
<td>3.1% (1/32)</td>
</tr>
<tr>
<td>Antithrombin Deficiency</td>
<td>12.0% (3/25)</td>
<td>3.1% (1/32)</td>
</tr>
<tr>
<td>Hyperhomocysteinemia</td>
<td>4.0% (1/25)</td>
<td>- (0/32)</td>
</tr>
<tr>
<td>Hereditary thrombophilia and / or antiphospholipid antibodies identified together</td>
<td>24.0% (6/25)</td>
<td>- (0/32)</td>
</tr>
<tr>
<td>Anticardiolipin antibody IgM + antithrombin deficiency</td>
<td>4.0% (1/25)</td>
<td>- (0/32)</td>
</tr>
<tr>
<td>Anticardiolipin antibody IgG + antibody anti-β2-GPI</td>
<td>4.0% (1/25)</td>
<td>- (0/32)</td>
</tr>
<tr>
<td>Lupus anticoagulant + antithrombin deficiency</td>
<td>8.0% (2/25)</td>
<td>- (0/32)</td>
</tr>
<tr>
<td>Deficiency of protein S + deficiency of antithrombin</td>
<td>8.0% (2/25)</td>
<td>- (0/32)</td>
</tr>
</tbody>
</table>

1pregnant women with SLE; 2pregnant women with at least one successful pregnancy.

and/or antiphospholipid antibodies (Table 2).

The relative risk for the presence of antiphospholipid antibodies in pregnant women with SLE was 13.20 (CI95%= 1.81 - 96.46). The presence of serum markers for hereditary thrombophilia had a relative risk of 7.26 (CI95%= 1.77 - 29.86). The relative risk was 7.92 (CI95%= 2.62 - 23.94) when considering the occurrence of serum markers for hereditary thrombophilia and/or antiphospholipid antibodies.

Isolated hereditary thrombophilia was present in 24.0% of the pregnant women diagnosed with SLE (6/25) and 6.1% (2/32) of pregnant women in control group. Two or more hereditary thrombophilias and/or the presence of antiphospholipid antibodies together were found in 24% (6/25) of patients EG (Table 3). Among pregnant women in the SG, the most frequent isolated hereditary thrombophilias were as follows: antithrombin deficiency (12%), protein C and S deficiencies, and hyperhomocysteinemia (4%). In screening for the presence of antiphospholipid antibodies, the presence of lupic anticoagulant alone was demonstrated in 20% of the sample group (5/25) (Table 3).

Discussion

The prevalence of thrombophilic factors in pregnant women with SLE is not well established in the literature. Thus, the investigation of the prevalence of these factors in this group of patients helps to clarify its importance both in these clinical profiles and in preventing obstetric complications. The presence of antiphospholipid antibodies in patients with SLE has been described, but markers for hereditary thrombophilia are not well characterized, and varying methodologies make it difficult to compare data.

The prevalence of antiphospholipid antibodies in SLE patients is approximately 40%, and between 12% and 44% of patients are positive for anticardiolipin, 15% to 34% are positive for lupus anticoagulant and 10% to 19% are positive for the anti-β2-glycoprotein I (β2-GPI anti). These data agree with the results of the present study, where 40% (10/25) of patients had markers for antiphospholipid antibodies. Anticardiolipin antibody was found in 12% (3/25) of patients, lupus anticoagulant was found in 24% (6/25) and anti-β2 GPI was found in 4% (1/25), which is the only antibody whose value was below that described in the literature.

Thrombotic events occur more frequently in lupus patients with antiphospholipid antibodies than in patients without SLE or other autoimmune disease who have these antibodies. A study conducted in 2009 showed similar results and found a higher frequency of thrombosis and pregnancy loss in patients with SLE, which were associated with the presence of markers for thrombophilia in patients with acquired primary APS.

The presence of markers of antiphospholipid
antibodies was found to be 3.1 times higher in women experiencing pregnancy loss, especially after 20 weeks gestation. This was an independent risk factor for additional losses in a pregnancy cohort study of 166 pregnant women in the Hopkins Lupus Center.22

Maternal lupus activity and the presence of antiphospholipid antibodies were associated with concomitant major causes of obstetric complications,12,22 It is estimated that approximately 20% of pregnancies in women with SLE result in fetal loss.23 The rate of preterm birth (birth before 37 weeks gestation) is also increased in this group of patients. The incidence appears to vary between 23% and 28%,23 Preterm birth is often spontaneous, and it is mainly due to premature rupture of membranes, but there is also a significant portion of cases in which labor is induced to protect the health of mother and/or baby (early fetal distress or preeclampsia).19

In relation to serum markers of hereditary thrombophilia, protein C deficiency, S and antithrombin, we found an incidence of 40% (10/25) among pregnant women with lupus, however several authors,9,24,25 did not find the presence of these markers in non-pregnant patients with SLE, suggesting that they were not associated with an increased thrombotic risk. However, we observed changes in these protein systems.10

We cannot say that patients with protein C and S deficiency are carriers of inherited deficiency since pregnancy is a hypercoagulable state with increased coagulation factors and simultaneous decrease of natural anticoagulant and fibrinolytic proteins. These important changes occur naturally in order to minimize the risk blood loss, but increases the occurrence of thromboembolic events. However as there were no subsequent dosages of natural anticoagulants, it is suggested that these deficiencies may be transient.26-28

We did not observe the presence of FV Leiden mutation in any patient. However, Brouwer et al.24 found that FV Leiden in non-pregnant patients with SLE increases the risk of venous thrombosis and is associated with an OR of 3.5.

The presence of hyperhomocysteinemia was found in only 4% (1/25) of women in the study group, a result which is lower than the one presented in the literature in non-pregnant patients with lupus, which is approximately 37%, being this related to renal impairment.8 High concentrations of this amino acid are common in SLE patients and are predictive of late thrombosis, stroke and hypertension.21

Studies show that women with thrombophilia (protein S deficiency, and antithrombin C, hyperhomocysteinemia and factor V Leiden mutation) during pregnancy have a higher prevalence of obstetric and perinatal complications such as miscarriage, fetal loss and preeclampsia.6,7 Thus, the high incidence of these markers in women with lupus may be related to the increased number of cases of obstetric complications in this group of women.

Whereas women with SLE have 2 to 4 times higher rates of complications during pregnancy than non-pregnant women with SLE,29 the identification of markers for hereditary and/or acquired thrombophilia in pregnant women with lupus may be clinically useful to determine which patients have a higher risk of obstetric complications.

We emphasize that our study population portrays some characteristics which make it quite different from the populations studied by other authors. The Brazilian racial miscegenation could induce genetic characteristics not found in populations studied in other trials regarding hereditary/acquired thrombophilia.29,30

We either did not find any study that focused in the association of SLE pregnant women and inherited thrombophilia. We were only able to find data on the prevalence of acquired thrombophilia (antiphospholipid antibodies) and its impact on pregnancy in patients with SLE. This may suggest that more studies regarding the impact of inherited thrombophilia in pregnant women with SLE should be carried on.

References


