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The detection of anti-β2-glycoprotein I antibodies is associated with increased risk of pregnancy loss in women with threatened abortion in the first trimester

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Abstract

Objective: This study was designed to evaluate whether the detection of serum antiphospholipid autoantibodies may be useful in predicting pregnancy outcome in women with threatened abortion in the first trimester.
Study design: A group of 77 pregnant women of between 8 and 12 weeks' gestation with vaginal bleeding was tested for serum antiphospholipid, lupus anticoagulants, anticardiolipin, antinuclear antibodies, and anti-β2-glycoprotein I antibodies, and was followed up until the spontaneous end of pregnancy. A control group composed of 15 healthy women with uncomplicated gestation was tested contemporarily for the same antibody panel.
Results: Of the 77 patients with threatened abortion, 32 (41.5%) progressed to deliver at term and 45 (58.5%) experienced early pregnancy loss. Among the antibodies evaluated, only anti-β2-glycoprotein I was significantly more frequent in those women whose pregnancy resulted in spontaneous abortion (22/45, 49%) than in those who progressed to term (6/32, 19%) or in the control group (2/15, 13%; $p = 0.004$). This difference was specific to the IgM isotype ($p = 0.001$). After adjustment by multivariate analysis, the odds ratio for pregnancy loss associated with a positive β2-glycoprotein I antibody test was 5.18 ($p = 0.001$).
Conclusion: The detection of anti-β2-glycoprotein I antibodies is associated with an increased risk of pregnancy loss in women with threatened abortion in the first trimester.

Keywords: Lupus anticoagulants; Anticardiolipin antibodies; Antinuclear antibodies; Threatened abortion; Anti-β2-glycoprotein I

1. Introduction

Maternal-fetal immunity plays an important role during the entire course of pregnancy, but mainly in early gestation, when the immune cross-talk between the embryo and the maternal decidua is fundamental for successful implantation. The impairment of this local network may cause a defective placentation and/or early pregnancy loss [1].

Recurrent miscarriage before the 10th week of pregnancy frequently remains unexplained [2–4], and its origin is multifactorial. Among the risk factors, the presence of antiphospholipid (APL) antibodies has been frequently associated with a poor pregnancy outcome [5,6]. In fact, the abnormal generation of autoantibodies directed against phospholipids is characterized by peculiar clinical manifestations, such as venous and arterial thrombosis, recurrent miscarriage, and thrombocytopenia [7–9].

Antiphospholipid antibodies are a heterogeneous family of immunoglobulins directed against plasma proteins, including

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lupus anticoagulants (LAC), anticardiolipin (aCL), antiprothrombin (aPT), and the most recently recognized anti-β2-glycoprotein I (β2-GPI) antibodies [10–12]. Therefore, they represent a possible cause of pregnancy loss through the promotion of microvascular placental thrombosis, which is frequently associated with infarction, perivillous fibrin deposits, and chronic inflammatory lesions [13]. However, the role of APL antibodies and in particular of anti-β2-GPI is still controversial and undefined [14,15].

The aim of the present study was to assess the prevalence of APL and other autoimmune antibodies in pregnant women with threatened abortion, and to evaluate whether their presence is associated with a higher probability of evolution to miscarriage.

2. Materials and methods

2.1. Subjects

We evaluated prospectively a cohort of 77 nulliparous women (age range 24–39 years) who consulted for vaginal bleeding between 8 and 12 weeks' gestation. They were included in the study at the time of hospitalization and followed up till the end of pregnancy. All patients gave their written informed consent before entering the study, which was approved by the local Human Investigation Committee. Participants were enrolled at the Emergency Service of a tertiary clinical care center and were managed at the discretion of the attending physician. A control group was composed of 15 healthy women with no history of previous miscarriage, tested at 8–12 weeks' gestation and followed to term (Table 1).

Gestational age was evaluated on the basis of the last menstrual period and confirmed by ultrasound (Real Time Ultrasound Scan Equipment, Siemens Sonoline ELEGRA® Millennium Edition [Erlangen, Germany] with a transvaginal probe at 4.5–7.0 MHz) at hospitalization. Fetal heart activity was detected by ultrasound in 36 of the 77 patients.

The main outcome measure of the study was early spontaneous miscarriage, defined as pregnancy loss within 16 weeks' menstrual age, excluding anembryonic and ectopic pregnancy, and elective abortion. Patients with severe uterine anomalies, thyroid dysfunction, glucose intolerance, kidney, or liver disease, pre-existing hypertension, a history of thrombosis, or autoimmune diseases such as systemic lupus erythematosus were not included in the study. A history of one, two, and three previous miscarriages was reported by 34 (44.1%), 8 (10.4%), and 2 patients (2.6%), respectively. None of the patients received any medication during hospitalization, and bed rest was always recommended.

2.2. Collection of samples

Blood samples were drawn by venipuncture from the antecubital vein, with minimal venous stasis. For LA determination, blood samples were collected in vacuum tubes containing 3.2% trisodium citrate anticoagulant (nine parts blood to one part anticoagulant). Platelet-free plasma, if possible, was prepared by double centrifugation at $2500 \times g$ for 15 min at room temperature and assayed immediately [16]. Blood samples for the measurement of APL, aCL, β2-GPI antibodies, and antinuclear antibodies (ANA) were taken into plastic tubes and were prepared by single centrifugation at $2500 \times g$ for 15 min at room temperature. Serum was subsequently divided into aliquots and stored at –20 °C.

2.3. Antibody tests

All laboratory analyses were performed using kits purchased from Orgentec Diagnostika (Mainz, Germany). Plasma samples were tested for the presence of LAC activity, according to recommended criteria from the International Society on Thrombosis and Haemostasis Subcommittee on Lupus Anticoagulants-Phospholipid-dependent antibodies [17]. LAC levels were evaluated by using a "TEST™ LAC screen and TEST™ LAC confirm" kit, which employs the reagents of the dilute Russell's viper venom test (dRVVT). The sample: standard ratio classified the test result as normal (a ratio between 0.8 and 1.2), feeble-positive (1.2–1.5), moderate-positive (1.5–2), or heavily positive (greater than 2).

Samples were tested for the presence of aCL antibodies (IgG, IgM, and IgA isotypes) with a standardized ELISA [18,19], and results were expressed as standard units for IgG (GPLU), IgM (MPLU), and IgA (APLU). The lower detection limit for IgA and IgG was determined at 1.0 APLU/ml and 1.0 GPLU/ml, respectively, whilst IgM aCL determination had a sensitivity of 0.5 MPLU/ml. Positive results were defined as >10 GPLU/ml for IgG,

Table 1
Characteristics of the study groups

	Control ($n = 15$)	Threatened abortion	
		Delivery at term ($n = 32$)	Pregnancy loss ($n = 45$)
Maternal age (years)	33.5 ± 2.8	28.7 ± 1.8	28.3 ± 1.4
Gestation length (weeks)	39.4 ± 0.2	39.1 ± 0.2	10.3 ± 1.1*
>2 previous miscarriages	0/15	1/32 (3%)	1/45 (2%)

* $p < 0.001$ vs. control group (ANOVA and Tukey test).