

CORRELATION BETWEEN BETA 2-GLYCOPROTEIN ANTIBODIES AND ANTIPHOSPHOLIPID ANTIBODIES IN PATIENTS WITH REPRODUCTIVE FAILURE

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ABSTRACT

PROBLEM: Antiphospholipid antibodies (APA) are important in the etiology of reproductive failure. Studies have shown that binding proteins are necessary for the detection of APA. One of these, beta 2 glycoprotein, has been shown to be necessary for detection of anticardiolipin antibodies. It is felt that some APA may be directed to the binding protein itself, or to a combination of the binding protein and phospholipid.

METHOD OF STUDY: In this study, a comparison of APA versus anti beta 2 glycoprotein antibodies was performed on the sera of 123 women under 40 years old with a history of reproductive failure. Antibodies to six phospholipid epitopes were measured: cardiolipin, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidic acid, phosphatidylglycerol and phosphatidylserine.

RESULTS: Of the 123 women that were tested, 33/123 had one or more positive IgG antibodies to phospholipids, of which 9/33 were to cardiolipin. However, only 1/123 had IgG antibodies to beta 2 GP and she was APA negative. Thirty eight of one hundred twenty three (38/123) women had one or more IgM antibodies to phospholipids, with 0/123 directed to cardiolipin IgM. In contrast, only 8/123 had IgM antibodies to beta 2 GP. Five of the eight (5/8) patients had IgM APA; 4/5 had IgM antibodies to PE and one to PI.

CONCLUSION: There is no correlation between beta 2 GP and APA status in this population. To date, our most sensitive test for detecting phospholipid autoimmune mediated IVF failure still appears to be the ELISA for APA.

Key Words:

Antiphospholipid antibodies, Cardiolipin antibodies, Beta 2 glycoprotein antibodies, reproductive failure, failed IVFs.

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AJRI 1998: 40:000-000

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INTRODUCTION

Antibodies to negatively charged phospholipids in sera of women with immune mediated reproductive failure are believed important etiologic factors (1). Initial studies focused on the presence of antibodies to cardiolipin which had been shown to cause clotting abnormalities in some patients with Systemic Lupus Erythematosus (SLE), and in subset of women who suffer recurrent miscarriage (2,3). Miscarriage is believed due to thrombotic event; anticardiolipin antibodies (aCL) can cause platelet membrane and endothelial wall damage, interfere with protein C (natural anticoagulant) activation, and inhibit prostacyclins. Cardiolipin is found primarily within the inner mitochondrial membrane, unlike the other phospholipids which are found on the cell surface. Among patients with recurrent miscarriages or failed IVF, the majority of APA is directed to epitopes other than cardiolipin (approximately 90%) (4).

Antibodies to various phospholipid epitopes have been best characterized by solid phase enzyme linked immunosorbent assay (ELISA); phospholipid is coated onto a polystyrene plate, and the antibody detected by binding to phospholipid form-

ing a “sandwich” with a color marker which is quantified (5). Binding proteins are necessary for APA detection as evidenced by the perceived absence of APA when newborn or fetal calf serum are withheld from the assay. Beta 2 glycoprotein (beta 2 GP) is the specific protein involved in the binding of aCL to solid phase cardiolipin (6,7). Hypothetical mechanisms include; a) the aCL recognizes a cardiolipin beta 2 GP complex, b) the beta 2 GP is the target for aCL (not the phospholipid) and c) the actual epitope is part of the native structure of beta 2 GP (8,9). The dependence of antibodies to beta 2 GP may be disease specific. One study has shown that patients with SLE who manufacture aCL actually had antibodies against the beta 2 GP component (10). In another study a positive correlation between aCL and anti beta 2 GP in SLE was identified, but not between aCL and beta 2 GP in patients with end stage renal disease (11).

To further complicate diagnosis, false positive aCL to epitopes such as syphilis, dsDNA and others are not uncommon. Corroborating evidence for confusion include studies which show a correlation between lupus anticoagulant activity of aCL and the presence of beta 2 GP versus the absence of correlation between beta 2 GP and aCL levels in SLE (12). Another recent study claims a significant correlation between previous thrombosis and beta 2 GP, but not between fetal losses and a beta 2 GP (13). Patients with APA likely represent a heterogeneous group with antibodies directed to either the phospholipid and/or phospholipid binding proteins.

We examine the potential correlation of antibodies to beta 2 GP with that of several negatively charged phospholipids in patients who have been unsuccessful with in vitro fertilization.

METHODS

STUDY GROUP

One hundred twenty three (123) consecutive patients, all under 40 years of age, with a history of reproductive failure as demonstrated by one or more failed IVFs. None of the patients had male factor as an etiology for their infertility, nor an overt history of thromboembolic disorders. These women did not have a history of recurrent pregnancy loss.

APA ELISA

Patient sera were tested for antibodies to two isotypes (IgG, IgM) of six different phospholipids (cardiolipin, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidylglycerol, and phosphatidic acid) by solid phase ELISA as previously described in detail (4). Antiphospholipid antibody seropositivity (APA+) was defined by the detection of APA measuring O.D.s from the mean to IgM and/or IgG isotypes. Borderline values were defined as >2 SD above the mean, and positive values were

defined as >3 SD above the mean of normal controls. The control group for the APA assay consisted of 40 non-infertility patients who had no history of clinical or subclinical autoimmune disease, or recurrent pregnancy loss.

BETA 2 GLYCOPROTEIN ELISA

All patient sera were tested for beta 2 GP by a solid phase ELISA to IgG and IgM isotypes, as supplied by INOVA DIAGNOSTICS (San Diego, CA). Briefly, purified beta 2 GP was coated on plastic microwell plates and stabilized. All sera was diluted 1:100 with a supplied sample diluent. Next, 100 microliters of the test calibrators, controls and diluted patient samples were added in duplicate to test wells. The wells were covered and incubated for 30 minutes at room temperature. The plates were then washed by adding 300 mcl of buffer to each well. The buffer was flicked out of the wells and the wash repeated twice. A HRP IgG conjugate was added for 30 minutes followed by another wash step (300 mcl of wash buffer added to each well, flicked off and repeated twice). Following this step, 100 mcl of chromogen was added to each well, and the trays were incubated in the dark for 20 minutes. Finally, the stop solution was added and the absorbance read as the mean OD at 450 nm. The results were calculated by linear correlation of the mean absorbance of the calibrator curve against the log of their concentrations. Negative values were less than 20 SGU (Standard beta 2 GPI(glycoprotein I) IgG and IgM units).

RESULTS

Of the 123 women that were tested, 33/123 had one or more positive IgG antibodies to phospholipids, of which 9/33 were to cardiolipin. However, only 1/123 had IgG antibodies to beta 2 GP and she was APA negative. Thirty eight of one hundred twenty three (38/123) women had one or more IgM antibodies to phospholipids, with 0/123 directed to cardiolipin IgM. In contrast, only 8/123 had IgM antibodies to beta 2 GP. Five of the eight (5/8) patients had IgM APA; 4/5 had IgM antibodies to PE and one to PI. (fig. 1)

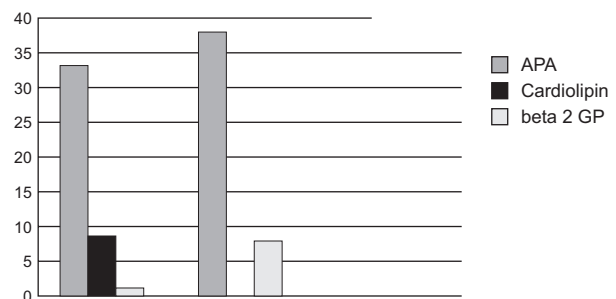


fig 1. Prevalance of APAs and Beta 2-GP Abs in patients with reproductive failure.

DISCUSSION

Our study revealed no correlation between APA and Beta 2 GP antibodies. The Beta 2 GP antibody prevalence was about 7% (9/123) with 4 associated with PE IgM and 1 with PI IgM, and 4 Beta 2 GP antibodies (1-IgG, 3 IgM) without any APA.

In previous studies the prevalence of APA in patients with recurrent miscarriages was greater than 50%; whereas, aCL comprised only 6% (4). This study shows that only 7% of 123 patients had antibodies to CL. This suggests that there are similarities in the two populations.

Unlike some of our predecessors, we were unable to identify a correlation between aCL and Beta 2 GP antibodies (10,11). Teixeira, et. al., observed something similar (12). It is possible that in the population we studied, there maybe a relationship with oxidized low density lipoproteins as other have reported (14,15).

We know that aCL have been associated with specific autoimmune diseases such as SLE and with overt thromboembolic phenomena, including peripheral thrombosis and recurrent pregnancy loss (1). The mechanism of action is thought to be an increase in the hypercoagulable state via inability to activate protein C, inhibition of prostacyclin, and endothelial wall and platelet membrane damage (17). Beta 2 GP has been associated with aCL and may be the actual epitope to which aCL are actually binding. Lupus anticoagulant activity associated with these antibodies may also be beta 2 GP dependent (16).

Although clotting aspects are an important etiology in recurrent miscarriages, this may not be the same for patients who suffer from multiple failed IVF (since the placental vasculature is immaturely established in the very early stages of pregnancy). The role of APA in reproductive failure appears to be more diverse. The adhesion properties of phospholipids play a major role in the physiology of reproduction; APA can interfere with the phospholipid adhesion which may result in reproductive failure (17). Sessions and Horowitz demonstrated the significance of phospholipids in the formation of syncytia in myoblast cultures, and Leyden and Rote showed that phospholipids (especially PS and PE) are important in the formation of syncytiotrophoblasts from cytotrophoblasts, and that antibodies to these phospholipids interfere with syncytia formation (17,18).

This study shows once again, that women unsuccessful with IVF have significant APA positivity, and that the incidence of aCL is low. There is no correlation between beta 2 GP antibody and APA status in this population. Therefore, beta 2 GP antibody is not clinically beneficial to detect phospholipid related autoimmune abnormalities in IVF failure

patients.

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