

# Antibodies to cardiolipin in patients with primary biliary cirrhosis

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**ABSTRACT:** Antibodies to cardiolipin have been recognized in up to 65% of patients with systemic lupus erythematosus. It has been claimed that they are significantly associated with intravascular thrombosis and with obstetrical complications. Thus far they have been found to be less prevalent in other diseases. Because of the high concentration of cardiolipin in mitochondrial membranes and the presence of antimitochondrial antibodies in patients with primary biliary cirrhosis, the authors investigated the prevalence of antibodies to cardiolipin in a group of 31 patients with primary biliary cirrhosis. It was found that the prevalence and levels of anticardiolipin antibodies of IgG and IgA isotype are as high in patients with primary biliary cirrhosis as in 35 consecutive patients with systemic lupus erythematosus. None of the patients with primary biliary cirrhosis gave any history of venous or arterial thrombosis. The rate of miscarriage was less than that reported for the general population. The availability for study of a second group of patients with high levels of anticardiolipin antibodies should make it possible to determine whether the association of these antibodies with thrombosis and fetal wastage in patients with systemic lupus erythematosus is a direct relationship or an epiphenomenon. *Can J Gastroenterol* 1989;3(3):98-102. *Pour resumé voir page 99*

**Key Words:** Anticardiolipin antibodies, Antimitochondrial antibodies, Primary biliary cirrhosis, Systemic lupus erythematosus

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ANTIBODIES TO PHOSPHOLIPID ANTIGENS have been recognized since 1942 when Pangborn (1) described the isolation from beef heart of cardiolipin (diphosphatidyl glycerol) and showed that it was the antigen active in the Wasserman reaction for syphilis. Anticardiolipin antibodies are frequently found in association with the circulating 'anticoagulant' in patients with systemic lupus erythematosus (SLE)(2) which leads to a prolongation of the partial thromboplastin time and which is an immunoglobulin interfering with phospholipid dependent coagulation tests (3).

A great deal of attention has recently been focused on anticardiolipin antibodies in SLE. It has been claimed that they are associated with a significantly increased risk of arterial and venous thrombosis (4,5). During pregnancy they appear to be predictors of fetal distress or death (6). Estimates of the frequency of lupus anticoagulant in patients with SLE have varied between 6 and 65%. Harris and colleagues (7) found anticardiolipin antibodies in 62% of patients with SLE,

## Anticorps dirigés contre la cardiolipine chez les patients atteints de cirrhose biliaire primitive

**RESUME:** Les anticorps contre la cardiolipine ont été identifiés chez 65% des patients souffrant de lupus érythémateux aigu disséminé. On a soutenu qu'ils étaient associés de façon significative à la thrombose intravasculaire et aux complications obstétricales. Jusqu'à présent, on semble les trouver moins fréquemment dans d'autres maladies. A cause de la forte concentration de cardiolipine dans les membranes mitochondriales, et de la présence d'anticorps anti-mitochondriaux chez les patients atteints de cirrhose biliaire primitive, nous avons étudié la prévalence d'anticorps anti-cardiolipine chez un groupe de 31 patients souffrant de cette affection. D'après les résultats, la prévalence et les niveaux d'anticorps anti-cardiolipine d'isotype IgG et IgA sont aussi élevés chez les patients atteints de cirrhose biliaire primitive que chez 35 patients consécutifs souffrant de lupus érythémateux aigu disséminé. Aucun des patients atteints de cirrhose biliaire primitive ne présentait une histoire de thrombose veineuse ou artérielle. Chez les patientes, le taux d'avortement était inférieur à celui de la population générale. Avoir accès, pour fin d'étude, à un second groupe présentant des concentrations élevées d'anticorps anti-cardiolipine permettrait de déterminer si l'association de ces anticorps aux thromboses, et à la résorption du fœtus chez les patientes atteintes de lupus érythémateux aigu disséminé, constitue une relation directe ou un épiphénomène.

while Sturfelt et al (8) reported their presence in 54% of 59 unselected SLE patients. However, in a recent study (9) of 60 consecutive patients lupus anticoagulant was found in 6.7% and anticardiolipin antibodies in 25%.

Recent studies have shown that lupus anticoagulant and anticardiolipin antibodies may be found in diseases other than SLE. Lupus anticoagulant has been found in 4% of young adults with cerebrovascular disease (10) and in 27% of patients with syphilis (11). It has also been reported in 37% of psychiatric patients treated with phenothiazines (12), in whom it is presumably drug induced. Hull and co-workers (13) recently reported a study of anticardiolipin antibodies in 70 patients with Behcet's syndrome and detected the antibodies in 13, of whom eight had a history of vascular pathology. Anticardiolipin antibodies have also been reported in single cases of Degos' disease (14) and Guillain-Barré syndrome (15). Recently, Canoso et al (16) reported anticardiolipin antibodies in all of 43 patients with acquired immune deficiency syndrome (AIDS) or AIDS-related complex as well as five of 10 men positive for antibodies to human immunodeficiency virus (HIV). None of these patients had a history of venous or arterial thrombosis.

Because of the high levels of cardiolipin in mitochondrial membranes and

the high frequency of antimitochondrial antibodies in patients with primary biliary cirrhosis, the authors decided to study the prevalence and concentration of anticardiolipin antibodies in patients with this condition.

The purpose of this paper is to report the detection of high levels of antibodies to cardiolipin in patients with primary biliary cirrhosis. The authors found that the prevalence of these antibodies, their isotype and their level in serum was comparable to that found in a group of 35 unselected patients with SLE. An increased incidence of venous or arterial thrombosis or of fetal wastage among the primary biliary cirrhosis patients was not found.

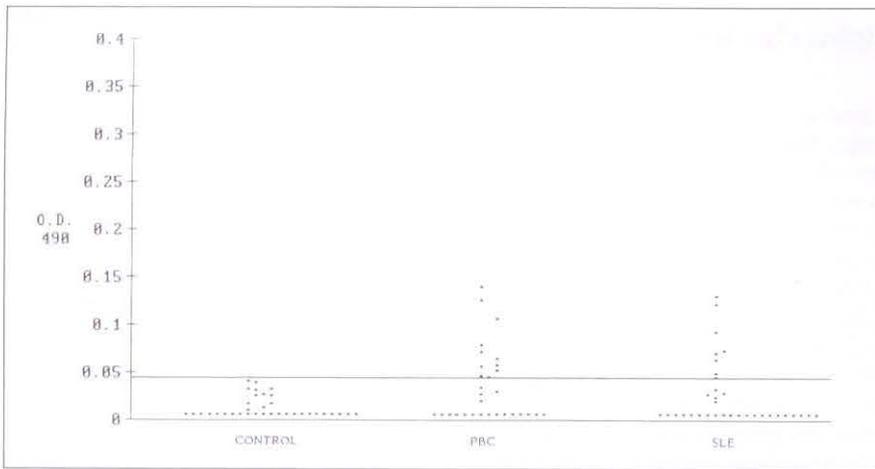
### PATIENTS AND METHODS

Thirty-one sera from the files of the immunopathology laboratory at the Victoria General Hospital, Halifax, Nova Scotia, were studied. All were positive for antimitochondrial antibody at a dilution of 1:100 or greater and all patients had a diagnosis of definite or probable primary biliary cirrhosis. Twenty patients were symptomatic and 11 were asymptomatic. In 21 patients the diagnosis was confirmed by liver biopsy. In the remaining 10 liver biopsy was either refused (two patients), nondiagnostic (one patient) or not performed because of elevated prothrombin and partial thromboplastin

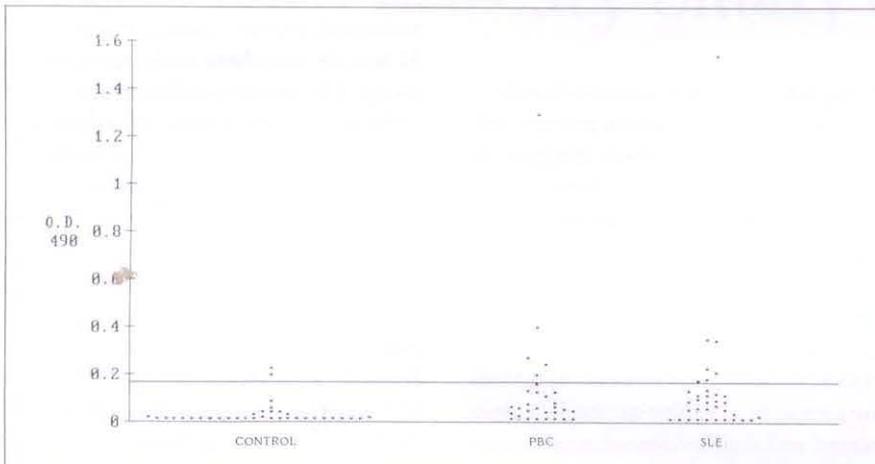
times due to end stage liver failure (seven patients). All 10 patients were diagnosed as having primary biliary cirrhosis based on their clinical course and persistent elevation of alkaline phosphatase and gammaglutamyl transferase. For comparison, the sera of 35 consecutive patients (mean age  $\pm$  SD 41.9  $\pm$  11.95 years) attending the lupus clinic and 35 control specimens from normal staff (mean age 35.4  $\pm$  12 years) of the hematology laboratory at the Victoria General Hospital were examined (all ages are given at the time serum was taken). All lupus patients fulfilled three or more of the American Rheumatism Association revised criteria for the diagnosis of SLE (17).

The mean age of the patients with primary biliary cirrhosis was 59.6  $\pm$  11.3 years. There were 26 females and five males with primary biliary cirrhosis, and 32 females and three males in the lupus group. The patients with primary biliary cirrhosis were interviewed by telephone. The female patients were questioned as to the number of pregnancies, miscarriages and stillbirths. All patients were asked a series of questions designed to elicit a history of vascular thrombosis or embolism. Specifically, each patient was asked whether he or she had ever suffered an episode of venous or arterial occlusion requiring medical or surgical treatment. All of the patients' charts were also reviewed to confirm the results of the telephone interview. None of the patients was receiving phenothiazines at the time that blood was taken. None was suffering from syphilis or HIV infection.

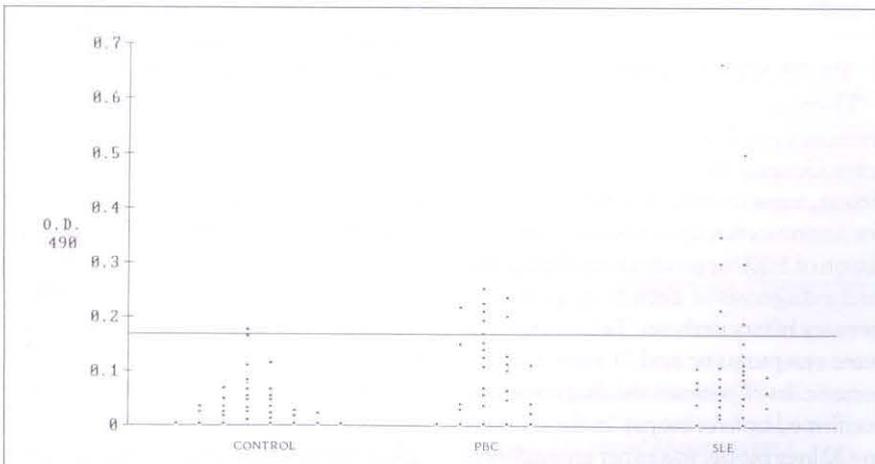
**Antibodies to cardiolipin:** All sera were frozen immediately after being taken and stored in aliquots at  $-70^{\circ}\text{C}$ . Each sample tested was thawed once only before measurement of antibody. Anticardiolipin antibodies were measured by an amplified ELISA assay as described by Carr et al (18). In brief, Linbro 96 well microtitre plates were coated with 45  $\mu\text{g}/\text{mL}$  cardiolipin (Sigma C 1649) in 95% ethanol. The solution was evaporated under a stream of nitrogen and the plates postcoated with 3 mg/mL gelatin in phosphate buffered saline (PBS) for 2 h at room temperature. After washing the plates with PBS, test sera diluted 1/100 in gelatin/PBS were added to the wells and incubated for 90 mins at room



**Figure 1** IgG anticardiolipin as measured by optical density (490 nm) in controls and patients with primary biliary cirrhosis or SLE. The horizontal line represents the mean plus three standard deviations for the normal controls



**Figure 2** IgM anticardiolipin as measured by optical density (490 nm) in controls and patients with primary biliary cirrhosis or SLE. The horizontal line represents the mean plus three standard deviations for the normal controls



**Figure 3** IgA anticardiolipin as measured by optical density (490 nm) in controls and patients with primary biliary cirrhosis or SLE. The horizontal line represents the mean plus three standard deviations for the normal controls

temperature. Plates were washed, and alkaline phosphatase-conjugated heavy chain specific antisera to human IgG, IgM or IgA (Sigma, St Louis, Missouri) were added. The plates were then incubated for 1 h at room temperature. After washing, the primary and secondary substrate of the amplified system were added. Absorbance was read after 10 mins at 490 nm on an automated EL 310 ELISA reader (Biotek, Cambridge, Massachusetts). Sera were regarded as positive if the test gave an optical density value of more than three standard deviations above the mean for normals. Uncoated wells were used as controls to detect non-specific binding, this was then subtracted from the optical density value.

**Antimitochondrial antibodies:** Antimitochondrial antibodies were assayed by an indirect immunofluorescence method using cryostat sections of mouse kidney for screening and subsequently using human kidney for titration. Bound antibodies were identified using FITC labelled antihuman immunoglobulin.

**Statistical analysis:** Fisher's exact test (two-tailed) was applied to compare the number of positives in each group.

### RESULTS

Figures 1, 2 and 3 show the levels of anticardiolipin antibodies of each isotype detected in the three sets of sera.

For all isotypes of antibody, the levels of anticardiolipin were as high in the primary biliary cirrhosis group as they are in the SLE group. Table 1 shows the analysis of antibody isotype in each of the groups studied. For anticardiolipin antibody of IgG and IgA isotype in the primary biliary cirrhosis group, the number of positive patients was significantly higher than normals but not statistically different from the SLE group.

When the 31 patients with primary biliary cirrhosis were divided into the 21 in whom the diagnosis was confirmed by liver biopsy and the 10 in whom the diagnosis was established clinically, it was found that six of the former group and four of the latter group were positive for IgG antibodies to cardiolipin.

There was no correlation between antibodies to double stranded and single stranded DNA and the presence of anticardiolipin antibody in the primary bil-

TABLE 1

Isotypes of anticardiolipin antibody: Number of patients with optical density greater than three standard deviations above the normal mean

Immunoglobulin isotypes	Normal	SLE	PBC
IgG	0	7 *P=0.014	10 P=0.001
IgM	2	5 P=0.26	6 P=0.26
IgA	1	7 P=0.034	8 P=0.056
Total	35	35	31

PBC Primary biliary cirrhosis; \*P values against normal controls

TABLE 2

Outcome of pregnancy in primary biliary cirrhosis patients

	Number of female patients	Total number of pregnancies	Number of patients with spontaneous abortions	Number of spontaneous abortions
Anticardiolipin antibody positive	7	19	1	1
Anticardiolipin antibody negative	13	41	4	5
Total	20	60	5	6

ciary cirrhosis group (data not shown). Of the patients with SLE, four were positive for antimitochondrial antibodies. Two of these were also positive for anticardiolipin, while two were negative.

Munoz et al (19) have suggested that in patients with primary biliary cirrhosis, IgM may bind nonspecifically in the ELISA assay for anticardiolipin antibodies. In the group of patients with primary biliary cirrhosis three of 16 showed high nonspecific binding for IgM and have been categorized as antibody negative. There was no nonspecific binding with the SLE group.

Of the 31 primary biliary cirrhosis patients, 23 were reached by telephone. None of these patients gave a history of venous or arterial thrombosis. Of the eight patients (two male, six female) not contacted, three had died of end stage liver failure and five were alive but lost to follow-up. Of a total of six females dead or lost to follow-up, three were positive for anticardiolipin antibodies while three were negative.

The charts of all patients lost to follow-up were reviewed. Of the six females lost to follow-up, obstetrical histories were missing from all but one chart. This patient had eight normal deliveries and no abortions. One 73-year-old male was found to have had a bypass graft of the left femoral artery for occlusion after a

history of intermittent claudication. This patient was negative for anticardiolipin antibodies.

Twenty-one of the 23 patients interviewed by telephone were female. Among the seven patients with primary biliary cirrhosis who were anticardiolipin antibody positive there were 19 pregnancies with only one abortion. In the 13 anticardiolipin antibody negative patients, three had one spontaneous abortion while one patient had two. There was no case of fetal loss after 20 weeks. Thus, in the primary biliary cirrhosis group, anticardiolipin antibodies were not associated with an increased risk of fetal loss.

## DISCUSSION

There has been a major upsurge of interest in the antiphospholipid antibodies since the demonstration that they may be predictors of intravascular thrombosis (5) and obstetrical complications (6). The mechanism by which these adverse effects are produced is still obscure. Suggested mechanisms include blocking the release of arachidonic acid from the membrane of the endothelial cells by binding to phospholipids (20) and decrease in the release of plasminogen activator following venous occlusion (21).

The mechanism of the obstetrical complications is also uncertain, although it

has been reported that the lupus anticoagulant may affect the placental circulation by inhibiting the production of prostacyclin by the myometrium (22). Studies of the pathology of placental tissue associated with fetal loss in patients with SLE have shown that the placentae are small, often with multiple infarcts secondary to thrombosis (23).

In relation to both the thrombotic and the obstetric complications associated with antiphospholipid antibodies it is still uncertain "whether antiphospholipids play a pathogenic role...or are merely 'markers' for...disease" (4). SLE is characterized by the production of large numbers of autoantibodies and by excessive B-cell proliferation (24). While antiphospholipid antibodies appear to be risk factors for vascular and obstetric complications, some of the many other immunologic abnormalities found in SLE could be equally important.

The authors have shown that antibodies to cardiolipin occur as frequently in a group of patients with primary biliary cirrhosis as in SLE and that the level of antibody is similar in both groups. The authors have found no increase in the frequency of venous or arterial thrombosis. The authors found no increase in fetal wastage in the anticardiolipin positive patients, compared to those who were anticardiolipin negative, although it was recognized that the higher mean age of the primary biliary cirrhosis group makes this observation of questionable significance. It has been shown that the prevalence of anticardiolipin antibodies of IgG isotype increases in an ageing population (mean age 81) (25). However, the present patients with primary biliary cirrhosis, although somewhat older than the SLE group (mean age 59.6 years), showed anticardiolipin antibodies in all three immunoglobulin isotypes.

While it seems probable that the presence of anticardiolipin antibodies is a predictor for thrombosis and fetal wastage in some patients with SLE, and perhaps in some individuals with no other identifiable disease, the present study indicates that patients with primary biliary cirrhosis may have an equally high incidence and concentration of anticardiolipin antibodies without sustaining any of these complications.

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