Isotype profile and clinical relevance of anticardiolipin antibodies in Sjögren's syndrome

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Abstract

The purpose of this study was to determine the occurrence and clinical value of anticardiolipin antibodies in patients with Sjögren's syndrome. Thirty one patients with primary Sjögren's syndrome (all women, mean (SD) age 48.3 (11.2) years) and 32 patients with secondary Sjögren's syndrome with rheumatoid arthritis (all women, mean (SD) age 54.9 (11) years) were studied. IgG, IgM, and IgA anticardiolipin antibodies were determined by a standard enzyme linked immunosorbent assay (ELISA) technique. Anticardiolipin antibodies were found in five patients (16%) with primary Sjögren's syndrome and in seven patients (22%) with secondary Sjögren's syndrome. There was no correlation between anticardiolipin antibodies and the clinical features of the antiphospholipid syndrome (thrombotic events, fetal loss, thrombocytopenia) or extraglandular manifestations of Sjögren's syndrome (arthritis, skin lesions, myositis, polyneuropathy, central nervous system disease, pulmonary and renal disease) in either group. Among the various serological features studied, anticardiolipin antibodies correlated with antinuclear antibodies and antibodies to RNP only in patients with primary Sjögren's syndrome. These results indicate that although anticardiolipin antibodies are often found in serum samples from patients with Sjögren's syndrome, their clinical significance remains unclear.

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It is now realised that Sjögren's syndrome is, after rheumatoid arthritis, the most common chronic inflammatory connective tissue disease, affecting between 1 and 3% of the population.^{1 2} It can occur as a distinct entity, primary Sjögren's syndrome, or in association with other autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, or more rarely scleroderma, polymyositis, primary biliary cirrhosis, chronic active hepatitis, or cryoglobulinaemia.^{3 4}

In Sjögren's syndrome the most characteristic clinical feature is inflammation and destruction of exocrine glands, principally the lacrimal and salivary glands, resulting in dry eyes and mouth. In addition there may be a wide spectrum of extraglandular manifestations including arthritis, skin vasculitis, myositis, central nervous system disease, polyneuropathy, interstitial pulmonary fibrosis, and renal failure.^{5 6}

This syndrome is also characterised by

immune system hyperactivity manifested by hypergammaglobulinaemia and the appearance of different autoantibodies in serum samples. There is also some evidence for the impairment of cellular immunity.⁷

Among the various autoantibodies present in the serum samples from patients with Sjögren's syndrome, anticardiolipin antibodies have been described.^{8–13} These antibodies are associated with the increased occurrence of thrombotic events, fetal loss, and thrombocytopenia in patients with systemic lupus erythematosus or primary antiphospholipid syndrome.^{14 15} Little is known, however, about the clinical significance of anticardiolipin antibodies in Sjögren's syndrome.

The aim of this study was to evaluate the occurrence and clinical significance of different isotypes of anticardiolipin antibodies in patients with primary and secondary Sjögren's syndrome.

Patients and methods

PATIENTS

Sixty three patients (all women) attending the department of connective tissue diseases, Warsaw, Poland with a diagnosis of Sjögren's syndrome were evaluated in this study. All patients fulfilled the criteria of Fox *et al* for the classification of Sjögren's syndrome.¹⁶ Thirty one patients had primary Sjögren's syndrome and none of them met the criteria for any other connective tissue disease. The other 32 patients had secondary Sjögren's syndrome associated with classical or definite rheumatoid arthritis (radiologically stage II or greater) classified according to the American Rheumatism Association criteria for rheumatoid arthritis.¹⁷ The mean (SD) age of patients with primary Sjögren's syndrome was 48.2 (11.2) years (range 30–90) and the mean (SD) disease duration was 73.8 (50.6) months. In patients with secondary Sjögren's syndrome the mean (SD) age was 54.9 (11) years (range 29–71), and the mean (SD) disease duration was 144.2 (111.3) months.

METHODS

Xerophthalmia was objectively confirmed by Schirmer's test or by Rose Bengal staining, or both. Xerostomia was objectively confirmed by sialometry and sialography.

Lip biopsy specimens of minor salivary glands were assessed according to the Chisholm-Mason classification and at least class 2 inflammatory changes were required for the diagnosis of Sjögren's syndrome.⁶

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Anticardiolipin antibodies of the IgG, IgM, and IgA classes were estimated by the standard enzyme linked immunosorbent assay (ELISA) method of Gharavi *et al*¹⁸ with further modifications.¹⁹ Results for IgG and IgM anticardiolipin antibodies were expressed as high, moderate, or low according to the accepted standards adopted by the 1986 International Workshop.²⁰ The working dilution of conjugate for IgA anticardiolipin antibodies was 1:500 and results were considered to be positive when they were greater than the mean result plus four standard deviations for 40 normal control subjects.

Other antibodies were measured by the following methods: antinuclear antibodies by immunofluorescence using a rat liver substrate; antibodies to double stranded DNA using *Crithidia luciliae* antigen; antibodies to RNP and Sm by the passive haemagglutination method; antibodies to Ro and La by counter-current immunoelectrophoresis; and IgM rheumatoid factor by the Rose-Waaler and latex tests. Cryoglobulins were detected by the method of Bronet *et al*.²¹ In all serum samples circulating immune complexes were measured by immunoelectrophoresis.²² Hypocomplemen-

taemia was evaluated by 50% haemolysis. False positive tests for syphilis were estimated by the Venereal Disease Research Laboratory (VDRL) and fluorescent treponeme antibody absorption tests.

STATISTICAL ANALYSIS

Statistical analysis was performed using Fisher's test.

Results

In all patients the diagnosis of Sjögren's syndrome was confirmed by one or more of the following: the presence of xerophthalmia (in 28 patients with primary Sjögren's syndrome (90%) and in 28 patients with secondary Sjögren's syndrome (88%)), xerostomia (in 27 patients with primary Sjögren's syndrome (87%) and in 26 patients with secondary Sjögren's syndrome (81%)), or typical Sjögren's syndrome changes in histopathological specimens of minor salivary glands (in 31 patients with primary Sjögren's syndrome (100%) and in 28 patients with secondary Sjögren's syndrome (88%)).

Table 1 shows the clinical manifestations of the disease in the two patient groups. Other clinical and haematological features such as central nervous system disease, polyneuropathy, pulmonary fibrosis, lymphadenopathy, splenomegaly, renal failure, thrombocytopenia, vessel thrombosis, and fetal loss were observed in a minority of patients in the two groups.

Anticardiolipin antibodies were present in 5 of 31 patients with primary Sjögren's syndrome and in 7 of 32 patients with secondary Sjögren's syndrome. Table 1 shows the isotype distribution of anticardiolipin antibodies in the two patient groups.

Table 2 presents the clinical and haematological associations of anticardiolipin antibodies. In patients with primary Sjögren's syndrome and secondary Sjögren's syndrome there was no correlation between either the clinical features of the antiphospholipid syndrome or extra-glandular manifestations of the main disease.

Table 3 shows the association of anticardiolipin antibodies with other immunological disorders present in serum samples from patients. Only in patients with primary Sjögren's syndrome was the correlation of anticardiolipin antibodies with antinuclear antibodies and antibodies to RNP statistically significant ($p < 0.01$).

Table 1 Isotype profile of anticardiolipin antibodies in patients with primary and secondary Sjögren's syndrome

Anticardiolipin antibodies isotype	Primary Sjögren's syndrome (n=31)	Secondary Sjögren's syndrome (n=32)
IgG	2	2
IgM	0	2
IgA	1	1
IgG+IgM	0	2
IgG+IgA	2	0
IgM+IgA	0	0
Total	5 (16%)	7 (22%)

Table 2 Clinical and haematological associations of anticardiolipin antibodies in patients with primary and secondary Sjögren's syndrome. No significant correlations were found ($p > 0.05$). Results given as number of manifestations/number of patients positive for anticardiolipin antibodies

Clinical feature	Primary Sjögren's syndrome (n=31)	Secondary Sjögren's syndrome (n=32)
Vein thrombosis	1/1 (IgG)	3/0
Arterial thrombosis	0	1/0
Fetal loss	4/0	5/1 (IgG+IgM)
Thrombocytopenia	0	5/1 (IgM+IgM)
Non-erosive arthritis	19/3 (IgG+IgA, IgG)	
Skin lesions	14/1 (IgG)	3/1 (IgG+IgM)
Raynaud's phenomenon	7/2 (IgG+IgA, IgG)	1/0
Rheumatoid nodules		11/1 (IgG)
Myositis	7/2 (IgG)	8/1 (IgG+IgM)
Central nervous system disease	3/0	3/1 (IgA)
Polyneuropathy	3/0	1/0
Pulmonary disease	2/0	2/0

Table 3 Correlation of anticardiolipin antibodies with immunological disorders in patients with primary and secondary Sjögren's syndrome. Results given as number of manifestations/number of patients positive for anticardiolipin antibodies

Immunological disorder	Primary Sjögren's syndrome (n=31)	Secondary Sjögren's syndrome (n=32)
Antinuclear antibodies	15/5*	5/1
Antibodies to double stranded DNA	1/0	0
Antibodies to RNP	3/3*	4/0
Antibodies to Sm	0	1/0
Antibodies to Ro	26/4	8/2
Antibodies to La	18/3	7/1
Rheumatoid factor	22/3	24/6
Immune complexes	23/5	26/6
Cryoglobulins	1/0	3/1
False positive VDRL test†	0	1/1

* $p < 0.01$.

†VDRL=Venereal Disease Research Laboratory.

Discussion

The purpose of this study was to determine the prevalence of anticardiolipin antibodies in serum samples from patients with primary and secondary Sjögren's syndrome and to determine whether they correlated with extraglandular manifestations of the disease. We also tried to determine whether anticardiolipin antibodies are associated with the features of antiphospholipid syndrome in patients with Sjögren's syndrome.

In this study anticardiolipin antibodies were found in 5 of 31 (16%) patients with primary Sjögren's syndrome and in 7 of 32 (22%) patients with secondary Sjögren's syndrome. In other studies the occurrence of anticardiolipin antibodies in patients with primary Sjögren's syndrome varied from 5 to 52%.^{9 10 12 23} They were identified in 7 to 13% of patients with rheumatoid arthritis^{8 11 24} and in 82% of patients with Sjögren's syndrome secondary to rheumatoid arthritis.²³ The isotypes of anticardiolipin antibodies in patients with primary Sjögren's syndrome have been previously studied by some workers^{9 10 12} but data for patients with secondary Sjögren's syndrome are lacking. Bao *et al*⁹ and Arroyo *et al*¹² found mainly IgG anticardiolipin antibodies in their patients with primary Sjögren's syndrome whereas Asherson *et al* found IgA anticardiolipin antibodies to be the most common isotype in patients with primary Sjögren's syndrome.¹⁰ None of these workers, however, was able to show any association of anticardiolipin antibodies in primary Sjögren's syndrome with an increased risk of clinical features of antiphospholipid syndrome.

In our patients there was no correlation between anticardiolipin antibodies and any extraglandular manifestations of the disease. The only patient in the group with primary Sjögren's syndrome who presented with venous thrombosis was positive for IgG anticardiolipin antibodies. In patients with secondary Sjögren's syndrome, one of five patients with thrombocytopenia, another one of five with fetal loss, and the only patient with a false positive test for syphilis were positive for IgG and IgM anticardiolipin antibodies. These results were not statistically significant.

Pennec *et al* have published results showing a correlation of IgG and IgM anticardiolipin antibodies with pulmonary fibrosis in patients with primary Sjögren's syndrome.¹³ In a follow up study they found anticardiolipin antibodies to be a risk factor for pneumonitis, arthritis, and renal failure. Careful interpretation of these data is necessary as the authors have taken the mean for healthy control subjects plus two standard deviations as their threshold for positive results.

Among the various immunological disorders which have been studied in these serum samples, only in patients with primary Sjögren's syndrome was there a correlation of anticardiolipin antibodies with antinuclear antibodies and antibodies to RNP ($p < 0.01$). These results are not surprising as antinuclear antibodies are commonly found in patients with primary Sjögren's syndrome and are now considered to be one of

the criteria for diagnosis of Sjögren's syndrome.¹⁶ On the other hand, the correlation between anticardiolipin antibodies and antibodies to RNP in primary Sjögren's syndrome is interesting, especially as antibodies to RNP are more characteristic of mixed connective tissue disease.

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