Isotype distribution of anticardiolipin antibodies in systemic lupus erythematosus: prospective analysis of a series of 100 patients

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Abstract

A prospective study of IgG and IgM isotypes of anticardiolipin antibodies (aCL) in a series of 100 patients with systemic lupus erythematosus was carried out. To determine the normal range of both isotype titres a group of 100 normal control serum samples was studied and a log-normal distribution of IgG and IgM isotypes was found. The IgG anticardiolipin antibody serum was regarded as positive if a binding index greater than 2·85 (SD 3·77) was detected and a binding index greater than 4·07 (3·90) was defined as positive for IgM anticardiolipin antibody. Twenty four patients were positive for IgG aCL, 20 for IgM aCL, and 36 for IgG or IgM aCL, or both. IgG aCL were found to have a significant association with thrombosis and thrombocytopenia, and IgM aCL with haemolytic anaemia and neutropenia. Specificity and predictive value for these clinical manifestations increased at moderate and high anticardiolipin antibody titres. In addition, a significant association was found between aCL and the presence of lupus anticoagulant. Identification of these differences in the anticardiolipin antibody isotype associations may improve the clinical usefulness of these tests, and this study confirms the good specificity and predictive value of the anticardiolipin antibody titre for these clinical manifestations.

Several recent studies found that patients with antiphospholipid antibodies (aPL) are prone to repeated episodes of venous or arterial thrombosis, or both,[1–11] recurrent fetal loss,[6–8 11–15] and thrombocytopenia.[2–6 8 9 11 15 16] In addition, there have been reports of the possible association of aPL with haemolytic anaemia[17] and neurological events such as cerebrovascular accidents,[18] seizures, migraine,[19] and chorea.[20] Other authors, however, consider that no association exists and aPL are only an epiphenomenon.[21–23] In fact, few prospective studies of a large number of unselected patients have been carried out and no definitive conclusions have been reached.

Of the various aPL, anticardiolipin antibodies (aCL) have received more attention owing to their sensitive, reproducible, and reliable detection by radioimmunoassay[3] or enzyme linked immunosorbent assay (ELISA).[24] Several studies suggest that aCL are closely related to the lupus anticoagulant and the biological false positive standard tests for syphilis (BFP-STS).[6] Although these antibodies are not restricted to patients with systemic lupus erythematosus (SLE), they are often found in these patients.

Our main objectives were to determine the prevalence of IgG and IgM anticardiolipin antibody isotypes in 100 consecutive patients with SLE in order to compare their possible association with clinical and laboratory manifestations and disease activity.

Patients and methods

PATIENTS

Clinical laboratory features of 100 consecutive and unselected patients (93 female, seven male) with SLE were prospectively studied during the years 1986–1987. All fulfilled four or more of the 1982 American Rheumatism Association (ARA) criteria for the classification of SLE.[25]

DISEASE ACTIVITY AND SUBSETS

Individual SLE clinical features were considered if they fulfilled the definitions of the ARA glossary.[26]

To assess disease activity all records were evaluated without knowledge of the anticardiolipin antibody status. Disease was judged to be clinically active when the following signs or symptoms were present: typical dermatitis; arthritis; serositis; central nervous system abnormalities (recent onset of chorea, seizures, psychosis, organic brain syndrome in the absence of offending drugs or known metabolic derangements, embolic cerebrovascular accidents); thrombocytopenia ($<100 \times 10^9/l$); haemolytic anaemia; vasculitis (biopsy); or nephritis (recent onset of haematuria (>10 red blood cells/high power field) or casts, or proteinuria >500 mg/24 h, or a 25% increase in serum creatinine). Sixty patients were classified as 'active' and 40 as 'inactive' based on these criteria.

Venous or arterial thrombosis, or both, was diagnosed in 10 patients. Diagnosis of venous thrombosis (seven patients) was based on clinical presentation and confirmed by venogram. Two patients with clinical features of cerebrovascular accidents or transient ischaemic attacks and evidence of cerebral infarction on computed tomographic scans, and two patients with clinical, electrocardiogram, and laboratory evidence of myocardial infarction were defined as having arterial thrombosis. None of the 93 women had a fetal loss during the study. Thrombocytopenia was present in 16 patients (platelet count $<100 \times 10^9/l$ on two occasions at least two weeks apart). Nine patients were considered to have haemolytic anaemia because they had a fall of at least 30 g/l in their blood haemoglobin, coinci-

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dent with a rise in unconjugated bilirubin of at least 10·3 μmol/l and a reticulocyte count above 5% at the time of the haemolytic episode. A positive Coombs' test was not considered essential, though most patients were tested. Neutropenia was present in five patients (neutrophil count lower than $0·5 \times 10^9/l$ on two occasions at least two weeks apart). Serum samples for the detection of anticardiolipin antibody were collected during the clinical events.

CONTROLS
The normal control group consisted of 100 healthy blood donors from the blood bank of the hospital clinic matched for age and sex. All showed normal coagulation assays and negative serological test for syphilis.

ANTICARDIOLIPIN ANTIBODY ELISA
Anticardiolipin antibodies were measured by an ELISA as described by Loizou *et al*[24] and Gharavi *et al*[27] with minor modifications of our own. Briefly, the flat bottomed wells of microtitre plates (Nunc, Denmark) were coated with 30 μl/well of cardiolipin (Sigma) suspended in ethanol at a concentration of 50 μg/ml and left to dry overnight at 4°C. The plates were treated for non-specific binding by incubation with 110 μl of 10% fetal calf serum (FCS; Flow) in phosphate buffered saline (PBS, pH 7·2) solution for two hours. The wells were then washed four times with 120 μl of PBS, and 100 μl of a 1:100 dilution of the serum in PBS-FCS solution was added to triplicate test wells; similarly, 100 μl of PBS-FCS was added to the blank control wells. The plates were incubated for one hour at room temperature. After washing the plates with PBS 100 μl of a goat antihuman IgG or IgM (Tago Inc) diluted 1:4000 was added to each well and incubated for one hour at 37°C. The plates were washed again with PBS, and 100 μl of alkaline phosphatase conjugated antibody (rabbit anti-goat IgG; Sigma) diluted 1:1000 in PBS-FCS was added to each well. The plates were then placed in a humidifier incubator at 25°C for one hour. They were washed with diethanolamine buffer (pH 9·8), and 100 μl *p*-nitrophenyl phosphate (1 mg/ml) prepared in diethanolamine immediately before use was added to each well. The plates were incubated in the dark at room temperature for one hour. The reaction was stopped by addition of 3 M NaOH (50 μl) to all wells and the optical absorbance was read at 405 nm on an ELISA microplate reader (Organon). Results were expressed as binding index (BI) calculated from optical absorbance (OA) values as follows:

$$BI = \frac{OA \text{ (test samples)} - OA \text{ (blank)}}{OA \text{ (referred normal pool)} - OA \text{ (blank)}}$$

COAGULATION ASSAYS
The following tests were carried out in all patients to detect the lupus anticoagulant according to the methods previously reported: prothrombin time, activated partial thromboplastin time, kaolin clotting time, diluted Russell's viper venom time, and tissue thromboplastin inhibition test.[28][29] The reagents used were rabbit brain thromboplastin for prothrombin time and tissue thromboplastin inhibition test, bovine thromboplastin for diluted Russell's viper venom time, and platelet factor 3 plus activator from rabbit brain tissue for activated partial thromboplastin time. The positive value for every coagulative test was defined as 3 + standard deviations above the mean normal value of the control group. To rule out a deficit in a coagulation factor each assay was performed also with a mixture of patient and control plasma (1/1, vol/vol). Patients were considered to have lupus anticoagulant when at least two assays were positive.

SEROLOGICAL TEST FOR SYPHILIS
A macroscopic rapid plasma reagin (Knickerbocker) was used in an 18 mm circle card test.

OTHER LABORATORY STUDIES
Antinuclear antibodies were determined by indirect immunofluorescence with mouse liver as substrate. Anti-double-stranded DNA antibodies were determined by Farr's ammonium sulphate precipitation technique.[30] Complement components (C3, C4) were estimated by radial immunodiffusion and CH_{50} by Lachmann and Hobart's haemolytic technique.[31]

STATISTICAL ANALYSIS
Conventional χ^2 analysis and Fisher's exact test were used to determine the statistical significance of clinical and laboratory findings. Stepwise regression analysis was used to correlate anticardiolipin antibody positivity with 16 variables (sex, age, SLE activity, cutaneous involvement, arthritis, serositis, central nervous system disease, renal involvement, venous or arterial thrombosis, or both, thrombocytopenia, haemolytic anaemia, neutropenia, lymphopenia, antinuclear antibodies, anti-DNA antibodies, and complement components) in all patients.

The sensitivity, specificity, and predictive value of aCL at low, moderate, or high titres for venous or arterial thrombosis, or both, thrombocytopenia, haemolytic anaemia, and neutropenia were determined according to the method of Galen and Gambino.[32]

Results
ANTICARDIOLIPIN ANTIBODY ISOTYPE DISTRIBUTION
Study of 100 normal control serum samples showed a log-normal distribution of both IgG and IgM anticardiolipin antibody titres (figs 1 and 2). Values were considered normal when the logarithms of the binding indexes were below the 98 centile of cumulative normal distribution. The IgG aCL were regarded as positive if a binding index greater than 2·85 (SD 3·77) was obtained. A binding index greater than 4·07 (3·90) was defined as positive for IgM aCL. When these conventions were used 36 patients were positive for IgG or IgM aCL, or
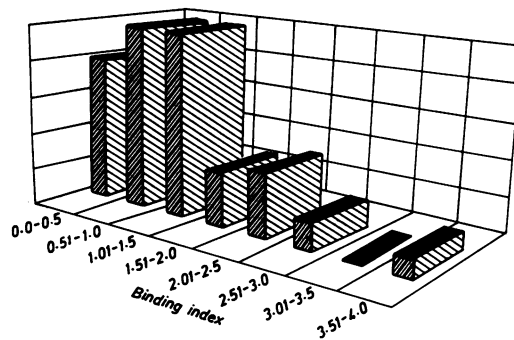
Figure 1: IgG anticardiolipin antibody distribution in normal control serum samples.
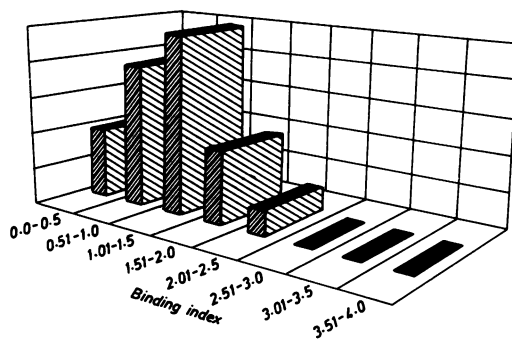

Figure 2: IgM anticardiolipin antibody distribution in normal control serum samples.

Table 1: Isotype spectra of anticardiolipin antibody positive patients

Patient No	IgG aCL*	IgM aCL
1	+	−
2	+	+++
3	++	−
4	+	−
5	+++	+++
6	−	+++
7	−	+++
8	+++	−
9	++	+++
10	+	−
11	++	−
12	−	+++
13	++	+
14	−	+++
15	−	+
16	+	−
17	−	+++
18	++	−
19	−	+++
20	+++	+
21	+++	−
22	−	+
23	−	++
24	−	++
25	+	−
26	−	+++
27	+	+++
28	++	+
29	+++	+++
30	+	−
31	++	−
32	+++	−
33	++	−
34	++	−
35	+	−
36	−	+++

*aCL=anticardiolipin antibodies.
+=low titre (IgG: 2·85–3·29; IgM: 4·07–4·95); ++=moderate titre (IgG: 3·30–5·05; IgM: 4·96–6·00); +++= high titre (IgG: ≥5·06; IgM: ≥6·01).

both. The isotype spectra were as follows: 24 patients were positive for IgG aCL and 20 for IgM aCL. All were arbitrarily divided into three subgroups consisting of low, moderate, or high titres of aCL. Only eight patients had both IgG and IgM aCL (table 1). None of the control sera was positive for IgG or IgM aCL.

Statistically significant association was found between the presence of aCL and lupus anticoagulant (p=0·01), but not between aCL and BFP-STS (rapid plasma reagin).

IgG ANTICARDIOLIPIN ANTIBODY

When compared with the 76 patients without IgG aCL the patients with IgG aCL were found to have an increased incidence of thrombosis (p=0·001), thrombocytopenia (p=0·03), and SLE activity (p=0·005). Stepwise regression analysis of IgG anticardiolipin antibody titres with the 16 variables previously described showed significant correlations with thrombosis (p=0·001) and thrombocytopenia (p=0·03), but not with SLE activity.

Table 2 shows the sensitivity, specificity, and predictive value of the IgG anticardiolipin antibody test at low, moderate, and high anticardiolipin antibody titres for thrombosis and thrombocytopenia. At low titre the specificity of the IgG anticardiolipin antibody test for thrombosis and thrombocytopenia was greater than 80%, the sensitivity for thrombosis was 70% and for thrombocytopenia 47%, but the predictive values were quite low. At moderate and high titres, however, the specificity of the test for both thrombosis and thrombocytopenia varied from 89% to 96% and its predictive value was in

Table 2: Sensitivity, specificity and predictive value of the IgG anticardiolipin antibody test for thrombosis and thrombocytopenia

	Sensitivity (%)		Specificity (%)		Predictive value (%)	
	A*	B*	A	B	A	B
Low titre	70	47	81	81	29	33
Moderate titre	50	35	89	89	33	40
High titre	20	18	96	96	33	50

*A=thrombosis; B=thrombocytopenia.

the range 33–50%. Interestingly when the binding index was ≥7·93 the predictive values for thrombosis and thrombocytopenia were 67%.

IgM ANTICARDIOLIPIN ANTIBODY ASSOCIATIONS

In comparison with the 80 patients without IgM aCL, those with these antibodies had an increased incidence of haemolytic anaemia (p=0·01) and neutropenia (p=0·005). In addition, stepwise regression analysis of IgM anticardiolipin antibody titres with 16 variables showed significant correlations with haemolytic anaemia (p=0·015) and neutropenia (p=0·01).

Table 3 shows sensitivity, specificity, and predictive value of the IgM anticardiolipin antibody test at low, moderate, and high antibody titres for haemolytic anaemia and neutropenia. At moderate and high titres the specificity of the test for both variables exceeded 80%, the sensitivity for haemolytic anaemia was 44% and for neutropenia 60%. When the binding index was ≥11 the predictive value for haemolytic anaemia was 60%, and when the binding index was ≥15 the predictive value was 100%. In

Table 3: Sensitivity, specificity and predictive value of the IgM anticardiolipin antibody test for haemolytic anaemia and neutropenia

	Sensitivity (%)		Specificity (%)		Predictive value (%)	
	A*	B*	A	B	A	B
Low titre	56	80	84	83	25	20
Moderate titre	44	60	88	87	27	20
High titre	44	60	90	89	31	23

*A=haemolytic anaemia; B=neutropenia.

addition, when the binding index was ≥15 the predictive value for neutropenia was 50%.

OTHER ASSOCIATIONS
Lupus anticoagulant was detected in 30 patients, and significant association with thrombosis (p=0·05) and thrombocytopenia (p=0·001) was found. In addition, BFP-STS (rapid plasma reagin) was present in 17 cases and no clinical or biological correlation was found.

Discussion
The need to establish an anticardiolipin antibody titre for identification of patients with anticardiolipin antibody associated clinical features is of paramount importance.[8] The results of this study indicate that control and SLE groups have a non-normal (log-normal) distribution of anticardiolipin antibody titres. To exclude the largest number of false positive anticardiolipin antibody sera only titres greater than 3·77 SD (binding index ≥2·85) were included for IgG aCL and greater than 3·90 SD (binding index ≥4·07) for IgM aCL.

The prevalence of aCL in our population with SLE is similar to that reported by other authors[10 14] using an ELISA. IgG aCL were detected in 24% of an unselected group of patients, IgM aCL in 20%, and either IgG or IgM aCL in 36%. A much higher prevalence was found by Harris et al using radioimmunoassay.[3] The differences in the reported prevalences of anticardiolipin antibody positivity may be due to differences in the sensitivity of the radioimmunoassay and ELISA techniques, in the cut off positive level, or may reflect the effects of patient selection and treatment.

The association of aPL (usually IgG aCL or lupus anticoagulant) with thrombosis, recurrent fetal loss, and thrombocytopenia has been reported in several studies.[1-16] In general, these studies have determined the antiphospholipid antibody status of randomly selected patients with SLE and clinical and laboratory features recorded by review of their medical charts. In our prospective study we confirmed the previously reported association of IgG aCL with thrombosis and thrombocytopenia. The association with recurrent fetal loss, however, was not examined because no patient had an intrauterine death during the study. Nevertheless, analysis of 12 patients from this series with previous fetal losses (data not included) showed no association with the anticardiolipin antibody titre at the moment of the study. In our opinion the lack of such an association is not surprising.

Firstly, it may be due to the existence of a variable period of years between the fetal loss and the determination of the anticardiolipin antibody titre. Secondly, phospholipids other than cardiolipin may be related to the recurrent fetal loss. Thirdly, other antibodies such as anti-Ro or antilymphocyte may occasionally be involved in the pathogenesis of fetal losses.[33]

We found a significant association between the presence of IgM aCL and haemolytic anaemia and neutropenia. Although Coombs' positivity is common in patients with SLE who have aCL,[3] the association with haemolytic anaemia has previously been recognised only by Delezé et al[17] and Alarcón-Segovia.[34] These authors consider that aPL may react with the cell wall of either erythrocytes or platelets and can cause their destruction either by complement or by receptor mediated entrapment by the reticuloendothelial system. Possibly, IgM aCL would best cause spleen clearance of erythrocytes and their complement mediated damage could hence be entertained. A similar mechanism would cause neutropenia.

No association between aCL and neurological events other than cerebrovascular accidents was found in our study. This lack of association may be due to the small number of patients (14 patients had migraine and two seizures). Nevertheless, both patients with cerebrovascular accidents had a positive anticardiolipin antibody titre.

Statistically significant association was found in our study between the presence of aCL and lupus anticoagulant, but not between aCL and BFP-STS. Similar results have been reported by other authors,[35] and they may be due to differences in phospholipid epitope specificity.[6]

In conclusion, we observed a high incidence of aCL in our SLE population and showed a relation between IgG aCL and thrombosis and thrombocytopenia and also between IgM aCL and haemolytic anaemia and neutropenia. Identification of these differences in the anticardiolipin antibody isotype associations may improve the clinical usefulness of these tests. In addition, the findings of this study confirm that the anticardiolipin antibody titre has a good predictive value for those clinical manifestations and may be a good predictor in patients with SLE.

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