Fluctuations of anticardiolipin antibody levels in patients with systemic lupus erythematosus: a prospective study

HENK JAN OUT,¹ PHILIP G DE GROOT,² PAULA HASSELAAR,^{1,2} MARJA VAN VLIET,^{1,2} AND RONALD H W M DERKSEN¹

From the ¹Division of Immunopathology (Department of Internal Medicine) and the ²Department of Hematology, University Hospital Utrecht, The Netherlands

SUMMARY In 53 patients with systemic lupus erythematosus sequential blood samples obtained during a four year period (range 6–47 months) were screened for anticardiolipin antibodies (ACAs). Disease activity and treatment with prednisone were also assessed and related to ACA concentrations. During follow up only 21 patients for ACA IgG (40%) and 25 for ACA IgM (47%) remained in the ACA category (negative, low positive, high positive) found at the first sample taken at entrance. Marked increases from negative to high positive concentrations were sometimes seen and were not accompanied by typical events such as thrombosis or thrombocytopenia (the ACA syndrome). Shifts in ACA concentrations could not always be explained by changes in prednisone dose. Also, in patients with low dose prednisone treatment or none at all (n=22) 10 patients (45%) changed ACA IgG category and 12 patients (55%) fluctuated in ACA IgM categories during follow up. As a consequence of the variability in ACA titres relations of ACAs with the ACA syndrome depended on the blood sample studied. In the second sample, randomly taken half way through follow up, no significant relations with the ACA syndrome could be found. Anticardiolipin antibody IgG was significantly associated with disease activity in 11/47 patients (23%) and in the group as a whole. During remission ACA IgG was significantly associated with the ACA syndrome, whereas during moderate/severe disease activity in the same patients that correlation was not significant. Anticardiolipin antibody IgM was much less influenced by disease activity, and in only 4/47 patients (9%) could a significant relation with disease activity be shown. Associations of ACA IgM with the ACA syndrome were significant during both lupus flares and remission.

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease characterised by a variety of circulating autoantibodies. Of these, antibodies against negatively charged phospholipids, such as anticardiolipin antibodies (ACAs), have received a lot of attention over the past decade. Many studies showed correlations between the presence of ACAs and particular clinical features, such as fetal loss, thrombosis, and thrombocytopenia.^{1–5} The term 'anticardiolipin syndrome' has been introduced for this entity.⁶

Longitudinal data on ACAs are scarce. Suggestions have been made that ACA titres may vary with

disease activity^{7,8} and that they may disappear spontaneously.⁹ It has been shown that immunosuppressive drugs influence ACA concentrations.¹⁰

Relations between ACAs and the ACA syndrome are usually established in cross sectional studies. Also, classification of patients as ACA positive or negative is based on single blood samples. To investigate whether such classification is valid we prospectively studied 53 patients with SLE over a four year period (range 6–47 months) and focused on the relation between ACA titres, lupus disease activity, and prednisone treatment.

Patients and methods

PATIENTS AND SAMPLES

Clinical data and blood samples of 53 patients with

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Correspondence to Dr H J Out, Division of Immunopathology, Department of Internal Medicine, University Hospital Utrecht, PO Box 85500, 3508 GA, Utrecht, The Netherlands.

SLE, according to American Rheumatism Association criteria,¹¹ were collected from September 1984 until August 1988 at each visit to our outpatient clinic and during hospital admission. There were 49 women (median age 36 years, range 18–67) and four men (median age 33 years, range 30–37). The median duration of follow up was 26 months (range

6–47 months), during which at least seven blood samples (range 7–22, median 12 samples) were taken. Blood samples for the preparation of serum were collected by venepuncture into glass tubes. The blood was allowed to clot at room temperature for two hours and centrifuged at 175 g for 10 minutes to obtain serum.

Table 1 Systemic lupus erythematosus activity scoring system*

Activity	Score
Renal	
Proteinuria >0.5 g/24 hours (onset within 4 months or duplication)	2
Haematuria (onset or duplication, >5 RBC ⁺ /HPF ⁺)	1
Casts (red blood cell or granular): presence onset	1
	2
Creatinine clearance: drop >25%, within 4 months	2
Cerebral	
Cerebrovascular accident	4
Insults	4
Psychosis	4
Chorea/athetosis	4
Brain nerve paralysis	4
Skin/hair/mucous membranes	
Alopecia	1
Active discoid lesions	1
Butterfly rash	2
Other active rashes	1
Active oral ulceration	2
Joints	
Arthralgia or myalgia, or both	1
Arthritis (≥2 joints) or tendinitis, or both	1
Serous membranes	
Sighing pain	1
Rubbing	2
ECG ⁺ and/or X-thorax and/or echocardiographic changes	2
Haematological	
Anaemia <101 g/l	1
<81 g/l	2
Leucopenia <4.0×10 ⁹ /l	1
<3.0×10 ⁹ /l	2
Thrombocytopenia <150×10 ⁹ /l	1
<50×10 ⁹ /l	2
<25×10 ⁹ /l	3
Vascular	
Minor vasculitis (purpura, periungual infarcts)	2
Major vasculitis (ulcers, mononeuritis)	4
Miscellaneous	
Uveitis or chorioretinitis, or both	3
Myositis: CPK ⁺ >150 U/l	2
>500 U/l	3

*Only active lesions or new onset of symptoms are scored.

⁺RBC=red blood cells; HPF=high powered field; ECG=electrocardiogram; CPK=creatine phosphokinase.

DISEASE ACTIVITY

Disease activity and prednisone treatment were recorded at each sample point. Disease activity was assessed with a scoring system consisting of typical clinical and laboratory features of SLE (Table 1) and classified as inactive (scores 0–1), mild (score 2–3), moderate (score 4–5), or severe (score ≥6).

Table 2 shows lupus symptoms at entrance and during follow up, classified according to organ system. The most common manifestations of active disease were arthritis, cytopenia, symptoms of the skin and mucous membranes. Sixteen patients had renal disease.

ANTICARDIOLIPIN ANTIBODY ASSAY

Anticardiolipin antibodies (IgG and IgM) were measured with an enzyme linked immunosorbent assay (ELISA) according to the procedure described by Loizou *et al*¹² with minor modifications.¹³ A positive result indicated a value in units more than 2SD over the value obtained with pooled control serum derived from 71 healthy volunteers. All results were expressed as the ratio of patient ACA concentrations to that of normal values plus 2SD in order to make comparisons of ACA assays performed on different days and to make calculations of mean values for whole group analysis. A ratio <1 represented negative ACA concentrations, values between 1 and 2 were regarded as low positive, and ratios of more than 2 indicated high positive ACA titres.

RELATION BETWEEN ACAS AND THE ANTICARDIOLIPIN SYNDROME

Correlations of ACA IgG and ACA IgM for fetal

Table 2 Lupus symptoms of 53 patients with systemic lupus erythematosus at entrance and during follow up classified according to organ system

Symptoms	Patients (n)
Renal	16
Cerebral	6
Skin/hair/mucous membranes	35
Joints	34
Haematological	33
Serous membranes	7
Vascular	17
Miscellaneous	2

Table 3 Number of patients (%) in each anticardiolipin antibody (ACA) and disease activity category at the first sample

Disease Activity	ACA IgG			Total	ACA IgM		
	Neg	Low pos	High pos		Neg	Low pos	High pos
0-1 (none)	13	6	3	22 (42)	17	3	2
2-3 (mild)	11	6	3	20 (38)	12	5	3
4-5 (moderate)	4	1	2	7 (13)	6	1	0
≥6 (severe)	1	3	0	4 (7)	2	1	1
Total	29 (55)	16 (30)	8 (15)	53 (100)	37 (70)	10 (19)	6 (11)

loss, thrombosis, and thrombocytopenia were assessed at three random points: at the start, half way through follow up, and the last taken sample. Also, comparisons of correlations with the ACA syndrome were made using the first samples in both no disease/mild disease and moderate/severe activity, in 32 patients who had had at least one lupus flare.

STATISTICS

Correlations within each patient between disease activity, ACA IgG, and ACA IgM were made with Pearson's correlation coefficient. Correlations of ACAs with features of the ACA syndrome were calculated using the χ^2 test. To investigate the interrelations between ACAs and disease activity for the studied population as a whole six measure points of each patient were selected from the available data using a table with random numbers. For this whole group analysis χ^2 tests were done. Values of $p < 0.05$ were considered significant.

Results

FIRST SAMPLE

Twenty four patients (45%) were positive for ACA IgG and 16 patients (30%) had positive ACA IgM concentrations (Table 3). Most patients (42/53 (80%)) had mild disease activity or none at all. Four patients received more than 40 mg prednisone (Table 4). Of these, three patients were positive for ACA IgG and two for ACA IgM.

FOLLOW UP

Table 5 shows the ACA concentrations of each patient during follow up compared with the ACA category found at the first sample. When all patients together are considered 13/29 patients (45%) with negative ACA IgG samples at entrance and 14/37 (38%) with initially negative ACA IgM concentrations developed (low) positive samples during follow up. For both ACA IgG and ACA IgM two patients developed high positive samples. Similarly, 14/24 patients (58%) starting with positive ACA IgG concentrations and 7/16 (44%) with positive ACA IgM concentrations at entrance showed negative samples during the course of the disease.

Fluctuations were also observed in patients with no or mild disease activity (n=20): three of 10 negative patients (30%) became positive (of which one high positive) for ACA IgG and 6/15 negative patients (40%) had positive (including one high positive) ACA IgM concentrations during follow up. Five of 10 initially ACA IgG positive patients (50%) showed negative samples during follow up. In all four patients starting with high positive ACA samples and with no disease/mild disease activity during follow up ACA titres remained positive.

To exclude variations in prednisone dose as the cause for ACA shifts all patients with no (n=10) or low dose (≤ 10 mg) prednisone (n=12) treatment were evaluated separately. Of nine ACA IgG negative patients at entrance, three (33%) became positive, whereas six of 12 (50%) ACA IgM negative

Table 4 Prednisone treatment at the first sample

Prednisone (mg)	ACA* IgG			ACA IgM		
	Neg	Low pos	High pos	Neg	Low pos	High pos
None	8	7	3	13	2	3
≤10	8	4	3	9	5	1
10-40	12	2	2	13	2	1
>40	1	3	0	2	1	1
Total	29	16	8	37	10	6

*ACA=anticardiolipin antibody.

Table 5 Number of patients in each anticardiolipin antibody (ACA) category during follow up

Group	First sample	ACA IgG				ACA IgM			
		Patients (n)	Follow up			Patients (n)	Follow up		
			Neg	Low pos	High pos		Neg	Low pos	High pos
All patients together (n=53)	Neg	29	29	13	2	37	37	14	2
	Low pos	16	12	16	3	10	6	10	6
	High pos	8	2	5	8	6	1	4	6
Patients with no or mild lupus activity (n=20)	Neg	10	10	3	1	15	15	6	1
	Low pos	8	5	8	1	3	0	3	2
	High pos	2	0	0	2	2	0	0	2
Patients with no or minor (≤ 10 mg) prednisone treatment (n=22)	Neg	9	9	3	0	12	12	6	1
	Low pos	9	6	9	1	6	3	6	4
	High pos	4	0	1	4	4	0	2	4

This table shows the follow up results of all patients classified as negative, low positive, or high positive at the first sample. The results are given as the number of patients with samples in each ACA category, including the first sample. As during follow up one patient could have samples classified in more than one ACA category the total number of patients obtained by adding all categories exceeds the number at the first sample.

patients developed positive titres, including one patient with high positive levels. Six of nine (67%) initially low positive patients became negative for ACA IgG, and 3/6 (50%) for ACA IgM.

CORRELATION OF ACAS AND DISEASE ACTIVITY

Anticardiolipin antibody IgG and disease activity were significantly correlated in 11 of 47 patients (23%); six patients with persistently negative ACA IgG concentrations and absent lupus activity were excluded because in those circumstances associations would be expected. Significant correlations between ACA IgM and disease activity were found

in only four of 47 patients (9%) with fluctuating ACA IgM concentrations or lupus activity, or both.

Anticardiolipin antibody IgG and ACA IgM were correlated in 17 of 41 patients (41%), excluding six patients with persistently negative ACA IgG and ACA IgM concentrations (n=12). Figure 1 illustrates graphically the relation between ACA IgG, ACA IgM, prednisone treatment, and disease activity in two female patients with SLE. In one patient ACAs and disease activity were correlated significantly, whereas the other patient showed a drop of high positive ACA IgG concentrations to normal during no disease/mild disease activity.

Fig. 1 Follow up results for two female patients with systemic lupus erythematosus. Left: Anticardiolipin antibody (ACA) IgG, ACA IgM, and disease activity are correlated significantly ($p<0.02$). No prednisone was given. Anticardiolipin antibody ratios >1 are positive, ACA ratios >2 are regarded as high positive. Right: No significant correlations. Initial spontaneous drop in ACA IgG concentrations from high positive to negative values. Normal ACA IgG concentrations are seen when high dose prednisone is given.

Table 6 Correlation of anticardiolipin antibody (ACA) IgG and ACA IgM (in p values, χ^2) with the ACA syndrome at different sample points in 53 patients with systemic lupus erythematosus

Sample* point	ACA IgG			ACA IgM		
	Fetal† loss	Thrombosis	Thrombocytopenia	Fetal† loss	Thrombosis	Thrombocytopenia
First sample	0.035	0.008	0.008	0.010	0.002	0.005
Second sample	NS	NS	NS	NS	NS	NS
Third sample	NS	0.026	NS	NS	0.019	NS
First sample taken during:						
No/mild dis.act.	TSN‡	0.003	NS	TSN	0.013	NS
Mod./sev. dis.act.	TSN	NS	NS	TSN	0.033	NS

*First sample taken at the start of the study; second sample taken half way through follow up; third sample taken at the end of the study.
†Twenty seven patients had a history of at least one pregnancy.
‡TSN=numbers too small for statistical analysis (n=13).

RELATION OF ACAs WITH THE ANTICARDIOLIPIN SYNDROME
Table 6 gives the relations between major manifestations of the ACA syndrome and ACAs at different random sample points.

With the first sample significant relations between ACAs and all features of the ACA syndrome were found. The second sample, however, taken half way through follow up did not show any significant relations at all. With the third sample an association only with thrombosis was found and not with fetal loss or thrombocytopenia.

When ACA concentrations were considered in the first sample obtained during no disease/mild disease activity and compared with ACA titres in the same patients during moderate/severe lupus activity (n=32), ACA IgG was not significantly associated with the ACA syndrome during lupus flares, whereas at remission ACA IgG showed a relation with thrombosis (p=0.003). For ACA IgM differences in disease activity did not influence significant relations.

Table 7 Whole group analysis. Mean values of anticardiolipin antibody (ACA) IgG and ACA IgM at each category of disease activity

	Disease activity			
	None	Mild	Moderate	Severe
ACA IgG*	1.4943	1.2585	1.3196	1.8886
ACA IgM†	1.5998	1.1566	0.9814	0.9494

*p=0.012 (χ^2) correlation between disease activity and ACA IgG.
†p=0.197 (χ^2) correlation between disease activity and ACA IgM.
Anticardiolipin antibody IgG and ACA IgM were correlated significantly (p<0.001, χ^2).

WHOLE GROUP ANALYSIS
Table 7 shows the results of whole group analysis, based on six random samples from each patient. χ^2 Analysis showed significant relations between ACA IgG and disease activity (p=0.012), and between ACA IgG and ACA IgM (p<0.001), but not between disease activity and ACA IgM (p=0.197).

Discussion

This prospective study of 53 patients with SLE shows the occurrence of shifts in ACA concentrations. During follow up only 21 patients (40%) for ACA IgG and 25 (47%) for ACA IgM remained in the ACA category (negative, low positive, high positive) initially found. Marked increases from negative to high positive concentrations were sometimes observed (Fig. 1) and were not accompanied by symptoms like thrombosis or thrombocytopenia. High dose prednisone may explain the variability in ACA concentrations (Fig. 1), but in patients with low dose prednisone treatment or none at all 10 patients (45%) changed ACA IgG category and 12 patients (55%) fluctuated in ACA IgM categories (Table 5). Because of the variability of ACA concentrations the time of evaluation affects the significance of the relation between ACAs and features of the ACA syndrome (Table 6).

These findings may explain the conflicting results found by various investigators for the prevalence of ACAs in SLE, ranging from 22%¹⁴ to 38%¹⁵ to 54.2%,¹⁶ and the subsequent interrelations of ACAs with the ACA syndrome. Weidmann *et al* did not find any association between ACAs and thrombosis or spontaneous abortions.¹⁷ Cronin *et al* found a correlation between ACA IgM and fetal loss and thrombocytopenia, but no association of ACA IgG with the ACA syndrome.¹⁸ Also, other investigators

were unable to find relations between ACAs and thrombosis.^{15 16}

Changes in ACA concentrations may be explained by variations in disease activity. In 11/47 patients (23%) and the group as a whole ACA IgG was significantly associated with lupus activity. This implies that screening for ACA IgG during lupus flares increases the chance for finding positive concentrations. Our results, however, indicate that during active disease a subset of ACA IgG positive patients with SLE, not characterised by the ACA syndrome, is included. We did not find a relation between ACA IgG and thrombosis using samples obtained during active disease, whereas in the same patients this association was significant during remission. In contrast with ACA-IgG, ACA IgM was found to be significantly associated with lupus activity in only 4/47 (9%) patients and not in the group as a whole. In these four patients ACA IgG was also significantly correlated with disease activity. Both during remission and moderate/severe disease activity a significant relation between ACA IgM and thrombosis was found.

We conclude that changes in ACA concentrations are a common phenomenon in patients with SLE, confirming the observations made in two recent reports.^{8 19} Disease activity and prednisone treatment can only partially explain these fluctuations. Associations of ACAs with fetal loss, thrombosis, and thrombocytopenia are mostly established retrospectively and the potential pathogenicity of ACAs for these events has not been proved. Prospective studies are necessary to establish the risks of ACA positive patients with SLE developing the ACA syndrome. As positive ACA titres found in patients with SLE may have consequences on treatment—for example, during pregnancy—we suggest that screening for ACAs should take place in at least two samples taken during low dose prednisone treatment or in its absence. This will increase the chance of finding ACA positive patients possibly at risk. When these samples are obtained during active disease positive ACA IgG concentrations are of limited value in identifying a subset of patients with SLE with clinical events associated with the ACA syndrome. It seems that the IgM isotype is more a marker of this syndrome during lupus flares and we suggest that in active disease ACA IgM should be considered first in evaluating possible future risks for the development of the ACA syndrome.

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