Laboratory and clinical study

ANTICARDIOLIPIN ANTIBODY IN LUPUS ERYTHEMATOSUS

M. Marschalko, A. Ruzsovits and A. Horvath

ABSTRACT

Introduction. Anticardiolipin antibodies are associated with vascular thrombotic events in different dermatological, infectious and autoimmune diseases.

Methods. In the present study the frequency of IgG anticardiolipin antibody was determined by ELISA in discoid, systemic and subacute lupus erythematosus. IgG anticardiolipin ELISA was made during a 5-year follow-up in 102 sera of 16 patients with SLE and in 32 sera of 5 patients with subacute cutaneous lupus erythematosus. The result of anticardiolipin ELISA test was compared with the ds DNA antibody ELISA result, thrombocyte count, and clinical thrombotic events in patients with systemic lupus erythematosus.

Results. Anticardiolipin antibody was shown in 64 out of 231 /27,7%/ patients with systemic, 3 out of 32 patients /9,4 %/ with subacute cutaneous and 2 out of 26 patients /7,7%/ with discoid lupus erythematosus. 41 out of 337 control persons /12,2%/ had anticardiolipin antibody.

In patients with systemic and subacute cutaneous lupus erythematosus the levels of anticardiolipin antibody were followed during the 5- year follow-up.

Conclusion. There was no significant correlation between anticardiolipin and anti-ds DNA antibody determinations, between anticardiolipin ELISA and platelet count and anticardiolipin ELISA and thrombotic clinical events in patients with systemic lupus erythematosus.

KEY WORDS

anticardiolipin, antiphospholipid, antibody, thrombotic events, thrombocytopenia, anti-ds DNA antibody

INTRODUCTION

Antiphospholipid antibodies have been found in sera of patients with primary antiphospholipid syndrome, autoimmune disorders, as well as in the sera of healthy people (1,2,3,4).

The clinical significance of the antiphospholipid antibodies is widely examined. Although the association of the antiphospholipid antibodies and the thrombotic events is well known, their pathogenetic role in the disease process and in the thrombotic events is not clearly understood (5).

Table 1. Results of anticardiolipin (ACA) ELISA tests in LE patients during the 1991-96 period; 16 SLE (patients 1-16) and 5 SCLE patients (17-21). Normal values up to 16 U/ml

ACA RESULT DURING THE FOLLOW-UP PERIOD							
Name	**Age Dg**	**1991**	**1992**	**1993**	**1994**	**1995**	**1996**
1 NZS	32 SLE		20 12	7			11
2 KA	54 SLE		17 5	9 5 2 5			5
3 NL	32 SLE		44 25	8 8 5		18 19 13	5
4 P J	46 SLE	340 330	90	150 160 57 130 8		63	72
5 LL	56 SLE	98 9		5 10		16	
6 LE	61 SLE	60 60 90 17		27 7 2 10 20	5		
7 MM	56 SLE	13 29 50 50	9 6	5		5 5 17	
8 K I	53 SLE	18 7		15 4 20	41	11	
9 D I	43 SLE	32 32	32 12 9	3	65	12 10	14
10 DF	55 SLE	10 5	8	2			
11 CSE	51 SLE	100	98			14	12
12 DR	52 SLE		8 5	3	11		
13 SZA	20 SLE			45 45	20 7		5
14 HM	42 SLE		11	6	25		5
15 PK	35 SLE			12 4	5	5 10	
16 BI	32 SLE			3 27 27	21		13
17 BKM	45 SCLE	12	6 3 11 3	3	5		6
18 PG	33 SCLE				5	5 10 5	3
19 ZVY	47 SCLE	8 16	16 5	6 1 5	5	10 17 9	9
20 LA	39 SCLE				5 5	5	
21 KJ	69 SCLE	5	12	2 20			

The aim of the present study was:

To determine the frequency of IgG anticardiolipin antibody (ACA) in different lupus erythematosus (LE) groups.

To investigate the clinical significance of ACA in systemic LE (SLE) and in subacute cutaneous LE (SCLE) during a 5-year follow-up.

Patients and methods

Sera of 337 control persons, suffering from different dermatological diseases - except for autoimmune ones - and sera of 289 LE patients (231 SLE, 32 SCLE and 26 DLE) were investigated. Diagnosis of SLE was based on the revised ARA criteria; diagnosis of SCLE and DLE was based on the characteristic clinical and histological findings and the results of the antinuclear antibody determinations.

Follow-up study: 102 sera of 16 SLE patients, 32 sera of 5 SCLE patients were collected during a 5 year follow-up and were tested repeatedly.

IgG type ACA was determined by ELISA (6,7).

Briefly: ELISA plates were coated with cardiolipin antigen /Sigma/ 50 µg/ml. Plates were blocked by the addition of 100 µl of PBS/FCS for 2 h at room temperature. Sera were diluted to 1/50 in 10% foetal calf serum. The plates were incubated for 1 h at room temperature. 100 µl of peroxidase conjugated goat antihuman IgG /Human, Hungary/ diluted 1:1500 was added to each well and the plates were incubated at room temperature for 1 h. Reference sera were obtained from Statens Serum Institute, Copenhagen. Extinction values were read at 492 nm. For each plate a standard curve was constructed

Table 2. Comparison of anticardiolipin (ACA) and anti-ds DNA antibodies in 85 sera from follow-up patients. The difference is significant: chi square test: 1.046; p:0.6

	ACA +	ACA -	total
anti DNA+	12	16	28
anti DNA-	18	39	57
total	30	55	85

and the concentration of each sample was determined. Test samples were reported as having raised ACA level when their extinction values exceeded 16 U/ml. Details of the test was reported previously (8), its specificity proved to be 89,4%, its sensitivity for SLE proved to be 51%.

Anti-ds DNA antibodies were measured by ELISA test. ELISA plates were coated overnight at 4°C with DNA from chicken erythrocyte. The test samples (diluted 1/200 in PBS Tween) were incubated for 1 h at 37°C. 100 µl peroxidase conjugated goat antihuman IgG was used at 1:1500. Extinction values were read at 492 nm. The extinction values were compared to the calibration curve gained by the dilution of a known reference positive serum. Positive results were evaluated at or above 20 U/ml.

Statistical analysis was done by using the chi-square test.

RESULTS

ACA in control group and in LE patients:

Out of 337 control persons 12,2% (41 patients) had ACA.

Sera of 231 patients with SLE were tested, 64 (27,7%) were positive. Out of 32 SCLE patients 3 (9,4%) were positive and out of 26 DLE patients 2 (7,7%) were positive.

Table 3. Comparison of ACA test and clinical symptoms. No positive correlation: chi square test: 0.011; p: 0.9

symptoms	present	absent	total
ACA+	4	10	14
ACA-	1	4	5
total	5	14	19

Result of ACA ELISA during the follow-up period:

16 patients with SLE and 5 patients with SCLE were followed up during the 1991-1996 period. Blood was taken minimum 3 times (patient 20), maximum 12 times (patient 19) (Table 1).

According to the results of ACA ELISA the patients could be divided into two groups:

Group 1: The results of ACA ELISA was negative at every occasion in 6 patients (patients 10, 12, 15, 17, 18, 20).

Group 2: The results of ACA ELISA varied during the period under observation in 15 patients. In several patients the fluctuations in the amount of the antibody were substantial (patients 4, 5, 6, 11), while in others the fluctuations between individual assays were small. No patient was positive on every occasion.

Comparison of the presence of ACA and anti-ds DNA antibodies.

In 85 out of the 102 sera of the followed-up patients the result of the anti-ds DNA and ACA was compared (Table 2).

There was a significant difference between the two tests: chi square test: 1.046; p: 0.6.

Correlation between ACA ELISA results and clinical signs.

The clinical symptoms which are thought to be associated with ACA, such as thrombotic events, vasculitis, haemolytic anaemia, thrombocytopenia, heart and central nervous symptoms were evaluated in the patients' history, in 16 patients with SLE, and in 4 patients with SCLE. In 10 patients there were no clinical signs and symptoms suggestive of the presence of antiphospholipid antibodies. In 10 SLE patients past histories contained at least one such event. If ACA was present at least once it was evaluated as positive. There was no significant correlation between the clinical signs in the patients' histories and the presence or absence of ACA. (Chi square test: 0.95; p: 0.75).

3 patients with SCLE and 16 patients with SLE (5 actually had symptoms, while 14 did not) were evaluated. The results of the ACA ELISA and the presence of the actual clinical symptoms were compared (Table 3). There was no positive correlation between the presence of the actual clinical signs and the presence of ACA. (Chi square test: 0.011; p: 0.9).

Table 4. Comparison of anticardiolipin (ACA) antibodies and platelet count. No positive correlation: chi square test: 0.38; p: 0.5-0.7

	platelet <100	platelet 100-150	platelet >150	total
ACA+	2	30	32	64
ACA-	2	1	2	5
total	4	31	34	69

Comparison of the results of ACA ELISA and platelet count.

During the follow-up period platelet counts and ACA ELISA results were compared in 69 samples of patients with SLE. The thrombocyte count did not correlate with the amount of ACA (Table 4). Chi square test 0.38; p: 0,5-0.7.

DISCUSSION

ACA has been shown to occur more frequently in LE than in control persons: it was found in 10-60% of patients with SLE (5,9,10,11,12).

In our study we found ACA in 12,2% of the control study population, in 7,7% of patients with DLE, in 9,4% of patients with SCLE, and in 27,7% of patients with SLE.

It was suggested that antiphospholipid antibodies may play a role in the vascular thrombotic events, in the thrombocytopenia and in haemolytic anemia of SLE (13,14,15) and they were found to be associated with disease activity (16).

During a 5-year follow-up interval the level of ACA fluctuated in most of our patients with SLE and SCLE. There were only 6 out of the followed-up patients (3 patients with SLE, 3 patients with SCLE) in whom ACA was not detected during this period. In most of the patients the fluctuation in the level of ACA was mild, in 4 patients there was a substantial fluctuation. Fluctuations in the amount of IgG and IgM ACA were found in a larger patient group, therefore it was suggested that the patients' condition should be evaluated on the basis of repeated assays (16).

However, we did not find any direct correlation between the clinical symptoms - past history, present state - (arterial and venous thrombotic events, vasculitis, thrombocytopenia, haemolytic anemia) anti-ds DNA level or the presence of ACA.

Our results are in disagreement with the results of those who found correlation between the clinical signs and the level of ACA. Ninomiya, Vianna, and coworkers found correlation between the incidence of thrombosis, fetal loss, thrombocytopenia, and presence of ACA (12,14). Sebastiani and coworkers showed that thrombosis and abortion might be associated with ACA (15). Herranz and coworkers found correlation between ACA and epilepsy in SLE (17).

Love analyzed the published data on the significance of antiphospholipid antibodies in SLE, he found a significant association between the presence of these antibodies and a history of thrombosis, neurologic disorders, or thrombocytopenia (11). Asherson and coworkers found a strong association between the antiphospholipid antibodies and cerebrovascular occlusion in SLE patients (18).

However, some of the authors did not find correlation between the clinical signs and the presence of ACA, or at least only certain clinical symptoms correlated with ACA.

Abu-Shakra and coworkers showed that the presence of ACA was associated with prolonged aPTT, thrombocytopenia, and a positive Coombs test result, but not with any of the clinical symptoms of the antiphospholipid antibody syndrome on a large population of SLE patients (19). Golstein and coworkers did not find correlation between antiphospholipid antibodies and neurological thrombotic events (20), Sachse and coworkers showed that IgG ACA was associated with spontaneous abortion, thrombocytopenia, livedo reticularis, but not with thrombosis or central nervous system disorders (21). In a children population with SLE, ACA activity and thrombotic events did not correlate (22).

These results show that the association of ACA and the clinical symptoms of the antiphospholipid syndrome in SLE are controversial. The role of ACA in the thrombotic process of LE may be complex: the association may not be direct and several additional abnormalities or cofactors could be involved in thrombosis in these patients: beta 2 glukoprotein, protein C, protein S, autoantibodies to endothelial cell surface, antibodies to platelet activating factor, annexin V, or other type of antiphospholipid antibodies than ACA alone /antiphosphatidylserine/ may play a role (23,24).

CONCLUSION

In this study no significant correlation between ACA and anti-ds DNA antibody, between ACA ELISA and platelet count or ACA ELISA and thrombotic events has been detected in SLE patients. Such results may be explained by a different SLE patients' population, which is different from patients seen by internists or neurologists.

REFERENCES

1. *Moore JE, Mohr CF. Biologically false positive serologic tests for syphilis. JAMA 1952; 150: 467-73.*

2. *Alarcon-Segovia D, Sanchez-Guerrero J. Primary antiphospholipid syndrome. J Rheumatol 1989; 16: 482-8.*

3. *Harris EN. Antiphospholipid antibodies. Brit J Haematol 1990; 74: 1-9.*

4. *Johansson EA, Lassus A. The occurence of circulating anticoagulants in patients with syphilis and biologically false positive antilipoidal antibodies. Ann Clin Res 1974; 6: 105-8.*

5. *Merkel PA, Chang Y, Pierangeli SS, Convery K, Harris EN, Polisson RP. The prevalence and clinical associations of anticardiolipin antibodies with connective tissue diseases. Amer J Med 1996; 101: 576-83.*

6. *Loizou S, McCrea JD, Rudge AC, Reynolds R, Boyle CC, Harris EN. Measurement of anti-cardiolipin antibodies by an enzyme-linked immunosorbent assay (ELISA). Standardization and quantitation of results. Clin Exp Immunol 1985; 62: 738-45.*

7. *Harris EN, Gharavi AE, Patel SP, Hughes GRV. Evaluation of anticardiolipin test: report of an International Workshop held 4 April 1986. Clin Exp Immunol 1987; 68: 215-22.*

8. *Marschalkó M, Ablonczy É, Horváth A. Anticardiolipin ellenanyagok bõrgyõgyászati jelentõsége. Bõrgyógy Vener Szemle 1994; 70: 13-9.*

9. *Alarcon-Segovia D, Delezé M, Oria CV. Antiphospholipid antibodies and the antiphospholipid syndrome in systemic lupus erythematosus: a prospective analysis of 500 consecutive cases. Medicine 1989; 68: 353-65.*

10. *Loizou S, Cofiner C, Weetman AP, Walport MJ. Immunoglobulin class and IgG subclass distribution of anticardiolipin antibodies in patients with systemic lupus erythematosus and associated disorders. Clin Exp Immunol 1992; 90: 434-39.*

11. *Love PE, Santoro SA. Antiphospholipid antibodies: Anticardiolipin and the lupus anticoagulant in systemic lupus erythematosus /SLE/ and in non-SLE disorders. Prevalence and clinical significance. Ann Intern Med 1990; 112: 682-98.*

12. *Ninomya C, Taniguchi O, Kato T, Hirano T, Hashimoto H, Hirose S. Distribution and clinical significance of lupus anticoagulant and anticardiolipin antibody in 349 patients with systemic lupus erythermatosus. Intern Med 1992; 31: 194-9.*

13. *Harris EN. Anticardiolipin Wet Workshop Report. Fifth international symposium on antiphospholipid antibodies. Am J Clin Pathol 1994; 101: 616-22.*

14. *Vianna SL, Haga HJ, Tripathi P, Cervera R, Khamashta MA, Hughes GR. Reassessing the status of antiphospholipid syndrome in systemic lupus erythematosus. Ann Rheum Dis 1992; 51: 160-1.*

15. *Sebastiani GD, Passiu G, Galeazzi M, Porzio F, Carcassi U. Prevalence and clinical associations of anticardiolipin antibodies in systemic lupus erythematosus: a prospective study. Clin Rheumatol 1991; 10: 289-93.*

16. *Out HJ, Vliet M, Groot PG, Derksen RH. Prospective study of lupus anticoagulant activity and anticardiolipin antibody titre in patients with systemic lupus erythematosus. Ann Rheum Dis 1992; 51: 353-7.*

17. *Herranz MT, Rivier G, Khamashta MA, Blaser KU, Hughes GR. Association between antiphospholipid antibodies and epilepsy in patients with systemic lupus erythematosus. Arthritis Rheum 1994; 37: 568-71.*

18. *Asherson RA, Khamashta MA, Gil A, Vazquez JJ, Chan O, Baguley E, Hughes GRV. Cerebrovascular disease and antiphospholipid antibodies in systemic lupus erythematosus, lupus-like disease, and the primary antiphospholipid syndrome. Amer J Med 1989; 86: 391-9.*

19. *Abu-Shakra M, Gladman DD, Urowitz MB, Farewell V. Anticardiolipin antibodies in systemic lupus erythematosus: Clinical and laboratory correlations. Amer J Med 1995; 99: 624-8.*

20. *Golstein M, Meyer O, Bourgeois P, Palazzo E, Nicaise P, Labarre C, Kahn MF. Neurological manifesta-*

tions of systemic lupus erythematosus: role of antiphospholid antibodies. Clin Exp Rheumatol 1993; 11: 373-9.

21. Sachse C, Luthke K, Hartung K, Fricke M, Liedvogel B, Kalden JR, Peter HH. Significance of antibodies to cardiolipin in unselected patients with systemic lupus erythematosus: clinical and laboratory associations. The SLE study group. Rheumatol Int 1995; 15: 23-9.

22. Massengill SF, Hedrick C, Ayoub EM, Sleasman JW, Kao KJ. Antiphospholipid antibodies in pediatric lupus nephritis. Amer J Kidney Dis 1997; 29: 355-61.

23. Nahass GT. Antiphospholipid antibodies and the antiphospholipid antibody syndrome. J Amer Acad Dermatol 1997; 36: 149-68.

24. Rand JH, Wu X, Andree HAM, Lockwood CJ, Guller S, Scher J, Harpel PC. Pregnancy loss in the antiphospholipid-antibody syndrome-a possible thrombogenic mechanism. New Engl J Med 1997; 337: 154-61.

AUTHORS' ADDRESSES

Marta Marschalko MD, PhD, Assoc. professor of dermatology, Depatment of Dermatology, Semmelweiss Medical University, Maria u. 41, 1085 Budapest, Hungary
Agnes Ruzsovits MD, same address
Attila Horvath MD, PhD, Professor and chairman, same address