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DNA-ANTIBODIES IN SERA FROM PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

Erik Christiansen, Susanne Ullman and Poul Halberg

From the Department of Clinical Chemistry, Medical Department III, Kommunehospitalet, and the Department of Dermatology, Rigshospital, Copenhagen, Denmark

Abstract. DNA-antibody determinations using the Farr technique and LE cell tests were performed on single serum samples from 55 patients with SLE according to well defined criteria. It was found that the DNA-antibody test was just as sensitive as the LE cell test, and studies of sera from patients with questionable SLE, discoid LE, other connective-tissue diseases, psoriasis, and from healthy persons, indicated that the DNA-antibody test is highly specific for SLE. Neither the DNA-antibody test nor the LE cell test could discriminate between SLE patients with active and inactive disease as estimated by clinical criteria or by the serum concentrations of complement C3 and C4.

Key words: DNA-antibodies; Lupus erythematosus, systemic; Farr technique

In clinical practice the demonstration of nuclear antibodies (usually by the indirect Coon's technique as ANF) is followed by an analysis of the types of nuclear antibodies present. This is done to increase the specificity of the nuclear serology. Antibodies against desoxynucleoprotein (DNP) are still most commonly demonstrated by the LE cell technique. Although for several years a number of techniques have been available for demonstrating antibodies against native double-stranded DNA (11, 14) it is the Farr technique which (5) has mainly been used since its introduction in 1968 (15). Some publications have indicated that this test is highly specific for systemic lupus erythematosus (SLE) and also that it may offer an indication of desease activity (8, 10). To test the diagnostic value of single observations, regardless of later fluctuations, a number of patients with well defined SLE underwent DNA-antibody determinations and LE cell tests simultaneously at one point of the course. In addition, the two tests were correlated to disease activity as estimated by clinical criteria and by the serum concentrations of complement C_3 and C_4.

MATERIAL AND METHODS

A total of 230 sera samples from patients and control persons were included in the study. Fifty-five patients satisfied the criteria of one or several of the SLE definitions proposed by the American Rheumatism Association (ARA) (2), Hahn et al. (6) and Dubois (4).

Eleven patients were classified as having questionable SLE. These patients all had nuclear antibodies in their sera and all had symptoms and signs compatible with SLE, but the score was insufficient to satisfy any of the definitions mentioned.

The patients with SLE and questionable SLE were seen regularly in a dermatological and a medical out-patient clinic, approximately half of them in each clinic. A few patients were referred from other clinics. Seven patients had lupoid hepatitis as defined by Dubois (4). Two of these patients met the ARA criteria and Hahn et al. criteria for SLE and were included among the 55 patients with SLE. Five patients with lupoid hepatitis had insufficient scores to meet the criteria and were therefore classified as questionable SLE. Twenty-nine patients had rheumatoid arthritis (classical or definite according to ARA's criteria (12)), 16 had discoid LE as defined by Lever (9) and 41 had other connective-tissue diseases (generalised scleroderma, polyarteritis nodosa, dermatomyositis, Sjögren's syndrome). The controls consisted of 30 unselected blood donors, 22 healthy nurses, laboratory technicians and physicians, and 26 patients with psoriasis. The patient sera were collected during the introduction of the anti-DNA test in the management of patients with SLE. The anti-DNA and LE cell tests recorded in this study are determinations originating from the first occasion both tests were performed simultaneously in one patient, regardless of fluctuations before and after that time. Most of the patients had complement C3 and C4 determinations done at the same time.

The measurements of DNA binding activity in sera were performed by the Farr technique (15) using ^{14}C-labelled DNA supplied by the Radiochemical Centre, Amersham. The results (means of duplicate determinations) were recorded in arbitrary units. Readings of less than 20 were considered normal, 20–30 questionably elevated, and more than 30 definitely elevated. LE cell tests were performed by the method of Hammer (7), but only findings of more than 6 classical LE cells per 20 000 leucocytes have been recorded

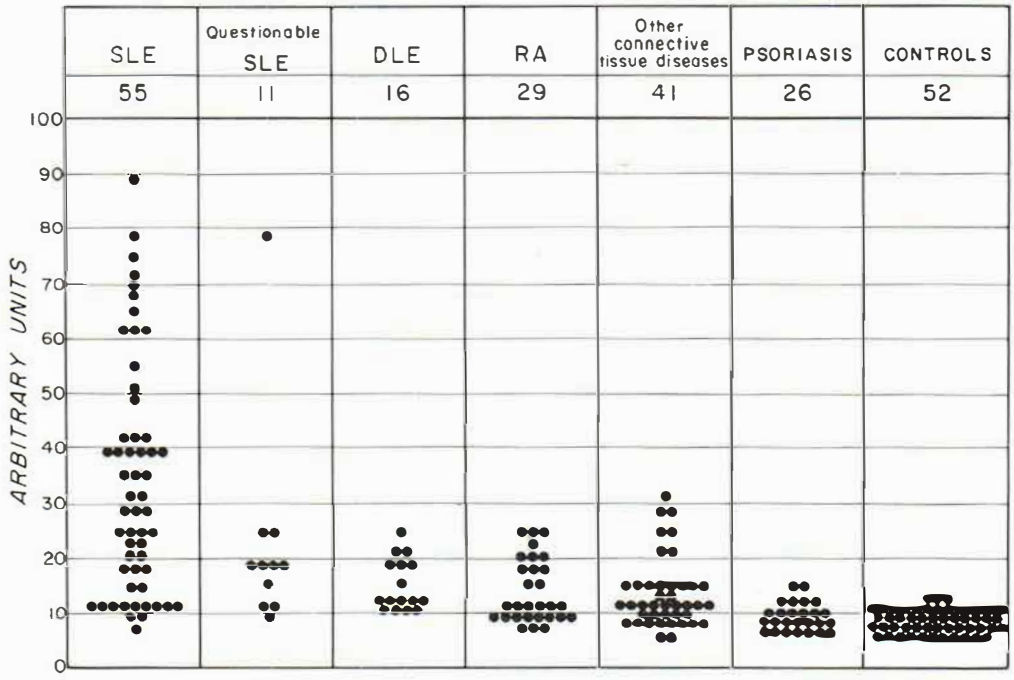

Fig. 1. DNA binding capacity in arbitrary units in 230 sera from patients and controls.

as positive in this study, extracellular material being disregarded. Seventy-eight per cent of the 55 SLE patients had two or more positive tests during the period of observation. Complements C3 and C4 were measured by an immune diffusion technique (1).

Disease activity was estimated according to the criteria of Schur & Sandson (13).

RESULTS

Fig. 1 shows the result of anti-DNA determinations in patients with SLE according to one or several criteria, questionable SLE, discoid LE, rheumatoid arthritis, a group of patients with connective-tissue diseases (generalised scleroderma, polyarteritis nodosa, dermatomyositis, Sjögren's syndrome), psoriasis and controls. Forty-nine per cent of the SLE patients and one of the 11 patients with questionable SLE had definitely elevated readings (more than 30). With the exception of one patient with scleroderma, all other patients and controls had readings of less than 30. One of the 7 patients with lupoid hepatitis had a very high value. This case has been classified as questionable SLE. The other 6 patients with lupoid hepatitis had normal or questionably elevated readings.

Table I shows the results of DNA binding and LE cell tests in patients with SLE according to the ARA, Hahn et al. and Dubois criteria, respectively.

The number of patients who met the various criteria varied (60, 73, and 95%, respectively), but in all three groups both definitely abnormal DNA binding capacity and positive LE cell tests were

Table 1. *Anti-DNA readings and LE cell tests in 55 patients with SLE defined according to three different sets of criteria*

Criteria	Number of patients	Anti-DNA readings ⩽20	20–30	>30	LE cells –	+
A.R.A.		11	6	16	20	13
	33	33%	18%	49%	61%	39%
Hahn et al.		10	7	23	20	20
	40	25%	18%	57%	50%	50%
Dubois		15	10	27	27	25
	52	29%	19%	52%	52%	48%
Patients meeting 3 sets of criteria		6	4	16	12	14
	26	23%	15%	62%	46%	54%
Patients meeting 1 or 2 sets of criteria		11	7	11	18	11
	29	38%	24%	38%	62%	38%

Table II. *Comparison between LE cell test and DNA binding capacity in 55 patients with SLE*

	Positive LE cell test	Negative LE cell test
DNA binding >30	20	7
DNA binding <30	5	23

found in approximately 50%, except for a slightly higher percentage of abnormal DNA binding (57%) in those patients classified according to Hahn et al., and a slightly lower percentage of positive LE cell tests (39%) in the patients classified according to ARA. Forty-seven per cent of patients met all three sets of criteria. In this group, the percentages of definitely abnormal anti-DNA readings and positive LE cells tests were higher (62 and 54%, respectively) than in the remaining patients (38 and 38%, respectively).

Table II shows that the results of the DNA binding capacity test and LE cell test were similar in 43 of 55 (78%) patients with SLE. Seven of 55 patients (13%) had abnormal DNA binding capacity but a negative LE cell test and 5 of 55 patients (9%) had normal or questionably elevated DNA binding capacity but a positive LE cell test.

The DNA binding capacity was abnormal in 49%, the LE cell test in 45%, one or both tests in 58% of the patients with SLE.

The results, set out in Table III, show that neither the measurement of DNA binding capacity, nor the LE cell test could distinguish between patients with clinically active vs. clinically inactive SLE, nor between patients with subnormal vs. normal complement C3 and C4.

DISCUSSION

The aim of the present study was to elucidate the diagnostic value of DNA-antibody determination by means of the Farr technique in patients with SLE, and to compare it with the LE cell test. To avoid the influence of the patients' varying times of observation, only one single observation (the first) for each patient was included in the study. We found the sensitivities of the two tests to be equal. In 44 patients with SLE, Pincus et al. (10) found DNA binding capacity readings of more than 30 in 50% of the patients, which result is similar to our findings. In 185 sera from 86 patients with SLE, Hughes (8) found a DNA binding of more than 30 in 83%. However, the criteria were not stated and they may well have been more selective than those used in the present study. Davis et al. (3) found readings of more than 30 in 36 of 38 patients, 33 of whom met the criteria of the ARA. The results, however, were not necessarily limited to a single test for each patient.

Even though the number of patients who met the three sets of criteria applied in this study varied, the percentages of patients with abnormal DNA binding (and with positive LE cell tests) were practically identical. A slightly increased percentage of abnormal anti-DNA tests in the group classified according to Hahn et al. is explained by the fact that contrary to the ARA and Dubois' criteria, Hahn et al.'s criteria include the presence of a DNA-antibody. In a highly selected group of SLE patients who met all 3 sets of criteria, 62% of the patients had definitely abnormal DNA binding. SLE patients who met only one or two sets of criteria had abnormal DNA binding in 38% and only one of 11 patients with questionable SLE had an abnormal

Table III. *Abnormal and normal DNA binding capacity, positive and negative LE cell tests correlated to clinical disease activity and abnormal and normal complement C3 and C4 concentrations in serum*

	Clinically active SLE	Clinically inactive SLE	Subnormal C3	Normal C3	Subnormal C4	Normal C4
No. of patients	33	22	17	37	22	28
DNA binding >30	20	7	9	18	10	15
	36%	13%	17%	33%	20%	30%
DNA binding <30	13	15	8	19	12	13
	24%	27%	15%	35%	24%	26%
Pos. LE cell test	19	6	8	17	9	14
	35%	11%	15%	31%	18%	28%
Neg. LE cell test	14	16	9	20	13	14
	27%	29%	17%	37%	26%	28%

reading. This suggests that the test is specific and that the use of strict criteria is meaningful.

Only one of 148 sera samples from control persons, patients with various connective-tissue diseases, and psoriasis, had an abnormal DNA binding, which seems to indicate that the test is highly specific.

In 22% of the patients with SLE, the results of the DNA binding and the LE cell tests were dissimilar, i.e. the use of two instead of one test increases the possibility of finding serological evidence for the diagnosis of SLE.

It has been suggested that DNA-antibodies, unlike the DNP-antibodies, might be present preferentially in patients with active disease (8, 13). In this study neither the DNA binding capacity nor the LE cell test could discriminate between patients with active and inactive disease as estimated by clinical criteria or by the serum concentrations of complement C3 or complement C4. As a diagnostic test the DNA binding capacity seems to be as sensitive and as specific as the LE cell test. Using both tests at the same time may add some (but not very much) practical information. When choosing one of the two tests in clinical practice it should be taken into consideration that DNA binding is less time-consuming and easier to read than the LE cell test.

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S. Ullman, M.D.
Department of Dermatology
Rigshospitalet
Blegdamsvej 9
DK-2100 Copenhagen
Denmark