Clin. exp. Immunol. (1975) **22,** 9–15

THE VALUE OF TESTS FOR ANTIBODIES TO DNA IN MONITORING THE CLINICAL COURSE OF SLE

A LONG-TERM STUDY USING THE FARR TEST AND THE DNA COUNTERIMMUNOELECTROPHORETIC METHOD

J. P. EDMONDS, G. D. JOHNSON, B. M. ANSELL AND E. J. HOLBOROW

MRC Rheumatism Unit, Canadian Red Cross Memorial Hospital, Taplow

(*Received* 3 *February* 1975)

SUMMARY

Serial serum samples from fifteen patients with SLE, taken over periods varying from 6 months to 6 years, were tested for DNA binding capacity, DNA electroprecipitins (DNA–EP) and C'3 level to assess the value of these investigations in reflecting clinical disease activity. Patients with renal involvement showed a good correlation between high levels of DNA binding, low serum C'3 and disease activity and typically, their DNA–EP was negative. By contrast, patients without renal involvement in whom vasculitis was prominent, showed a poor correlation of DNA binding capacity to changes in the state of their disease although the DNA–EP test was persistently positive. It was also apparent that both the DNA–BC and C'3 can show marked variation in response to alterations in treatment without accompanying clinical change.

Although these serological tests, particularly the DNA binding capacity, are of recognized value in the diagnosis of SLE, they serve most usefully as guides to long-term management when they can be related to the clinical pattern of the disease.

INTRODUCTION

Although the clinical expression of SLE is known for its variability, one unifying feature in this disorder is the presence of antibodies directed against native DNA (Robbins *et al.*, 1957; Seligmann, 1957; Miescher & Strassle, 1957; Ceppellini, Polli & Celada, 1957), which appear to be almost specific for this condition and consequently of diagnostic value. Several methods for the detection of anti-DNA antibodies have been described and include gel diffusion (Tan *et al.*, 1966) complement fixation (Robbins *et al.*, 1957) haemagglutination (Jokinen & Julkunen, 1965) and radioimmunoassay. For its sensitivity and specificity, the Farr test (Farr, 1958; Wold *et al.*, 1968) which measures the primary binding capacity of serum for DNA, is now the method most widely used.

Correspondence: Dr J. P. Edmonds, Department of Medicine, Royal Postgraduate Medical School, Ducane Road, London W12 0HS.

We have recently described a counterimmunoelectrophoretic technique (Johnson, Edmonds & Holborow, 1973) modified after Davis (1971) for the detection of precipitating antibodies to DNA. In our group of patients with SLE, a strong clinical correlation was found between the presence of cutaneous vasculitis and a positive DNA precipitin test while a negative test was generally found in patients with renal disease.

The diagnostic value of a positive Farr test is now well recognized (Hughes, 1973), and there is evidence that, in general, high titres of anti-DNA antibody are found in active disease (Schur & Sandson, 1968), but there is less information on how accurately a fluctuating antibody level correlates with disease activity and to what extent this can be used as a therapeutic guide (Hughes, Cohen & Christian, 1971; Lightfoot, 1972).

We have examined serial measurements of DNA binding capacity, C'3 level and the DNA electroprecipitin (DNA–EP) test to assess their value in reflecting clinical disease activity. The results indicate the occurrence of patterns of disease, within the clinical spectrum of SLE, with distinguishing immunological features.

PATIENTS AND METHODS

Fifteen female patients with SLE were studied. All fulfilled the ARA criteria for SLE (Cohen & Canoso, 1972) and had been under the care of our unit for periods varying from 6 months to 6 years. Their clinical course and treatment was followed during the 2 years of this study and retrospectively where necessary. Between four and forty-eight serial serum samples, stored in our serum bank at −20°C, were available from each patient. Samples were tested for DNA binding capacity, serum C'3 level, and precipitating antibody to DNA.

The DNA binding capacity was measured by the Farr test using ammonium sulphate precipitation and DNA was labelled with tritium by the method of Carr *et al.* (1969). Based on probit analysis at the 95% confidence level, the normal upper limit was 10% binding.

The serum C'3 was measured by a commercially available radioimmunodiffusion method (Immuno-plate, Hyland Labs), the suggested lower normal level being 120 mg% (Shanbrom, Khoo & Lou, 1967).

Precipitating antibody to DNA was detected by two-stage electroimmunodiffusion in agarose with electrophoretic separation of the serum for 45 min before the DNA was added and the electrophoresis resumed for a further 15 min (Johnson *et al.*, 1973). A positive result was taken to be the appearance of a precipitin line, as shown in Fig. 1, at the end of the electrophoresis.

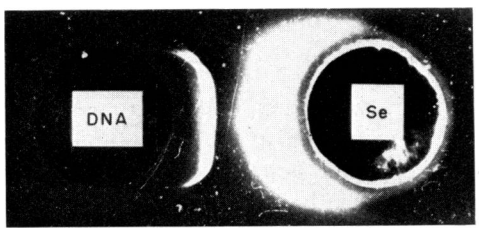

FIGURE 1. In DNA–EP-positive sera, initial electrophoretic separation of serum (Se) in the anodal well with subsequent electrophoresis of DNA in the cathodal well, gives a clear precipitin line around the antigen well, easily distinguished from non-specific precipitation around the serum well.

In attempting to correlate clinical activity with serial levels of DNA binding capacity and serum C'3, a chart, such as that shown in Fig. 2, was constructed for each patient. At the top are shown the major clinical episodes. Below is the serial level of serum C'3, a line being drawn at 120 mg%, the suggested lower normal limit. Below that is the serial DNA binding capacity, a line being drawn at 10% binding capacity, the upper normal limit by our method. At the bottom, the dosage of principal drugs is charted. The result of serial precipitating anti-DNA antibody tests are shown as DNA–EP+ (persistently positive), DNA–EP− (persistently negative), or DNA–EP± (variable), as in the study shown in Fig. 2.

TABLE 1. Clinical features in fifteen cases of SLE related to the occurrence of DNA electroprecipitating antibody

	Total	Persistently positive	Persistently negative	Variable
Number of patients	15	5	6	4
Nephropathy	9	0	6	3
Cutaneous vasculitis	9	5	1	3

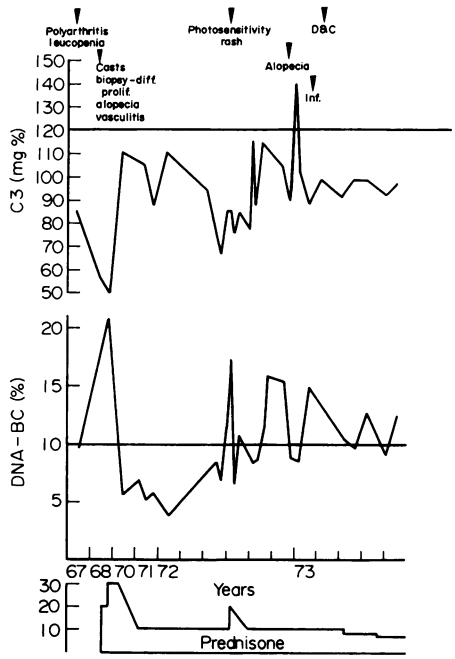

FIG. 2

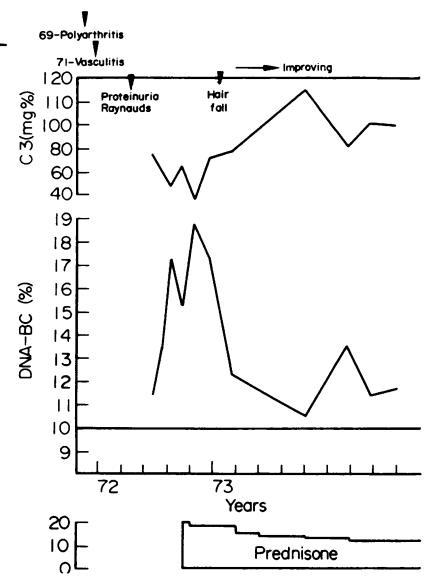

FIG. 3

FIGURE 2. Patient P.E. presented with arthritis and leucopenia in 1967 and later developed cutaneous vasculitis, alopecia and biopsy-proven renal involvement. She showed good correlation of clinical activity to serological abnormality with a rise in DNA binding capacity and fall in C'3 during episodes of photosensitivity rash, alopecia and infection. Mild proteinuria without deterioration in renal function. The DNA–EP test was intermittently positive (DNA–EP±).

FIGURE 3. Patient J.C. presented with arthritis and mild cutaneous vasculitis. Renal biopsy for proteinuria showed focal proliferative nephritis. This was the only patient who had a persistently negative DNA–EP and cutaneous vasculitis. She showed a good correlation of clinical and serological activity. The peak of DNA binding capacity and fall in C'3 in mid-1973 was associated with a flare of arthritis without clinical renal deterioration.

RESULTS

DNA precipitin test

We have previously published the results of this test (Johnson *et al.*, 1973) which are summarized in Table 1. Six patients had a persistently negative result. All this group had clinical renal disease with either a positive renal biopsy and/or proteinuria of over 3·5 g daily and/or urinary casts. One of the six had mild cutaneous vasculitis (patient J.C., Fig. 3), manifest as nail fold lesions. Five patients had a persistently positive result; none of these had evidence of renal disease and the one renal biopsy performed in this group was normal on light microscopy. By contrast, all had cutaneous vasculitis. Four patients had a variable result—positive on some occasions and negative on others. In this group, four had clinical

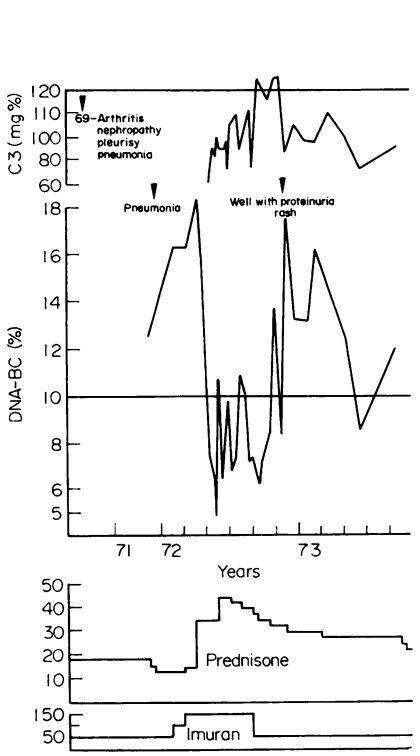

FIG. 4

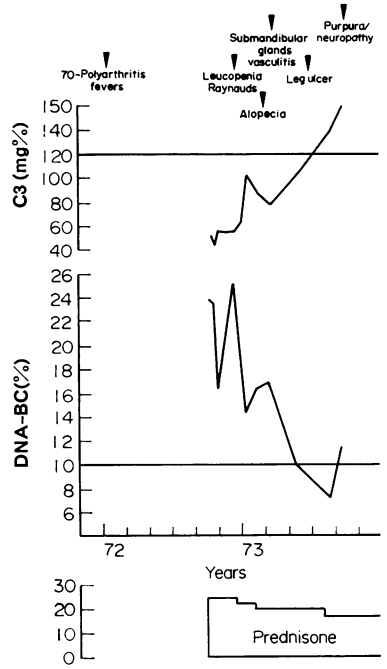

FIG. 5

FIGURE 4. Patient W.S. This patient had a biopsy proven focal proliferative nephritis and a persistently negative DNA–EP. Throughout 1972 she remained generally well but with some arthritis, a mild skin rash and proteinuria of 1–2 g daily. Although the dose of prednisone and azathioprine were increased to control these disease manifestations there was no change in her clinical state despite temporary suppression of the DNA–BC to normal levels.

FIGURE 5. Patient J.S. Although this patient showed persistent polyarthritis, her disease course has been dominated by vasculitis with skin lesions and neuropathy. There has never been clinical evidence of renal involvement and the DNA–EP has always been positive. She showed a poor correlation of clinical activity to serological abnormality with new vasculitic episodes occurring when the DNA–BC was low or normal.

renal disease, and three had cutaneous vasculitis. There was a correlation, therefore, between a persistently positive test and cutaneous vasculitis and between a persistently negative test and nephropathy with an overlap group showing combined features.

Clinical disease activity and serial levels of DNA–BC and serum C'3

It is not possible to offer a statistical analysis of these results because of the variability of the patterns seen and because continuous lines drawn between the serial levels of DNA binding capacity and serum C'3 on samples taken at varying time intervals, are of limited validity. Within these limitations, three patterns were seen.

The first pattern (examples of which are shown in Figs 2 and 3) was of generally good correlation of disease activity to DNA binding capacity and serum C'3. This pattern was found in one of the four patients with renal involvement who also had vasculitis and a variably positive DNA precipitin test (patient P.E., Fig. 2) and in four of the six patients with renal involvement who had a persistently negative DNA precipitin test (example, patient J.C., Fig. 3).

The second pattern shown in Fig. 4 was seen in two of the six patients with renal disease and a persistently negative precipitin test (example, patient W.S., Fig. 4) and in one of the four patients with renal disease, vasculitis and a variably positive precipitin test. The notable feature of these three patients was a marked alteration in DNA binding capacity, apparently more in response to changes in treatment than to clinical activity.

The third pattern shown in Fig. 5 was seen in all five of the group who did not have nephropathy but showed prominent cutaneous vasculitis and had a persistently positive precipitin test. This group showed poor correlation of DNA binding capacity to disease state.

DISCUSSION

The results of this study show that clinical disease activity in SLE does not always correlate accurately with a rise or fall in DNA binding capacity or serum C'3 levels. Of more clinical significance is the finding that the patients showed an incomplete separation into two main groups. Since several patients formed a borderline group, it must be stressed that this division reflects a trend which is useful in interpreting these serological tests in patients with SLE and possibly in affording some insight into the pathogenetic mechanisms involved.

The best correlation of serial levels of DNA binding capacity and serum C'3 to disease activity was seen in the patients with clinical renal involvement. This was also the group in which the DNA precipitin test was persistently negative. While these patients usually had high DNA binding capacity and low serum C'3 during periods of active disease, the manifestations of activity were not necessarily renal but included involvement of other systems.

The poorest correlation of DNA binding capacity and serum C'3 to disease activity was seen in patients without clinical renal involvement in whom cutaneous vasculitis was prominent and whose DNA precipitin test was persistently positive.

Studies on four patients showed that alterations in the dose of prednisone and azathioprine can affect both Farr binding and serum C'3 without apparent clinical changes. Since this was seen only in patients with renal involvement, it is possible that our methods of clinical assessment were not detecting continued or progressive renal damage occurring with rising levels of DNA binding capacity particularly when accompanied by a falling C'3 level.

We agree with previous studies (Schur & Sandson, 1968; Hughes *et al.*, 1971; Koffler *et al.*,

1969), which found that high levels of anti-DNA activity and low serum complement are usually associated with active disease. The reverse, however, is not always true and some patients, particularly those with vasculitis as a prominent feature, can remain active despite only slightly elevated or even normal DNA binding capacity. It was in some serum samples from this group of patients that we found a positive DNA precipitin test with a normal DNA binding capacity. One explanation of this unexpected result is that although these sera lack free anti-DNA antibody, they contain DNA–anti-DNA complexes, which dissociate under the condition of electrophoresis, releasing antibody to react positively in the DNA–EP test.

Lightfoot (1972) reported that a high or rising DNA binding capacity was eventually associated with a flare of disease in over 80% of patients but there was sometimes a long period between a rise in anti-DNA activity and the onset of a clinical exacerbation and in that situation, the Farr test could not be used as an accurate monitor of disease. Schur & Sandson (1968) found that the combination of very low complement levels and high titres of antibody to DNA was always associated with active renal disease. We have seen low complement levels with high DNA binding capacity in patients with renal involvement during periods of activity in systems other than the kidney, and low complement levels without an accompanying rise in DNA binding capacity in patients without renal disease during episodes of active vasculitis, suggesting that antigen–antibody systems, other than DNA–anti-DNA, are involved in the pathogenesis of these lesions.

The two main groups of SLE patients, those with and those without renal involvement, showed correlation both with a pattern of serial DNA binding capacity and serum complement levels, and with either persistently negative and/or persistently positive DNA–EP test. The latter is detecting a group of precipitating antibodies whose precipitating properties may be a reflection of their higher avidity. The importance of antibody affinity in determining the clinical result of circulating immune complexes has been suggested by Soothill & Steward (1971) who showed that inbred strains of mice, prone to nephritis following neonatal LCM virus infection, produced antibody of lower affinity to soluble antigens than did nephritis-resistant strains. The situation in SLE may be similar. Although as yet unconfirmed, Steward *et al.* (1974) have shown that in SLE patients with renal disease, the avidity of anti-DNA antibodies is lower than in patients without renal involvement.

There is now good evidence that antigen–antibody complexes involving native DNA are important in the pathogenesis of renal lupus (Koffler *et al.*, 1971). Our finding of a good correlation between serial levels of DNA binding capacity and clinical activity in patients with nephropathy suggest that this antigen–antibody system is of predominant importance in this group. If, as we have suggested, the precipitin test is detecting antibody of high avidity for DNA, the persistent positivity of this test in the nephritis-free patients may indicate that, by virtue of strong antigen–antibody binding and the consequent rapid clearance of such large complexes, the DNA–anti-DNA complexes are relatively benign in these patients. Immune complexes, involving antigens other than native DNA may be of more importance in the pathogenesis of non-renal disease, explaining the poor correlation of clinical events to serial DNA binding capacity in patients with prominent vasculitis. Although the native DNA–antinative DNA system appears to be the one most specific for SLE, it is likely that other nuclear autoantibodies also play a part in immune complex formation and the variability in the clinical expressions of SLE may eventually be explained both by differences in the antigenic specificity of the complexes and by the qualitative properties of the antibodies.

From a clinical standpoint, the DNA binding capacity is of recognized value in the diagnosis of SLE but in established disease appears to be more reliable in reflecting activity in patients with renal involvement than in those without. Depression of the serum C'3 level is also a useful monitor of active disease but in our experience is not necessarily indicative of clinically detectable renal involvement. The results of this study indicate that these serological tests are of most help in monitoring SLE, and consequently in guiding therapy, where they can be related to the clinical pattern of the illness.

The authors would like to thank Professor Bywaters and Dr Gumpel for allowing us to study patients under their care, Mrs Ella Stephenson for technical assistance with serum bank samples, and Mrs J. Andrews for secretarial help.

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