Frequency and clinical significance of antibodies to ribonucleoprotein in SLE and other connective tissue disease subgroups

D. M. GRENNAN, C. BUNN, G. R. V. HUGHES, W. W. BUCHANAN, AND W. C. DICK

From the Centre for Rheumatic Diseases and University Department of Medicine, Glasgow Royal Infirmary, and the Rheumatology Department, Royal Postgraduate Medical School, London

SUMMARY Antibodies to the ribonucleoprotein (RNP) component of extractable nuclear antigen were measured in patients with systemic lupus erythematosus (SLE) and other connective tissue subgroups by counterimmunoelectrophoresis. Antibodies to RNP were found in the sera of 32% of patients with a primary diagnosis of SLE, 29% of patients with features of SLE and erosive joint disease, none of 9 scleroderma patients, and in 75% of 8 patients with features of SLE and scleroderma. In the SLE patients overall there was an increased frequency of sclerodactyly and severe Raynaud's phenomenon in the patients with antibodies to RNP but no association of antibodies to RNP was found with the presence of erosive joint disease, Sjögren's syndrome, or the absence of renal disease in these patients.

Antibodies to ribonucleoprotein (RNP) have been shown hitherto in the sera of patients with a number of connective tissue diseases. The presence of high titres of antibodies to the RNP component of a saline extractable nuclear antigen (ENA) has been said to characterize a syndrome with distinctive clinical features and prognosis and given the name of 'mixed connective tissue disease' by Sharp *et al.* (1972). Antibodies to RNP have also been shown in the sera of patients with systemic lupus erythematosus (SLE) and scleroderma in varying frequencies in different series and have been said to be associated with atypical features in these diseases (Parker, 1973; Kurata and Tan, 1976). Most studies into the significance of these antibodies were carried out in the American continent. In our study we examined the frequency and clinical significance of these antibodies to RNP in patients with a primary diagnosis of SLE and of scleroderma, and in patients with features of SLE associated with an erosive arthritis or features of scleroderma. A counterimmunoelectrophoretic method was used to detect antibodies to RNP which has been shown

to be a sensitive and rapid technique for detecting antibodies to different antigens (Kurata and Tan, 1976).

Patients

The patients were categorized clinically before the results of the tests for RNP antibodies were known.

Sera of 63 patients with a primary diagnosis of SLE were examined. All but 2 of these satisfied three or more of the preliminary criteria of the American Rheumatism Association (ARA) (Cohen *et al.*, 1971) for a diagnosis of SLE excluding the criterion related to the presence of LE cells. In addition they had positive antinuclear antibody tests at a titre of 1/32 or higher on serological testing using a standard immunofluorescent technique (Beck, 1961). The remaining 2 patients had antinuclear antibodies and antibodies to native DNA as shown by the Farr test using *E. coli* native DNA as test antigen (Hughes, 1971). Both were young women and one had polyarthritis in association with glomerulonephritis shown on renal biopsy as a focal proliferative glomerulonephritis with deposition of immunoglobulin and complement on capillary loops. The other had a history of recurrent mild polyarthritis, fever, and truncal and facial rashes. In this group of 63 SLE patients were 5

patients with an erosive arthritis, and in 4 of the 5 rheumatoid-like irreversible deformities in the hands. Serological testing in all these patients showed the presence of antinative DNA antibodies. Another 6 of the SLE patients had sclerodactyly, 38 had Raynaud's phenomenon, 5 severe Raynaud's phenomenon, 25 had clinical evidence of renal involvement, and 13 had Sjögren's syndrome (see below). The frequency of these different clinical features was compared in the SLE patients with and without antibodies to RNP on serological testing (Table 1).

The frequency of positive serological tests for RNP antibodies in the 63 SLE patients was compared with their frequencies in 9 scleroderma patients, in 8 patients with features of both SLE and scleroderma, in 7 patients with features of SLE and erosive arthritis, and in 3 patients with features of polymyositis and scleroderma (Table 2). 6 of the 9 scleroderma patients had typical gross generalized cutaneous involvement, and either gastrointestinal, cardiac, or pulmonary involvement. The other 3 had sclerodactyly in association with the calcinosis, Raynaud's phenomenon, and telangiectasia of the 'CRST' syndrome, and oesophageal involvement in one. None of the patients in this group had clinical features other than polyarthritis suggestive of SLE and none an acute febrile onset of their illness. Serological tests showed antinuclear factor positive at a titre of 1/64 or more in 7 of the 9 patients.

Eight patients were included in the group with features of both SLE and scleroderma (Table 3). 6 had a primary diagnosis of SLE as discussed previously and were included in the group of 63

SLE patients. In addition, 2 others not included in the SLE group were included in this group (Cases 1 and 4). Illness in Case 1 developed acutely with polyarthritis, fever, and malaise which responded to treatment with corticosteroids, and was associated with a high-titre positive antinuclear factor suggesting a diagnosis of SLE. She was also noted to have sclerodactyly which has worsened despite improvement of the other features after corticosteroid therapy. Illness in Case 4 began with the polyarthritis, facial rash, and photosensitivity typical of SLE but she subsequently developed cutaneous tightening and tethering of face, hands, and forearms, suggestive of scleroderma.

The clinical features of the 7 patients with features of both SLE and an erosive arthritis are summarized in Table 4. The first 5 were all thought to have a primary diagnosis of SLE as discussed above and all were positive for antinative DNA antibodies on serological testing. They are included in the 63 patients with SLE. Neither of the last 2 patients was included in the group of 63 SLE patients although one had antinative DNA antibodies on serological testing while the other (Case 15) had a high-titre positive antinuclear factor compared with a relatively low-titre positive rheumatoid factor.

Sera were also examined from 3 patients with features of polymyositis with typical proximal muscle involvement, raised serum muscle enzyme, and muscle biopsy changes in association with features of scleroderma. The latter were oesophageal dilatation and absence of peristalsis particularly in the distal oesophagus, and tightness of facial skin around nose and mouth in all 3; and small bowel dilatation in 1. All 3 had Raynaud's phenomenon.

Table 1 Frequency (%) of different clinical features in SLE patients with and without antibodies to ribonucleoprotein (RNP) (no. positive/no. tested in parentheses)

	RNP+	RNP-
SLE+sclerodactyly	25 (5/20)	2 (1/43)*
SLE+erosive arthritis	5 (1/20)	9 (4/43)
SLE+severe Raynaud's	25 (5/20)	0 (0/43)*
SLE+Raynaud's	65 (13/20)	58 (25/43)
Renal SLE	35 (7/20)	42 (18/43)
SLE+Sjögren's syndrome	30 (6/20)	16 (7/43)

*Differences statistically significant.

Table 2 Frequency of antibodies to RNP in various disease groups

	No. tested	No. positive	%
SLE	63	20	32
Scleroderma	9	0	0
Features of SLE+scleroderma	8	6	75
Polymyositis/scleroderma	3	2	67
Features of SLE+erosive arthritis	7	2	29

See text for explanation of various disease groups.

SJÖGREN'S SYNDROME

The presence or absence of clinical Sjögren's syndrome was documented in all patients, and was diagnosed if either keratoconjunctivitis sicca (KCS) or xerostomia was present. KCS was diagnosed using standard criteria (Whaley *et al.*, 1973) in the presence of subnormal Schirmer II tear test and punctate or filamentary keratitis seen after instillation of Rose-bengal into the conjunctival sac and with the aid of a slit lamp. Xerostomia due to Sjögren's syndrome was diagnosed in the presence of xerostomia on clinical examination, subnormal salivary flow rates for the age of the patient, and histological abnormalities greater than grade I on salivary labial gland biopsy (Whaley *et al.*, 1973).

Methods

COUNTERIMMUNOELECTROPHORESIS

ENA was prepared from fresh calf thymus nuclei

Table 3 Patients with features of both SLE and scleroderma

Case no.	Sex	Age at onset (years)	Disease duration (years)	SLE features	Scleroderma features	Myositis	Raynaud's phenomenon	Renal involvement	KCS	Xerostomia	ANF	RF	DNAB (%)
1	F	31	1	Polyarthritis	Sclerodactyly	+	++	-	-	-	1000	Neg	9
2	F	42	6	Polyarthritis; alopecia; pleurisy	Sclerodactyly; pinched mouth	+	++	+	-	-	1000	32	81
3	F	20	3	Polyarthritis; nephritis	Sclerodactyly	+	+	+	-	-	1000	Neg	97
4	F	27	17	Polyarthritis; malar rash; photosensitivity	Tight skin facial area, hands, and forearms; telangiectasia	-	++	-	-	-	1000	32	0
5	F	32	8	Polyarthritis; malar rash; photosensitivity; alopecia; epilepsy	Sclerodactyly; dysphagia	-	++	-	-	-	256	16	33
6	F	17	2	Polyarthritis; malar rash; alopecia	Sclerodactyly	-	+	-	+	-	256	Neg	20
7	F	49	3	Polyarthritis; pleurisy	Sclerodactyly	-	+	-	-	-	1000	128	58
8	F	29	3	Polyarthritis; thrombocytopenia; nephritis	Sclerodactyly; tight facial skin; vitiligo	-	+	+	-	-	1000	Neg	14

KCS = keratoconjunctivitis sicca; RF = rheumatoid factor; ANF = antinuclear factor; DNAB = % DNA binding.
 + = present; ++ = severe; H = homogeneous; Sp = speckled; N = nucleolar

Table 4 Clinical features of patients with features of SLE and an erosive arthritis (1/titre ANF and RF shown)

Case no.	Sex	Age at onset (years)	Disease duration (years)	No ARA criteria for SLE	SLE disease features	Raynaud's phenomenon	Other	KCS	Xero-stomia	ANF	RF	DNAB (%)
9	F	43	11	3	Malar rash; pericarditis; Raynaud's	+	—	+	+	1000 (H)	256	74
10	F	17	12	3	Malar rash; Raynaud's; photosensitivity;	+	—	—	—	1000 (H)	32	80
11	F	42	3	4	Raynaud's; pleurisy; leucopenia; Raynaud's	+	±	+	+	1000 (H)	512	58
12	F	31	12	4	Malar rash; photosensitivity; alopecia;	+	—	+	—	1000 (H)	1024	47
13	F	52	3	4	Raynaud's; Alopecia; pericarditis; leucopenia;	+	Hypo-thyroid	—	+	1000 (H)	Neg	70
14	F	24	10	2	Raynaud's; Alopecia;	+	—	+	+	1000 (H)	2048	64
15	F	15	11	2	Raynaud's; Pericarditis; Raynaud's	++	—	—	—	1000 (H)	32	7

by the method of Sharp *et al.* (1972). The concentration of protein in the extract used was 5 mg/ml. All sera were heated for 30 minutes at 56°C before counterimmunoelectrophoresis.

Agarose (Indubiose) was prepared as a 1% solution in 0.025 M barbital buffer, pH 8.4. Plates were alcohol-cleaned and the agarose pipetted on to the slide. After this had solidified a set of wells were cut in 3 parallel rows, with 2 wells in each row. The wells in the left column were filled with antigen extract and the wells in the right with sera. The left column was used as the cathodal set and the right the anodal.

The sera were placed in their wells and electrophoresis started in 0.05 M barbital buffer, pH 8.4, using a current of 26 mA. After 45 minutes the antigen wells were filled and electrophoresis continued for another 30 minutes. Slides were examined for precipitin lines immediately after the run and these were recorded. The slides were left in a moist chamber overnight and re-examined for precipitin lines.

Sera with positive precipitin lines were retested after treatment of the antigen with ribonuclease (Koch-light) in a ratio of enzyme to substrate by weight of 15:1. Thus positive precipitin lines were recorded as RNP or as other if they were not destroyed by treatment of the antigen with ribonuclease. All clinical classifications were carried out before the results of precipitin tests were known.

DNA ANTIBODIES

Antinative DNA antibodies were measured by a DNA binding technique (Hughes, 1971). Labelled *E. coli* native DNA was used as the test antigen and results were expressed as % DNA binding, the normal range being 0–30%.

RHEUMATOID FACTOR

Rheumatoid factor was estimated using latex particles coated with bovine gammaglobulin (Denver Laboratories).

STATISTICS

Statistical significances where mentioned were analysed using the χ^2 test with Yates's correction.

Results

Table 2 shows the frequency of antibodies to RNP in the sera of the different connective tissue groups. 32% of the 63 SLE patients overall and 29% of SLE patients with erosive arthritis had antibodies to RNP, while no antibodies were detected in the sera of the 9 scleroderma patients. 6 of the 8 patients with features of both SLE and scleroderma and 2 of

the 3 with features of both polymyositis and scleroderma had antibodies to RNP.

The frequency of various clinical features in the 63 SLE patients with and without antibodies to RNP are given in Table 1. The frequency of erosive joint disease, Raynaud's phenomenon, and renal disease in the RNP positive and negative patients was similar. 5 patients, however, had particularly severe Raynaud's phenomenon in both summer and winter and all 5 were noted to have antibodies to RNP, whereas none of the 43 SLE patients without antibodies to RNP had severe Raynaud's phenomenon ($P < 0.01$). Similarly, 25% of the 20 patients with antibodies to RNP had sclerodactyly, whereas only 2% (one patient) of the 43 patients without antibodies to RNP were noted to have sclerodactyly ($P < 0.05$). Clinical features of Sjögren's syndrome were noted in 13 of the SLE patients overall: in 6 of the 20 (30%) patients with antibodies to RNP, and in 7 of the 43 (16%) patients without antibodies to RNP. These differences were not significant ($P > 0.05$).

Discussion

Like Kurata and Tan (1976) we found counterimmunoelectrophoresis a convenient method of measuring antibodies to RNP. Previous studies with this technique have not detected antibodies to RNP in sera from normal populations (Kurata and Tan, 1976). 46% of the 50 SLE patients investigated by Kurata and Tan using this method were found to have antibodies to RNP, which is comparable to the 32% positive of SLE patients in this study. However, Kurata and Tan (1976) also found antibodies to RNP in the sera of 31% of scleroderma patients, while the sera of all 9 of our scleroderma patients were negative. In our study a striking feature was the high frequency of antibodies to RNP in the sera of patients with features of both SLE and scleroderma and both polymyositis and scleroderma, which was in contrast to absent or low frequencies of antibodies in the other disease groups. Some of our patients seem to be similar to those described by Sharp *et al.* as having the 'MCTD' syndrome, although one of our patients with antibodies to RNP has active renal disease and another has epilepsy so that major organ involvement was seen in this group of patients.

In our patients with a primary diagnosis of SLE, antibodies to RNP appeared to be associated with atypical disease features such as sclerodactyly or severe Raynaud's phenomenon but not with the absence of renal disease, Sjögren's syndrome, erosive joint disease, nor with Raynaud's phenomenon. Thus our findings are in keeping with

previous ones (Parker, 1972), that antibodies to RNP in groups of patients with SLE are associated with an increased frequency of atypical features such as sclerodactyly or severe Raynaud's phenomenon. However, these results do not support the suggestion of Sharp *et al.* (1972) that ENA protects against renal disease, nor was there a significant association between antibodies to RNP and the presence of Sjögren's syndrome.

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