Clinical and laboratory characteristics of patients with speckled pattern antinuclear antibodies

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SUMMARY
Fifty patients whose sera contained a speckled antinuclear antibody (ANA) were interviewed and examined to determine if there was any relationship between their clinical manifestations and the presence of certain serological markers. The results suggest that speckled ANA is usually found in patients with definite connective tissue diseases, but a significant minority have incomplete or early stages of these diseases. Characterisation of the antibody to extractable nuclear antigen (ENA) and other serological markers does not normally assist in making a clinical diagnosis, but the detection of a speckled ANA should prompt further investigation and careful follow-up.

INTRODUCTION
Autoantibodies to nuclear antigens can be detected in a variety of ways. Immunofluorescent staining of tissue specimens and cultured cells can reveal antibodies reactive with nuclear components and various patterns of staining, i.e. homogeneous, nucleolar, speckled patterns have been identified and found to be diagnostically useful. In addition immunochemical tests using antigens extracted from cell nuclei (extractable nuclear antigens; ENA) can be used to identify a number of these antinuclear antibodies. A number of groups have reported the clinical significance of antibodies to extractable nuclear antigens (ENA), but these studies have generally been carried out on highly selected groups of patients

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drawn from specialist clinics. This selection tends to introduce a bias in favour of the speciality concerned. In an attempt to overcome this problem, we have examined the clinical and laboratory features of patients whose sera were received in a routine diagnostic immunology laboratory and gave a speckled antinuclear antibody staining pattern at a titre of one in 80 or greater, when tested by indirect immunofluorescence.

MATERIALS AND METHODS

Patients
Fifty consecutive patients on whom data were complete and who had significant titres of speckled antinuclear antibody (ANA) were selected for study. Of these 50 patients, 42 were female and eight male with a mean age of 45 years (range: 15 to 87 years). The duration of disease symptoms was between two and 31 years. Each patient was reviewed in person by a clinician (ACF, JDMcC or AJT) who completed a detailed questionnaire recording the time and nature of the onset of clinical symptoms and the course of the illness. Patients were examined for signs of disease with particular reference to the locomotor system and connective tissues. Systemic lupus erythematosus (SLE), progressive systemic sclerosis (PSS) and rheumatoid arthritis (RA) were all diagnosed according to criteria of the American Rheumatism Association (ARA). The results of all previous investigations were recorded and a blood sample was taken for estimation of antibodies to double-stranded deoxyribonucleic acid (ds DNA), single-stranded deoxyribonucleic acid (ss DNA), histone, centromere, cardiolipin and extractable nuclear antigens (ENA). Rheumatoid factor (RF), complement components C3 and C4, immunoglobulins G, A and M were also measured.

Laboratory methods
Antibodies giving a characteristic speckled ANA pattern were detected by indirect immunofluorescence using 5 μm cryostat sections of rat composite tissue as substrate and fluorescein conjugated rabbit immunoglobulin to human kappa and lambda chains. The titres of antibodies in individual G and M immunoglobulin classes were determined using both fluorescein conjugated rabbit immunoglobulins to human IgG and IgM.

Antibodies to extractable nuclear antigens (anti-ribonucleoprotein, – Sm, – Ro and – La) were detected and identified using counterimmunoelectrophoresis (CIE) and various saline cellular extracts as previously described. Antibody levels to double-stranded (ds) DNA were assayed using the Farr technique and a level of ds DNA antibody activity greater than 25 units/ml was considered as a positive result. Antibody to denatured single-stranded (ss) DNA was detected by CIE. Anticentromere antibody was detected by indirect immunofluorescence using HEp 2 cultured cells as antigen. Positive reference sera for antibodies to extractable nuclear antigens (ENA), ds DNA and centromere were obtained from the Centers for Disease Control, Atlanta, Georgia, USA. Antibody to histones was determined using indirect immunofluorescence and an acid-extraction histone-reconstitution assay. Rheumatoid factor (RF) was assayed using the differential agglutination test employing rabbit IgG as antigen and a quantitative latex assay which employed human IgG as antigen. The WHO International Reference preparation for rheumatoid factors was employed and a level of 30 IU/ml or greater was considered positive for this study. Antibody to cardiolipin was determined by a positive reaction against the Venereal Disease Research
Laboratory (VDRL) antigen with an accompanying negative reaction with the fluorescent treponemal antibody absorption test (FTA-ABS). Measurements of immunoglobulins G, A and M and complement C3 and C4 were assayed by laser nephelometry using internal standards (Beckman, UK).

**Statistical analysis**

Clinical and laboratory data were analysed using the ‘Statistical Package for the Social Sciences’ on a Vax computer at the Queen’s University of Belfast. The statistical methods applied were the chi-squared test, the independent t-test and its non-parametric equivalent, the Mann-Whitney U test.

**RESULTS**

The majority of patients in the study group (40%) were diagnosed as systemic lupus erythematosus (SLE) with progressive systemic sclerosis (PSS) as the second most common diagnosis (18%). Patients with other connective tissue disorders comprised a further 22% of the total. The remainder (20%) had miscellaneous disorders, which included pulmonary fibrosis (with and without systemic hypertension), thrombocytopenic purpura, haemolytic anaemia and a bleeding tendency. Two patients had hypergammaglobulinaemia (IgG) and one had systemic hypertension (Table I). None of these patients was on any

| Diagnosis                          | Number | Number in group |
|------------------------------------|--------|-----------------|
| Systemic lupus erythematosus       | 15     |                 |
| with Sjögren’s syndrome            | 3      |                 |
| with recurrent deep vein thrombosis| 1      | 20 (40%)        |
| with cutaneous vasculitis          | 1      |                 |
| Progressive systemic sclerosis     | 4      |                 |
| with Sjögren’s syndrome            | 2      |                 |
| with Sjögren’s syndrome and        | 1      | 9 (18%)         |
| rheumatoid arthritis              |        |                 |
| with rheumatoid arthritis          | 1      |                 |
| with polymyositis                  | 1      |                 |
| Mixed picture                      | 4      | 4 (8%)          |
| Vasculitis (small vessel)          | 2      |                 |
| with Sjögren’s syndrome            | 1      | 3 (6%)          |
| Rheumatoid arthritis              | 1      |                 |
| with Sjögren’s syndrome            | 2      | 3 (6%)          |
| Polymyositis                       | 1      | 1 (2%)          |
| Miscellaneous                      |        |                 |
| Pulmonary fibrosis                 | 4      |                 |
| Haematological disorders           | 3      | 10 (20%)        |
| Hypergammaglobulinaemia            | 2      |                 |
| Hypertension                       | 1      |                 |
| **Total**                          | 50     | 50 (100%)       |

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medication known to be associated with drug-induced lupus and in all cases antibodies to histones were not detected. Sjögren's syndrome, which was diagnosed clinically, was found in 18% of the patients. Sixty-two per cent of patients were receiving immunosuppressive therapy at the time of their review. These individuals were distributed evenly throughout the clinical groups irrespective of their antibody profile. Renal involvement was associated with antibody to ds DNA (50%) and decreased levels of C3 (67%). Twenty per cent of the patients with SLE and 22% of those with PSS had laboratory evidence of renal disease.

**Antibody profiles**

All the patients in the study had antibody to extractable nuclear antigens (ENA). The specificities of these antibodies are shown in Table II. The incidence of antibodies to ribonucleoprotein (RNP) alone, Ro and La, RNP, Ro and La, and Sm antigens were 54%, 24%, 14% and 8% respectively. Patients with antibody to Sm always had antibody to RNP and sometimes Ro. Antibodies to Sm and ds DNA were detected only in patients with SLE, whereas anti-RNP and anti-Ro with anti-La were present in a similar wide range of conditions. An important exception was PSS where all nine patients had antibody to RNP but none had antibody to Ro or La. All of these nine patients also had ss DNA antibody in their sera. Patients in the miscellaneous group had a higher percentage of antibodies to the mixture of RNP, Ro and La. Of the nine patients with Sjögren's syndrome, five (56%) had antibody to Ro and La. None of the 50 patients tested had antibodies to the VDRL antigen or to centromere. The SLE group had the highest incidence of IgG elevation and complement depression.

**Table II**

*Antibody profiles of patients grouped according to clinical groups*

| Clinical group                      | ds DNA (patient numbers) | RNP only (27) | Ro/La only (12) | RNP/Ro/La* (7) | Sm** (4) |
|------------------------------------|---------------------------|---------------|-----------------|----------------|---------|
| Systemic lupus erythematosus       | 100                       | 33            | 50              | 14             | 100     |
| Progressive systemic sclerosis     | 0                         | 26            | 0               | 29             | 0       |
| Mixed picture                     | 0                         | 7             | 8               | 14             | 0       |
| Vasculitis                         | 0                         | 4             | 17              | 0              | 0       |
| Rheumatoid arthritis               | 0                         | 7             | 8               | 0              | 0       |
| Polymyositis                       | 0                         | 4             | 0               | 0              | 0       |
| Miscellaneous                      | 0                         | 19            | 17              | 43             | 0       |

Figures within the table are percentages
*Antibody present to at least two of these antigens
**Sm positive (two patients Sm + RNP, two patients Sm, RNP + Ro)

**Statistical findings**

In view of the large number of possible associations of clinical features and serum markers, a comprehensive listing of these is not given – most were not significant.

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Some of the more positive associations are shown in Table III. If values below the 1% level are considered to be clinically significant, then only xerostomia and the presence of an LE skin rash were significantly associated with particular antibodies. There was no significant association between the presence of antibody to RNP and Raynaud’s phenomenon or the presence of antibodies to Sm antigen and renal or central nervous system disease.

### Table III

**Association between clinical features and the presence of serum markers**

| Clinical features                                      | Associated serum* marker | P value |
|--------------------------------------------------------|--------------------------|---------|
| Eye problems at presentation                           | La                       | 0.05    |
| Joint features at review                               | RNP                      | < 0.05  |
| Signs/symptoms at any time:                            |                          |         |
| 1. Involvement of cervical spine (C1-3)                | RF                       | < 0.05  |
| 2. Involvement of cervical spine (C4-7)                | RF                       | < 0.05  |
| 3. Myalgia                                             | RNP                      | < 0.05  |
| 4. Dysphagia                                           | RNP                      | < 0.05  |
| 5. Radiological evidence of abnormal oesophageal motility | RNP                      | < 0.05  |
| 6. Xerophthalmia                                       | Ro                       | < 0.05  |
| 7. Xerostomia                                          | Ro                       | < 0.01**|
| 8. Xerostomia                                          | La                       | < 0.01**|
| 9. Discoid LE skin rash                                | Sm                       | < 0.01**|

*Serum marker present at time of review
**Clinically significant

### DISCUSSION

In general, these results are in keeping with previously published work. However, our patients had a much wider range of diagnoses than those drawn purely from rheumatological departments. Connective tissue disorders were diagnosed in 80% of patients but the remaining 20% had a variety of immunological, pulmonary, cardiovascular and haematological complaints. All the patients whose sera gave a speckled ANA staining pattern had antibody to ENA. Antibodies were not associated with a particular clinical diagnosis, with the exception of the association of SLE with antibodies to ds DNA or Sm. Antibody to RNP alone was detected in all nine patients with PSS. This is in contrast to a number of studies where very few of these patients (0 – 5%) had antibody to RNP.\(^{13, 14}\) In a multicentre study on patients with PSS, serum antibodies to RNP characterised individuals with a PSS-overlap syndrome but did not occur in those with PSS alone.\(^5\) In contrast, the follow-up to Sharp’s original paper on mixed connective tissue disease reported that 45% of those with antibody to RNP finally developed PSS.\(^{15}\) The nine patients in our study with PSS were among the oldest in the group and had the longest disease duration, so the length of follow-up may explain these discrepancies. Antibodies to Ro and La were detected in 56% of patients with Sjögren’s syndrome which is consistent with previous reports.
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contrast, however, these antibodies were detected in 33% of patients with SLE who did not have Sjögren’s syndrome. Other studies have shown antibody to La in only 2–3% of such patients.3,16

The attempt to find statistically significant associations between clinical features and serological markers proved fruitless. Only xerostomia and a discoid LE skin rash were significantly associated with particular antibodies and these are unlikely to be of practical value. Despite this, our study demonstrates that the vast majority of patients with a speckled ANA do have significant disease and that the detection of this serological marker should prompt further investigation.

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