Antinuclear antibody–negative systemic lupus erythematosus–how common?

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SUMMARY A review of five years’ DNA-binding antibody results in a routine service laboratory revealed 38 patients who had high DNA-binding capacity (DNA-bc) but no antinuclear antibodies (ANA). On retrospective case note analysis, 22 patients (58%) were thought to have systemic lupus erythematosus (SLE), although only six (16%) fulfilled the preliminary classification criteria of the American Rheumatism Association (ARA). Our findings indicate that ANA-negative SLE is commoner than generally realised and lead us to recommend the measurement of DNA-bc in every case where clinically appropriate.

Current clinical teaching suggests that ANA are present in at least 98% of cases of SLE,¹,² and that the measurement of DNA-bc is more appropriate as a secondary, specific diagnostic test on ANA-positive sera. The usefulness of the ANA test as a screening procedure for the detection of sera with high DNA-bc has recently been evaluated³ and its worth established in this context. However, it is possible that some cases of SLE which are not associated with ANA will be missed if the ANA test alone is carried out.³ Antinuclear antibody-negative SLE has been implied, if not directly considered, in many studies of SLE over the years.⁴–⁶ Two series citing ten⁷ and eight⁸ cases, respectively, of ANA-negative SLE have drawn more direct attention to this entity and Maddison et al⁹ recently presented data on 66 cases of SLE with serological abnormalities but negative ANA tests. In each study the cases were collected over a number of years as being atypical members of larger series of ANA-positive lupus.

The present study approaches ANA-negative SLE from a different angle in beginning with an abnormal laboratory finding. Review of DNA-bc results since the inception of the assay in 1976 as a routine laboratory test in Aberdeen showed a number of sera with markedly raised titres of antiDNA antibodies, but no detectable ANA. A high DNA-bc is probably the most specific laboratory test for diagnosing SLE⁰¹,¹¹ although El-Ghobarey’s study¹² showed that only 87% of cases with DNA-bc greater than 30 units (U)/ml had SLE. We present here our findings following a retrospective case note analysis on 38 patients who demonstrated a high DNA-bc in the absence of ANA.

Subjects and methods

The subjects consisted of 38 patients who had been in the care of various clinicians in Grampian Health Board hospitals and were therefore drawn from a population of approximately 500 000. Ages ranged from two to 84 yr, (x = 50 yr). Twenty-two were female. All subjects had consistently negative ANA tests (x = 4.1 tests/subject), and had a recorded DNA-bc of 40 U/ml or greater. The DNA-bc assay had been performed on one to eight times per patient (x = 2.64) and was greater than 40 U/ml on one to four occasions per patient (x = 1.77). Fourteen patients had a single result over 40 U/ml; the assay had not been repeated in five of these.

Antinuclear antibody tests were carried out by the method of Beck¹² using an indirect immunofluorescence technique on a substrate of rat liver slices at a serum dilution of 1/16. The DNA-binding capacities were measured using the antiDNA kit (RC Amersham) based on the method of Wold et al,¹³ which is a specific radioimmunoassay using ammonium sulphate precipitation of ¹²⁵I-labelled, double-stranded DNA. The manufacturers suggest a normal range, subject to interlaboratory variation, of 0–10 U/ml, and 25 U/ml is the level above which the diagnosis is “largely confined” to SLE.¹⁴

After initial perusal of the case histories we eliminated those in which there was insufficient...
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results

Review of the case notes suggested that 22 (58%) of our cases had SLE and the clinical features in this group are shown in Table 2. The remaining 16 cases did not have enough evidence to support this diagnosis.

Comparison with the ARA list, modifying only the criteria regarding LE cells and proteinuria, showed that only six cases (16%) had four or more features of SLE, while the remaining 16 (42%) whom we thought to have SLE had either two or three of the features listed. When the clinical data on these 22 cases were compared to the expanded list, 16 had between four and nine ($x = 5.1$) features of SLE and the remaining six had three features each. All in this latter group had, by definition, markedly raised DNA-bc and their other clinical features are shown in Table 3.

Discussion

A condition with manifestations as protean as SLE does not readily lend itself to objective analysis. The ARA criteria go part of the way towards allowing comparison of different groups but it is intellectually disquieting to accept that a patient with a butterfly rash, arthritis, positive WR and raised DNA-bc has the very same disease as another with alopecia, Raynaud's phenomenon, haemolytic anaemia and recurrent mouth ulcers, while conversely, a third patient with arthritis, DNA binding antibodies, mild proteinuria, mononeuritis, vasculitic rash and thrombocytopenia cannot be classified as a case of SLE. These examples underline the need to apply the ARA criteria sensibly and to accept their limitations. In their review of the use of the ARA criteria Canoso and Cohen stated that a standard set of diagnostic criteria for SLE is unlikely to appear until a great deal more is known of the aetiology and pathogenesis.

In trying to evaluate our cases we have based assessment on the ARA criteria but have tried to broaden these to increase their clinical application. Initially two of the original criteria were modified. First, substitution of high DNA-bc, the most specific laboratory investigation, for the outdated LE cell test is widely accepted. Second, proteinuria greater than 3.5 g/day was replaced by "Albustix++", representing approximately 1.5 g/day of urinary protein. This semiquantitative method was employed as accurate quantification of proteinuria is seldom carried out; the lesser amount of protein loss was chosen as considerable degrees of renal damage can occur without a urinary protein loss ever approaching the demands of the ARA criteria. This point has been

evidence to support a diagnosis of SLE. The clinical details in the remainder were then compared to the American Rheumatism Association (ARA) preliminary classification criteria for SLE, substituting a high DNA-bc for positive LE cell test and "Albustix (Ames)++" for "proteinuria > 3.5g/day". The 14 ARA criteria were devised to allow more objective classification of patients for the purpose of clinical trials, population surveys etc, and a patient who exhibits four or more criteria, simultaneously or serially, is said to have SLE. Their use is widespread but of limited value in that many well established features of SLE are completely omitted while others appear in a very precise and over-restrictive way. It has been repeatedly stressed that fulfilment of four ARA criteria is not necessary to make a diagnosis. To allow a clinically more comprehensive assessment of our cases we further modified the original ARA list to that shown in Table 1, and compared the clinical details, in turn, to these amended criteria.

Table 1 Expanded list of SLE features based on ARA list

<table>
<thead>
<tr>
<th>Feature</th>
<th>*1 Rash (incl butterfly, livedo reticularis, vasculitis)</th>
<th>9 False + ve syphilis serology</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 Discoid lupus</td>
<td>10 Albustix ++</td>
</tr>
<tr>
<td></td>
<td>3 Raynaud's phenomenon</td>
<td>11 Granular urinary casts</td>
</tr>
<tr>
<td></td>
<td>4 Alopecia</td>
<td>12 Pleurisy/pericarditis</td>
</tr>
<tr>
<td></td>
<td>5 Photosensitivity</td>
<td>13 Neuropsychiatric disorder</td>
</tr>
<tr>
<td></td>
<td>6 Oral/nasal ulceration</td>
<td>14 Cytopenia</td>
</tr>
<tr>
<td></td>
<td>7 Arthritis</td>
<td>15 Fever</td>
</tr>
<tr>
<td></td>
<td>8 DNA-bc &gt; 40U/ml</td>
<td>16 Ocular disease</td>
</tr>
</tbody>
</table>

*Denotes changes from original ARA list.

Table 2 Clinical features of SLE in 22 patients

<table>
<thead>
<tr>
<th>Feature</th>
<th>DNA-bc &gt; 40U/ml</th>
<th>Raynaud's</th>
<th>Arthrits</th>
<th>Neurropsychiatric disorder</th>
<th>Haematological disorder</th>
<th>Fever</th>
<th>Pleurisy/pericarditis</th>
<th>Ocular problems</th>
<th>Rashes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>22 (100%)</td>
<td>3 (14)</td>
<td>15 (68)</td>
<td>14 (64)</td>
<td>8 (36)</td>
<td>8 (36)</td>
<td>7 (32)</td>
<td>7 (32)</td>
<td>5 (23)</td>
</tr>
</tbody>
</table>

Table 3 Clinical pattern in patients with only three listed features of SLE

<table>
<thead>
<tr>
<th>Feature</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raynaud's</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Arthrits</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Neurropsychiatric disorder</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Proteinuria</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Pleurisy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Neurropsychiatric disorder</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Cytopenia</td>
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previously raised\textsuperscript{16,17} and is supported, amongst others, by Pollak and colleagues\textsuperscript{18} whose patients with biopsy-proven lupus nephritis had proteinuria in excess of 200 mg/day in only 48 of 77 cases. The ARA criteria with these modifications gave six patients with the requisite four, or more, criteria and a further 16 patients with a formally inadequate two or three features. The discrepancy between clinical and classification assessments was clearly due to the exclusion from the ARA list of a number of well recognised features of SLE which were exhibited by some of our patients. Fever has been described in 80–100\%\textsuperscript{19,20} of SLE patients and was seen in a number of ours. A range of neuropsychiatric problems far wider than “psychosis and convulsions” is now recognised\textsuperscript{21,22} and it was of particular interest that depression which Bennett and his associates\textsuperscript{23} describe as the commonest disorder of mental function in SLE led to four of 22 in our clinical SLE group requiring formal psychiatric assessment. A variety of ocular problems is recognised\textsuperscript{24} and the 32\% incidence of eye disorders in our patients compares well with overall figures in previous studies.\textsuperscript{20,23} In assessing cutaneous manifestations we included, in addition to facial erythema, rashes such as livedo reticularis, vasculitis and maculopapular eruptions, all of which may be useful clinical pointers to a diagnosis of SLE.\textsuperscript{20,26}

Using our broadened list of clinical features of SLE (Table 1) we now have 16 patients with four or more features and a further six (Table 3) each with three features. Some additional points of interest in this latter group are: haematological disorder in patient 1 included temporally distinct thrombocytopenia and Coombs'-positive haemolysis; patient 2 had a spontaneous abortion and a puerperal exacerbation of arthritis; patient 3 had episodes of non-specific chest pain, required sedatives for “nerves” and had a period of weight-loss, diarrhoea and abdominal pain for which extensive investigation gave no diagnosis; patient 4 had recurrent paraesthesiae and a depressive illness culminating in a drug overdose, in addition to mild renal impairment for no apparent reason; patient 5 had periungal erythema, a diminished gas transfer factor with exertional dyspnoea and a history of musculoskeletal pains; patient 6 had recurrent myalgia and stiffness, headaches, steroid-responsive strokes, telangiectases, proteinuria of up to five grams per day with impaired creatinine clearance and a brother who during the last few months has developed SLE with ANA. Thus we support a diagnosis of SLE in 22 cases.

What of the other 16 patients with high DNA-bc but not thought to have SLE? In some the information in the case notes was simply inadequate to allow any conclusions. Three patients had liver disease, which can be associated with high DNA-bc.\textsuperscript{27} Some may be evolving cases of SLE in whom sufficient clinical features to allow diagnosis will only develop after a number of years. Of our six cases who fulfilled the ARA criteria, only four would have done so at the time of first detection of a high DNA-bc. Late development of ANA has been highlighted by Bohan\textsuperscript{28} who described five cases of active SLE developing ANA for the first time, ten months to seven years after the initial diagnosis. From the outset we eliminated from further study any patient who had ever had a positive ANA result. The 38 patients included in the study had remained ANA-negative for over-follow-up periods ranging from nine to 40 months. Detection of cytoplasmic antibodies (Ro and La)\textsuperscript{29,20} may be an important serological feature of ANA-negative SLE and we are hoping to study these in our patients.

More extensive studies will be necessary before it can be determined whether or not ANA-negative SLE is clinically distinct from the seropositive variety. The prominent characteristics in Fessel's\textsuperscript{8} and Maddison's\textsuperscript{9} groups, respectively, differed from each other and were also at variance with the original ARA group.\textsuperscript{15} Our patients were different again, displaying a relatively low incidence of skin manifestations, alopecia, photosensitivity and cellular urinary casts\textsuperscript{(Table 2).}

It remains to be seen how large the ANA-negative SLE group will become once it is actively sought. The prevalence of SLE is not known but using a suggested figure of one in 2000,\textsuperscript{30} our half-million catchment population should contain around 250 cases of SLE. The 22 ANA-negative cases detected to date would represent 8.9\%, a much larger figure than the one or two per cent which we are led to expect. Furthermore, we have excluded levels of DNA-bc less than 40 U/ml from our study thus leaving out an unknown number of cases with significantly raised DNA-bc.

The diagnosis of SLE is often difficult; only seven of our 22 cases had been diagnosed by their attending physician. However, the potential benefits of making the correct diagnosis at an early stage are obvious and we therefore recommend the measurement of DNA-bc in every case where clinically appropriate, regardless of the presence or absence of ANA.

We would like to thank the local Area Committee of Physicians for permission to include its members' patients in our study.

References

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