

## EXTENDED REPORT

## Anti-chromatin antibodies in systemic lupus erythematosus: a useful marker for lupus nephropathy

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**Background:** Anti-chromatin antibodies have recently been described in patients with systemic lupus erythematosus (SLE) and it has been suggested that their presence is associated with lupus nephritis.**Objective:** To assess the prevalence and clinical associations of these antibodies in SLE.**Methods:** The presence of anti-chromatin antibodies in 100 patients with SLE was investigated by an enzyme linked immunosorbent assay (ELISA). To determine the specificity of these antibodies, 100 patients with primary Sjögren's syndrome, 30 with primary antiphospholipid syndrome (APS), 10 with systemic sclerosis, and 100 normal controls were also tested.**Results:** Positive levels were detected in 69/100 (69%) patients with SLE. In contrast, they were found in only 8/100 (8%) of those with primary Sjögren's syndrome, in 1/10 (10%) with systemic sclerosis, in 2/30 (7%) with primary APS, and in none of the 100 healthy controls. Patients with anti-chromatin antibodies had a twofold higher prevalence of lupus nephropathy than those without these antibodies (58% v 29%,  $p < 0.01$ ). A significant correlation was found between the levels of anti-chromatin antibodies and disease activity score as measured by the European Consensus Lupus Activity Measurement (ECLAM;  $p = 0.011$ ).**Conclusions:** The measurement of anti-chromatin antibodies appears to be a useful addition to the laboratory tests that can help in the diagnosis and treatment of SLE. These antibodies are both sensitive and specific for SLE, and are a useful marker for an increased risk of lupus nephritis.

Systemic lupus erythematosus (SLE) is the prototypic autoimmune connective tissue disease. It may affect any organ of the body and display a broad spectrum of clinical and immunological manifestations. However, it is now thought that patients with SLE can be divided into more homogeneous subsets of pathogenic, therapeutic, and prognostic significance.<sup>1</sup>

The presence of certain autoantibodies is one of the factors associated with some symptoms of the disease and aids in the classification of patients with SLE into specific subsets. This is the case for antiphospholipid antibodies, which are clearly associated with the development of thrombotic events and obstetric morbidity,<sup>2</sup> anti-ribonuclear protein (RNP) antibodies that are markers of myositis and Raynaud's phenomenon,<sup>3</sup> and anti-SSA/Ro that are associated with congenital heart abnormalities in newborn infants.<sup>4</sup> One of the most severe events in the course of SLE is the development of glomerulonephritis,<sup>5</sup> and much effort has been spent to find a useful and early marker of this complication. Anti-dsDNA antibodies are often associated with lupus nephritis,<sup>6</sup> and the presence of anti-dsDNA is a hallmark of SLE.<sup>7,8</sup>

However, evidence has accumulated in recent years that anti-chromatin autoantibodies are correlated even better with lupus nephritis than anti-dsDNA.<sup>9–12</sup> Chromatin is the native histone-DNA complex found in the nucleus of eukaryotic cells, and it is organised into a repeating series of nucleosomes. Anti-chromatin-chromatin immune complexes can bind to the glomerular basement membrane *in vivo*.<sup>13</sup> Chromatin (or nucleosomes) is an antigen for T and B cells from patients with SLE.<sup>12,14</sup> Additionally, anti-chromatin antibodies are a ubiquitous feature of murine lupus,<sup>15</sup> and are necessary but not sufficient for the development of glomerulonephritis in one strain of mouse.<sup>16</sup> It was found that anti-chromatin always preceded the appearance of anti-dsDNA antibodies in two strains of mice, and the suggestion

was made that anti-dsDNA antibodies were a subset of anti-chromatin antibodies.<sup>15</sup>

In this study we investigated the prevalence of both anti-dsDNA and anti-chromatin antibodies in a large series of patients with SLE in order to assess their clinical significance and, particularly, their value as a marker of lupus nephropathy. To determine the specificity of these antibodies for SLE, a large number of patients with other connective tissue diseases were tested for anti-chromatin antibodies. It was also possible to follow the antibodies over time from serial bleeds of some patients with SLE.

## PATIENTS AND METHODS

## Patients and controls

Clinical and laboratory features of 100 consecutive and unselected patients (93 female, seven male; mean (SD) age 37 (14) years, range 11–70 years) with SLE were prospectively studied. All fulfilled four or more of the 1982 American College of Rheumatology (ACR) revised criteria for the classification of SLE.<sup>8</sup>

The disease control groups consisted of 100 patients with primary Sjögren's syndrome (classified according to the European criteria),<sup>17</sup> 30 with primary antiphospholipid syndrome (APS) (categorised according to the preliminary criteria for the classification of APS),<sup>18</sup> and 10 with systemic sclerosis (classified according to the ACR preliminary criteria).<sup>19</sup> The normal control group consisted of 100 healthy blood donors from the blood bank of our hospital.

**Abbreviations:** ACR, American College of Rheumatology; APS, antiphospholipid syndrome; CI, confidence interval; ECLAM, European Consensus Lupus Activity Measurement; ELISA, enzyme linked immunosorbent assay; OR, odds ratio; SLE, systemic lupus erythematosus

### Definition of clinical features and disease activity

The patients had been attending our institute either as in- or outpatients between 1999 and 2000. All had documented medical histories and underwent a medical interview as well as a routine general physical examination by a qualified internist. A serum sample from each patient was collected for the immunological tests. Clinical and serological characteristics of all these patients were collected in a protocol form. Salient features included in this protocol were: (a) gender, (b) age, (c) laboratory features, and (d) clinical manifestations at the time blood was drawn. Information collected in the protocol forms was transferred to a computerised database program. The study was performed according to the principles of the Declaration of Helsinki.

The clinical manifestations evaluated in this protocol were defined according to the recommendations of the ACR glossary committee.<sup>20</sup> Specifically, nephropathy was considered when patients presented (a) persistent proteinuria >0.5 g/day or greater than 3+ if measurement was not performed; or (b) cellular casts (may be red cell, haemoglobin, granular, tubular, or mixed); or (c) otherwise unexplained rise in serum creatinine >75 µmol/l. Renal biopsies were reviewed by two pathologists and categorised according to the modified classification proposed by the World Health Organisation<sup>21</sup>: type I—normal kidney; type II—mesangial glomerulonephritis (presence of mesangial deposits with mesangial hypercellularity); type III—focal proliferative glomerulonephritis (inflammatory changes affecting some glomeruli but leaving other unaffected); type IV—diffuse proliferative glomerulonephritis (virtually all glomeruli show inflammation); type V—membranous nephropathy (diffuse generalised thickening of the capillary wall and predominant intramembranous and/or subepithelial electron-dense deposits, without inflammatory changes).

Disease activity was assessed by the European Consensus Lupus Activity Measurement (ECLAM).<sup>22</sup>

### Detection of autoantibodies

Anti-chromatin antibodies of the IgG isotype were measured by a commercial semiquantitative enzyme linked immunosorbent assay (ELISA; INOVA Diagnostics Inc, San Diego, CA) according to the manufacturer's instructions. Antinuclear antibodies were determined by indirect immunofluorescence using triple tissue cryostat sections (liver-stomach-kidney) and Hep-2 cells as substrate (Euroimmun). Anti-dsDNA antibodies were determined by Farr's ammonium sulphate precipitation technique (Amerlex, Trinity Biotech, Ireland).<sup>23</sup> Antibodies to extractable nuclear antigens of the IgG isotype, including Ro(SSA), La(SSB), U1-snRNP, and Sm were detected by ELISA (Captia, Trinity Biotech, Ireland). Rheumatoid factor was detected by nephelometry (Behring). Anticardiolipin antibodies of the IgG and IgM isotypes were measured by an ELISA, as previously described.<sup>23</sup> The lupus anticoagulant activity was detected by coagulation assays, following the guidelines of the International Society on Thrombosis and Haemostasis (Scientific Subcommittee on Lupus Anticoagulants/Phospholipid Dependent Antibodies).<sup>24</sup>

### Statistical analysis

Conventional  $\chi^2$  and Fisher's exact tests were used for analysing qualitative differences, and Student's *t* test was used for comparison of means, assuming similar variance in independent samples. A value of  $p < 0.05$  was taken to indicate significance. When several independent variables appeared to have statistical significance in the univariate analysis, a logistic regression test was performed for multivariate analysis to rule out possible confounding variables. Only those variables showing statistical significance in the multivariate analysis were considered significant in this study. The odds ratio (OR) was calculated for assessing the risk of appearance of each

**Table 1** Prevalence of anti-chromatin antibodies in systemic autoimmune diseases and controls

Group	Anti-chromatin antibodies No (%)	p
Systemic lupus erythematosus (n=100)	69 (69)	<0.05
Primary Sjögren's syndrome (n=100)	8 (8)	—
Systemic sclerosis (n=10)	1 (10)	—
Primary antiphospholipid syndrome (n=30)	2 (7)	—
Healthy blood donors (n=100)	0 (0)	—

variable. A lower limit of the 95% confidence interval (CI) that exceeded 1.0 was taken to indicate statistical significance in the case of positive association and an upper limit lower than 1.0 in the case of negative association. Results of the analysis of continuous variables are indicated as mean (SD). Linear regression analysis was performed for comparison of continuous variables. This statistical analysis was performed by the SPSS and STATCALC programs using the information stored in the database program.

## RESULTS

### Prevalence of anti-chromatin antibodies

Positive levels of anti-chromatin antibodies (>20 U) were detected in 69/100 (69%) patients with SLE. In contrast, they were found in only 8/100 (8%) patients with primary Sjögren's syndrome, in 1/10 (10%) patients with systemic sclerosis, in 2/30 (7%) patients with primary APS, and in 0/100 (0%) healthy blood donors (table 1).

### Relationship between anti-chromatin antibodies and clinical features

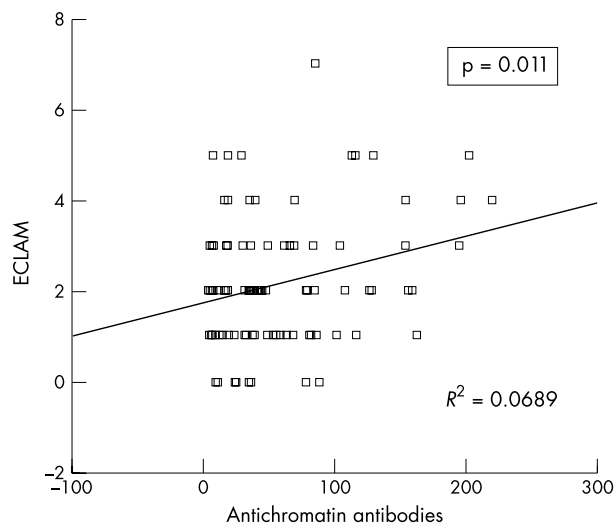
Fifty two (52%) patients with SLE had clinical evidence of lupus nephropathy at the time of the protocol study. Renal biopsies performed at the time of clinical diagnosis of renal involvement had disclosed type IV lesions in 19 patients, type III in 15, type II in 11, and type V in 7. Forty two of the patients with nephropathy were positive for anti-chromatin antibodies, yielding a sensitivity of these antibodies for lupus nephropathy of 81% and a specificity of 39%. Patients with anti-chromatin antibodies had a twofold higher prevalence of lupus nephropathy than those without these antibodies (58% v 29%,  $p < 0.01$ ; OR=3.4, 95% CI 1.3 to 9.3). The mean level of anti-chromatin antibodies in patients with lupus nephropathy was 68 U and in patients without nephropathy 42 U ( $p < 0.01$ ).

No differences in the prevalence of the other clinical manifestations were found among patients with and without anti-chromatin antibodies (table 2).

**Table 2** Prevalence of active SLE clinical manifestations in patients with and without anti-chromatin antibodies. Results are shown as No (%)

Manifestations	Anti-chromatin antibodies		p
	Positive (n=69)	Negative (n=31)	
Cutaneous	30 (43)	14 (45)	NS
Arthritis	22 (32)	10 (32)	NS
Serositis	2 (3)	0 (0)	NS
Haematological	4 (6)	2 (6)	NS
Nephropathy	40 (58)	9 (29)	<0.01
Central nervous system	1 (1)	0 (0)	NS
Thrombosis	1 (1)	0 (0)	NS

NS, not significant.



**Figure 1** Correlation between the levels of anti-chromatin antibodies and SLE disease activity score as measured by ECLAM.

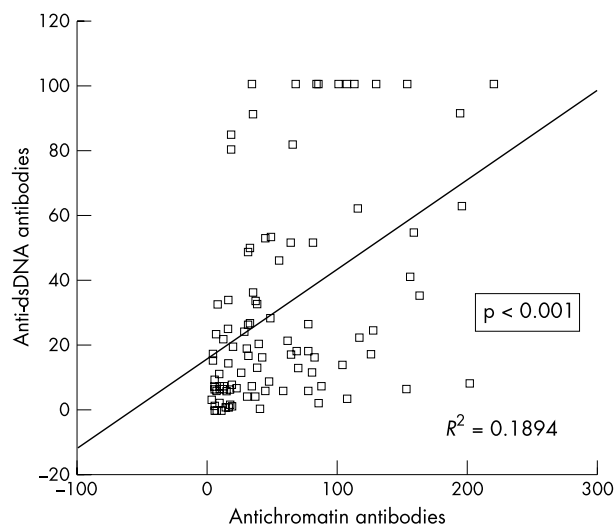
A significant correlation was found between the levels of anti-chromatin antibodies and disease activity score as measured by ECLAM ( $p=0.011$ ,  $R^2=0.0689$ ) (fig 1).

#### Relationship between anti-dsDNA antibodies and clinical features

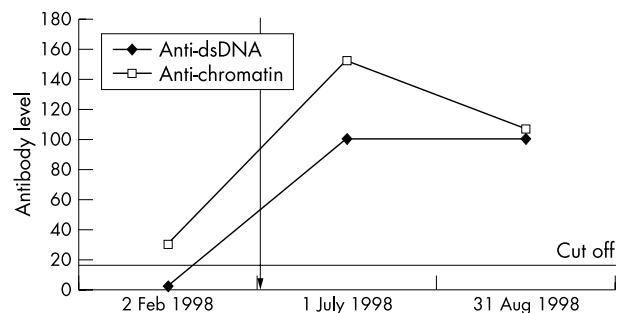
Positive levels of anti-dsDNA antibodies ( $>15$  U) were found in 55 (55%) patients with SLE but in none of the other groups of patients. Thirty nine of the 52 patients with nephropathy were positive for anti-dsDNA, yielding a sensitivity of these antibodies for lupus nephropathy of 75% and a specificity of 63%. Patients with anti-dsDNA antibodies were also found to have a higher prevalence of lupus nephropathy than those without these antibodies (71% v 32%,  $p<0.001$ ; OR=5.4; 95% CI 2 to 14.8). No differences in the prevalence of the other clinical manifestations were found among patients with and without anti-dsDNA antibodies.

#### Relationship between anti-chromatin antibodies and anti-dsDNA and other serum autoantibodies

Forty nine patients had positive levels of both anti-dsDNA and anti-chromatin antibodies (30/49 (61%) had lupus nephropathy), but 20 patients had anti-chromatin without anti-dsDNA



**Figure 2** Correlation between the levels of anti-chromatin antibodies and those of anti-dsDNA antibodies.



**Figure 3** Serial determinations of both anti-chromatin and anti-dsDNA antibodies in a patient who developed lupus nephropathy during the study period, showing that anti-chromatin antibodies appeared before anti-dsDNA antibodies.

antibodies (8/20 (40%) had lupus nephropathy), while only six had anti-dsDNA without anti-chromatin antibodies (3/6 (50%) had lupus nephropathy). Additionally, a significant correlation was found between the levels of anti-chromatin antibodies and those of anti-dsDNA antibodies ( $p<0.0001$ ,  $R^2=0.189$ ) (fig 2).

Serial determinations of both anti-chromatin and anti-dsDNA antibodies in three patients who developed lupus nephropathy during the study disclosed that anti-chromatin antibodies appeared before anti-dsDNA antibodies in one of them (fig 3).

No correlation was found between anti-chromatin antibodies and the presence of antinuclear antibodies, antiphospholipid antibodies, antibodies to extractable nuclear antigens, or rheumatoid factor.

## DISCUSSION

Lupus nephropathy is a common complication of SLE that can greatly influence the prognosis. Clearly, the mortality rate is higher for patients with SLE with nephritis than in those without renal disease,<sup>5 22 25</sup> and some 10–60% of patients with SLE with nephritis (depending on genetic, socioeconomic, and treatment differences) eventually develop end stage renal failure that requires dialysis or transplantation.<sup>26</sup> Therefore, much effort has been spent in finding a useful and early marker of this complication. In this study we found that anti-chromatin antibodies were clearly associated with the presence of lupus nephritis with a sensitivity of 81%, which was slightly higher than the 75% sensitivity of anti-dsDNA antibodies. Additionally, a significant correlation was found between the levels of anti-chromatin antibodies and disease activity.

Most studies have found that anti-chromatin/nucleosome antibodies are quite sensitive and specific for SLE,<sup>9–12 27</sup> but a few have found a high prevalence in other diseases.<sup>11 28 29</sup> In the current study anti-chromatin showed a sensitivity of 69% in the patients with SLE. There was a specificity of 100% in the normal group, and 92% in the disease control group. These results agree with the group of studies showing both high sensitivity and specificity. Also in agreement with these studies, anti-chromatin showed a higher prevalence than anti-dsDNA in patients with SLE. There are three reasons that some studies showed high anti-chromatin reactivity in patients with systemic sclerosis. In one case whole nucleosomes were used that contained small amounts of the Scl-70 antigen. The patients with systemic sclerosis who were anti-Scl-70 positive were low positive on this preparation.<sup>29</sup> In another case, the antigen was denatured H2A plus denatured H2B added to DNA (personal communication).<sup>28</sup> In this case the sera from patients with systemic sclerosis were reacting with epitopes on the denatured histones that did not reconstitute into their native conformations. In a third study<sup>11</sup> a cut off between positive and negative for anti-chromatin was chosen

that was two standard deviations above the average of a non-disease group. Because a non-disease group does not display a Gaussian distribution of binding, this was not the appropriate statistical method to choose a cut off (it was too low). When H1-stripped chromatin or nucleosome core particles are used as the antigen, and an appropriate cut off between positive and negative is used, virtually no patients with systemic sclerosis are positive for anti-chromatin antibodies.<sup>27,29</sup>

In two studies using ELISA to measure anti-dsDNA and anti-chromatin antibodies in both human and murine lupus, no samples were positive for anti-dsDNA and negative for anti-chromatin antibodies,<sup>9,15</sup> suggesting that anti-dsDNA were a subset of anti-chromatin. In this study, using the Farr assay to measure anti-dsDNA antibodies, six samples were anti-dsDNA positive but anti-chromatin antibody negative. The most likely explanation is that some antibodies recognise structures of DNA that can occur in protein-free DNA in solution but do not occur in the DNA wrapped around the histones in chromatin that is bound to the solid phase of the ELISA plate. None the less, in this study at least one patient did develop anti-chromatin antibodies before developing anti-dsDNA. Additionally, 49 of the 55 anti-dsDNA positive samples were also anti-chromatin positive. Both these findings are consistent with the concept that most of the anti-dsDNA antibodies in patients with SLE are a subset of anti-chromatin antibodies.

The measurement of anti-chromatin antibodies appears to be a useful addition to the laboratory tests that can help in the diagnosis and treatment of SLE. These antibodies are both sensitive and specific for SLE, and are a useful marker for an increased risk of lupus nephritis. Because a positive LE cell test was often correlated with more severe symptoms in lupus, and anti-chromatin (previously called antideoxyribonucleoprotein) antibodies are the main antibody causing LE cell formation<sup>29</sup>—together with anti-histone H1 antibodies<sup>30</sup>—it is not surprising to find correlations between disease and anti-chromatin. Additionally, the finding that immune complexes comprising chromatin and anti-chromatin can deposit in the glomerular basement membrane of the kidney,<sup>13</sup> and that anti-chromatin antibodies are a necessary component for the development of glomerulonephritis in one strain of mouse,<sup>16</sup> provides theoretical evidence that anti-chromatin antibodies can have pathological properties in some patients with SLE.

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