Rheumatology 2004;43:220–224 doi:10.1093/rheumatology/keh024
Advance Access publication 16 September 2003

Anti-nucleosome antibodies in patients with systemic lupus erythematosus of recent onset. Potential utility as a diagnostic tool and disease activity marker

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***Objective*. To compare the utility of anti-chromatin antibodies for the diagnosis of systemic lupus erythematosus (SLE) and as markers of disease activity.**

***Methods*. We included 73 consecutive patients (62 female) with SLE (four or more ACR criteria) of recent onset (< 1 yr since diagnosis). As control groups we included 130 healthy blood donors and 261 patients with 11 systemic autoimmune diseases (SAD). Disease activity was assessed using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI). A venous blood sample was drawn to measure three anti-chromatin antibodies [anti-nucleosome (anti-NCS), anti-double-stranded DNA (anti-dsDNA) and anti-histones (anti-HST)] by enzyme-linked immunosorbent assay.**

***Results*. The prevalence of anti-chromatin antibodies in SLE patients and healthy controls was 100 and 3% respectively for anti-NCS, 63 and 5% for anti-dsDNA, and 15 and 3% for anti-HST. Anti-NCS had a sensitivity of 100% and specificity of 97% for SLE diagnosis. When SLE and SAD patients were compared [excluding mixed connective tissue disease (MCTD)], the sensitivity of anti-NCS, anti-dsDNA and anti-HST antibodies for SLE diagnosis was 93, 71 and 40% respectively and the specificity was 97, 98 and 98%. Anti-chromatin antibodies were not useful in differentiating between SLE and MCTD patients. Anti-NCS antibodies showed the highest correlation with disease activity ($r = 0.45$, $P < 0.0001$), especially in patients negative for anti-dsDNA antibodies ($r = 0.58$, $P = 0.001$). Anti-NCS antibodies also showed strong association with renal damage (odds ratio 4.1, 95% confidence interval 1.2–13.6, $P = 0.01$).**

***Conclusion*. Anti-NCS antibodies could be a useful tool in the diagnosis and assessment of disease activity in SLE patients, especially in patients who are negative for anti-dsDNA antibodies.**

KEY WORDS: Anti-nucleosome antibodies, Anti-chromatin antibodies, Systemic lupus erythematosus, Diagnosis, Disease activity.

Systemic lupus erythematosus (SLE) is an autoimmune disease of unknown aetiology, characterized by the presence of autoantibodies against cellular antigens, mainly nuclear antigens, such as anti-histones (anti-HST), anti-single-stranded DNA and anti-double-stranded DNA (anti-dsDNA) antibodies [1]. Anti-dsDNA antibodies are considered the main diagnostic tool for SLE and a useful marker of disease activity; however, they are found only in 50% of SLE patients and do not always correlate with disease activity [2, 3]. On the other hand, antinuclear antibodies, the most prevalent antibodies, have low specificity for the diagnosis of SLE, because they are found in most systemic autoimmune diseases (SAD) and even in healthy individuals [4]. Thus, it is important to look for other autoantibodies that may be useful in the diagnosis and assessment of disease activity in SLE patients.

Recently, it was proposed that the nucleosome is the principal antigen in the pathophysiology of SLE, and that anti-nucleosome antibodies (anti-NCS) are associated with organic damage [5–7]. However, the sensitivity and specificity of anti-NCS antibodies for the diagnosis of SLE in patients with long disease duration have not been shown to be better than those of anti-dsDNA antibodies [8, 9].

In the murine models of lupus, anti-NCS antibodies arise before the development of other anti-chromatin antibodies and they have the highest prevalence from the early stages of life [10]; therefore, we hypothesized that anti-NCS antibodies could be useful in the diagnosis and assessment of disease activity in SLE patients of recent onset.

Patients and methods

We conducted a cross-sectional study among 73 consecutive patients who attended the lupus clinic at the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INCMNSZ) between August 1999 and November 2001, with SLE of recent onset (< 1 yr since diagnosis) according to American College of Rheumatology (ACR) criteria [11]. All patients were evaluated by the same rheumatologist, who assessed disease activity using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) [12]. A venous blood sample was drawn at the same visit and stored at -70°C to measure immunoglobulin G (IgG)-class anti-chromatin antibodies (anti-NCS, anti-dsDNA and anti-HST). As control groups we included (i) 130 healthy blood donors who tested negative for HIV, hepatitis B and C viruses and VDRL (Venereal Disease Research Laboratory slide test) (56% females, mean age 34 ± 9 yr) and (ii) 261 prevalent patients with 11 SAD, including 50 with mixed connective tissue disease (MCTD), 31

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Submitted 10 June 2003; revised version accepted 31 July 2003.

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with primary antiphospholipid syndrome, 70 with primary vasculitis (Takayasu arteritis, Wegener's granulomatosis, polyarteritis nodosa, Henoch–Schönlein purpura, Behçet disease and microscopic polyangiitis), 50 with primary Sjögren syndrome, 40 with inflammatory myopathy and 20 with systemic sclerosis, according to appropriate classification criteria [13–20].

Anti-chromatin antibodies were determined by immunoenzymatic assay (EIA), according to the manufacturer's recommendations [Orgentec Diagnostika (Germany) (anti-HST and anti-NCS antibodies) and The Binding Site (UK) (anti-dsDNA antibodies)]. Briefly, plates coated with purified human nucleosomes, histones or calf thymus dsDNA were incubated with 100 μl of 1:100 serum dilutions for 30 min at room temperature. Each plate included a calibration curve of five points, a positive control and a negative control. Plates were washed three times with the wash solutions of the kits. A prediluted anti-human IgG horseradish peroxidase conjugate (100 μl/well) was pipetted into the wells of the microplate and incubated for 30 min (dsDNA plates) or 15 min (histones and nucleosomes plates) at room temperature. After three washes, 100 μl of prediluted substrate was added and incubated for 30 min (dsDNA plates) or 15 min (histones and nucleosomes plates) at room temperature. Colour release was stopped by the addition of 100 μl of prediluted stop solution. Plates were read at 450 nm and a 650 nm reference filter was used. Reproducibility was evaluated using eight positive and negative controls ($r = 0.98$). Because contamination with Jo-1 in the human nucleosome EIA preparations has been reported (personal communication, Henry Chabre), we tested five anti-Jo-1-positive sera in the three anti-chromatin assays, and all samples were negative to anti-NCS, anti-dsDNA and anti-HST antibodies (data not shown). These results suggest that the preparation used for coating the plates was not contaminated with Jo-1.

The cut-off values of anti-chromatin antibodies were set above the mean $\pm$ 2 S.D. of a separate control group of 100 healthy individuals (55, 15 and 100 U/ml for anti-NCS, anti-dsDNA and anti-HST antibodies respectively). Because these values were not useful in differentiating between SAD and SLE patients, we used a ROC (receiver operating characteristic) curve considering the titres found in 73 SLE patients and 261 patients with rheumatic diseases. Antibody determinations were performed by one of the authors (JC), who was blinded to the clinical information. The study was approved by the Institutional Committee of Biomedical Research at the INCMNSZ.

Statistical analysis

To determine the diagnostic utility of anti-chromatin antibodies, we used the sensitivity, specificity, positive predictive value, negative predictive value and likelihood ratio. Continuous variables are expressed as mean $\pm$ S.D. Pairs of groups were compared using Student's t test for normally distributed continuous variables or the Mann–Whitney U test for those without normal distribution. The χ^2 test or Fisher's exact test was used for categorical variables as needed. For comparisons among three or more groups, an ANOVA (analysis of variance) test or its equivalent non-parametric test was used. As a secondary analysis, the correlation coefficient (Spearman) between anti-chromatin antibodies and disease activity was determined, using a modified SLEDAI in which anti-dsDNA antibodies and complement protein values were excluded to avoid overestimation of the coefficient. Statistical significance was set at $P \leq 0.05$, two-tailed.

Results

We included 73 SLE patients [62 females (85%)] with age 25.6 $\pm$ 8.8 yr (range 15–61, median 24) and SLE duration 5.5 $\pm$ 2.9 months (range 2–12, median 5). The SLEDAI score at clinical evaluation was 5.3 $\pm$ 5.9 (range 0–25, median 4). The most frequent clinical manifestations, defined according to ACR criteria, are summarized in Table 1.

Anti-chromatin antibodies

The prevalence of anti-NCS, anti-dsDNA and anti-HST antibodies in SLE patients was 100, 63 and 15% respectively; whereas in healthy controls it was 3, 5 and 3% for anti-NCS, anti-ds DNA and anti-HST antibodies respectively. Titres of anti-chromatin antibodies in SLE patients were 244 $\pm$ 88 U/ml (range 57–481 U/ml) for anti-NCS, 335 $\pm$ 425 U/ml (range 0–1540 U/ml) for anti-dsDNA and 48 $\pm$ 58 U/ml (range 0–237 U/ml) for anti-HST. Comparing SLE patients with healthy controls, anti-NCS, anti-dsDNA and anti-HST antibodies had a sensitivity of 100, 63 and 15% respectively, specificity of 97, 95 and 97%, positive predictive value of 95, 90 and 73%, negative predictive value of 100, 93 and 67%, and likelihood ratio values of ∞, 12.6 [95% confidence interval (CI), 5.5–24.5] and 5.0 (95% CI, 2.6–14.8) for the diagnosis of SLE (Table 2).

Table 1. Demographic and clinical[a] characteristics of SLE patients

Characteristic	$n = 73$
Demographic	
Male, female	11, 62
Age (yr): mean $\pm$ S.D.	25.6 $\pm$ 8.8
SLE duration (months): mean $\pm$ S.D.	5.5 $\pm$ 2.9
SLEDAI modified, mean $\pm$ S.D.	5.3 $\pm$ 5.9
Total criteria: mean $\pm$ S.D.	5.7 $\pm$ 1.3
Clinical: no. (%)	
Malar rash	39 (53)
Discoid lupus	10 (14)
Photosensitivity	33 (44)
Oral ulcers	37 (51)
Arthritis	63 (86)
Serositis	16 (22)
Renal	39 (53)
Neurological	4 (6)
Haematological	55 (75)

[a]According to ACR criteria.

Table 2. Anti-chromatin antibodies in SLE patients and healthy controls

Antibodies	SLE: mean $\pm$ S.D.[a] ($n = 73$)	Healthy: mean $\pm$ S.D.[a] ($n = 130$)	SN	SP	PPV	NPV	Likelihood ratio (95% CI)
Anti-HST	48 $\pm$ 58	27 $\pm$ 35	15	97	73	67	5.0 (2.6–14.8)
Anti-dsDNA	335 $\pm$ 425	3 $\pm$ 7	63	95	90	93	12.6 (5.5–24.5)
Anti-NCS	244 $\pm$ 88	10 $\pm$ 22	100	97	95	100	∞ (–)[b]

SN, sensitivity; SP, specificity; PPV, positive predictive value; NPV, negative predictive value.
[a]In U/ml.
[b]Because the sensitivity value was 100%, the likelihood ratio was considered to be infinite.

TABLE 3. Prevalence of anti-chromatin antibodies in patients with SAD

Disease	*n*	Anti-HST (%)	Anti-dsDNA (%)	Anti-NCS (%)
Mixed connective tissue disease	50	12	30	70
Primary antiphospholipid syndrome	31	6	6	3
Primary vasculitis[a]	70	0	0	0
Sjögren syndrome	50	0	4	4
Inflammatory myopathy	40	5	0	5
Scleroderma	20	0	0	5

[a]Includes Takayasu arteritis, Wegener's granulomatosis, polyarteritis nodosa, microscopic polyangiitis, Henoch–Schönlein purpura and Behçet disease.

Utility of anti-chromatin antibodies in differentiating SAD from SLE

Anti-chromatin antibodies were highly prevalent in MCTD patients, whereas in the other SAD studied they were found in $< 7\%$ of patients (Table 3). Comparing SLE patients and patients with SAD (excluding MCTD), the anti-NCS, anti-dsDNA and anti-HST antibodies had a sensitivity of 93, 71 and 40% respectively and specificity of 97, 98 and 98% for SLE diagnosis. When MCTD patients were included in the analysis, the sensitivity was identical, but the specificity decreased to 85, 92 and 96% for anti-NCS, anti-dsDNA and anti-HST antibodies respectively.

Among MCTD patients, the prevalence of anti-NCS antibodies was 70% (Table 3). The sensitivity and specificity of anti-NCS antibodies for identifying SLE from MCTD patients was 93 and 30% respectively. Therefore, anti-NCS antibodies were not useful for this task.

Correlation between anti-chromatin antibodies and SLE activity

All anti-chromatin antibodies correlated with SLE activity (Fig. 1). The correlation coefficient was stronger for anti-NCS antibodies ($r = 0.45$, $P < 0.0001$) than for anti-HST ($r = 0.28$, $P = 0.01$) and anti-dsDNA antibodies ($r = 0.25$, $P = 0.03$).

To determine the utility of anti-NCS antibodies as a marker of disease activity in patients negative for anti-dsDNA antibodies, we analysed a subgroup of 27 patients who tested negative for anti-dsDNA antibodies. We found that the correlation of anti-NCS with disease activity was stronger in this subgroup than in the whole group of SLE patients ($r = 0.58$, $P = 0.001$) (Fig. 2).

Association between anti-NCS antibodies and SLE manifestations

To establish the association between anti-chromatin antibodies and SLE manifestations, we converted the antibody titres to a dichotomous variable, defining high and low titres as values above and below the median respectively. High titres of anti-NCS antibodies were associated with proteinuria [odds ratio (OR) 4.1, 95% CI 1.2–13.6, $P = 0.01$], haematuria (OR 4.2, 95% CI 1.1–15.4, $P = 0.03$), malar rash (OR 4.7, 95% CI 1.1–19.1, $P = 0.03$), arthritis (OR 6.5, 95% CI 1.8–20.6, $P = 0.003$) and oral ulcers (OR 10.0, 95% CI 2.4–42.5, $P = 0.001$). High titres of anti-dsDNA antibodies were associated with oral ulcers (OR 5.4, 95% CI 1.1–27.3, $P = 0.02$) and high titres of anti-HST antibodies were associated with pleuritis (OR 2.1, 95% CI 1.6–2.7, $P = 0.05$) (Table 4).

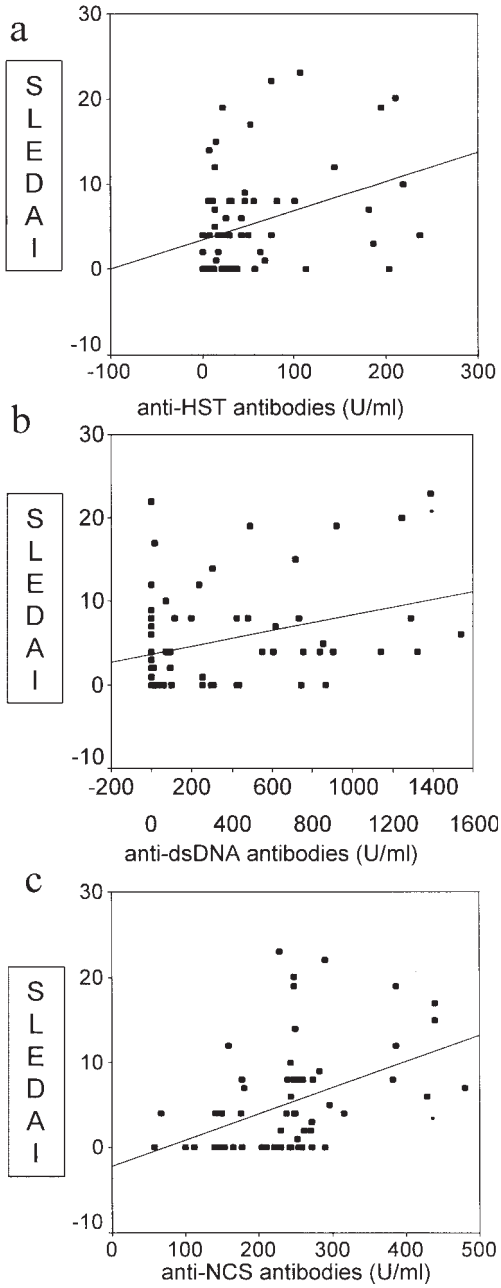

FIG. 1. (a) The correlation coefficient of the anti-HST antibodies was 0.28, $P = 0.01$, (b) anti-dsDNA 0.25, $P = 0.03$ and (c) the anti-NCS 0.45, $P < 0.0001$.

Global effect of anti-chromatin antibodies on SLE activity

In order to evaluate the impact of anti-chromatin antibodies on SLE activity, we studied a multiple linear regression model using disease activity as the dependent variable and the titres of anti-NCS, anti-HST and anti-dsDNA antibodies as independent variables. Considered together, anti-chromatin antibodies explained almost 40% of SLE activity ($r^2 = 0.364$, $P < 0.001$) (Fig. 3).

Discussion

SAD, including SLE, share several clinical and biological features that make their differential diagnosis difficult, especially in the

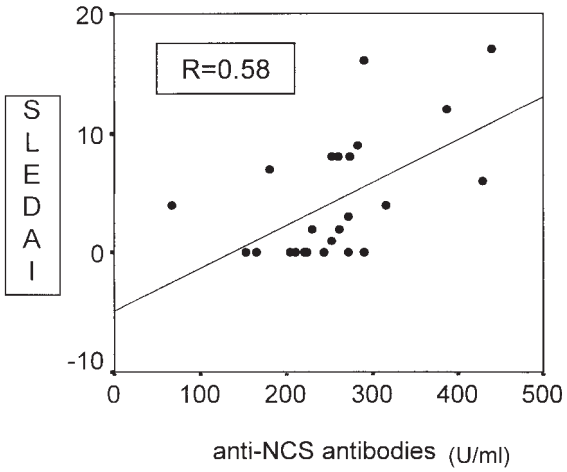

FIG. 2. The correlation coefficient between anti-NCS antibodies and disease activity, in anti-dsDNA negative SLE patients was 0.58 ($P = 0.001$).

TABLE 4. Association between anti-chromatin antibodies and clinical manifestations of SLE

Antibody and manifestation	High titre (%)	Low titre (%)	OR	95% CI	P^a
Anti-NCS					
Proteinuria	50	19	4.1	1.2–13.6	0.01
Haematuria	38	14	4.2	1.1–15.4	0.03
Malar rash	31	9	4.7	1.1–19.1	0.03
Arthritis	62	21	6.5	1.8–20.6	0.003
Oral ulcers	44	7	10.3	2.4–42.5	0.001
Anti-dsDNA					
Oral ulcers	23	3	5.4	1.1–27.3	0.02
Anti-HST					
Pleuritis	11	0	2.1	1.6–2.7	0.05

Titres of anti-NCS were converted to a dichotomous variable. Titres above/below the median were considered high/low.
[a]The χ^2 test or Fisher's exact test was used as needed.

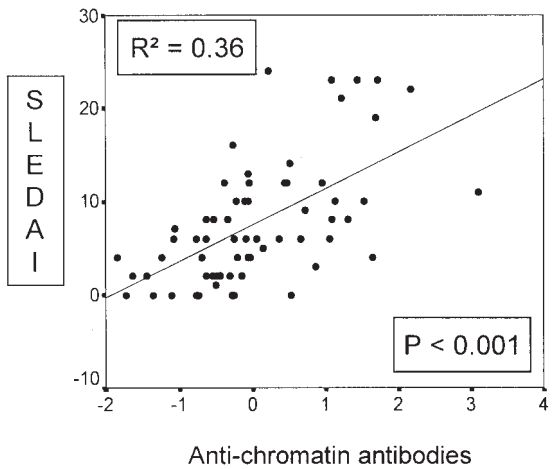

FIG. 3. The global impact of all anti-chromatin antibodies on SLE activity is shown in this multivariate model. Altogether anti-chromatin antibodies explained 36% of the disease activity.

early stages. Traditional tests used in the diagnosis of SLE, such as antinuclear antibodies, have low specificity because they identify antibodies against nuclear antigens generically [4]. Because the nucleosomes have been considered the principal antigen in the pathophysiology of SLE [5, 6, 21–23], we investigated the prevalence of anti-NCS antibodies in SLE patients with recent onset.

In this study, anti-NCS antibodies were detected in all SLE patients with recent onset. They showed a high correlation with disease activity, especially among SLE patients who tested negative for anti-dsDNA antibodies, and were strongly associated with renal damage. All the SLE patients we studied had anti-NCS antibodies, whereas anti-dsDNA antibodies were present in only 63%. Previous reports [8, 9], in which SLE patients with longer disease duration (mean 8 yr) were studied, did not show a higher prevalence of anti-NCS antibodies than anti-dsDNA antibodies. We cannot explain the discrepancy in the results between these previous studies and our study. Whether these differences depend on the effect of disease activity or SLE treatment on the antibody titres needs to be determined.

The high sensitivity and specificity of anti-NCS antibodies for SLE diagnosis in our study is consistent with findings in murine models of lupus, in which it has been demonstrated that the development of anti-NCS antibodies occurs before other anti-chromatin antibodies [10]. Our results show that the prevalence of anti-NCS antibodies is higher than that of anti-dsDNA antibodies in the initial stages. This is similar to what has been reported in murine models of lupus [10].

Considered together, anti-chromatin antibodies explained close to 40% of SLE activity. Anti-NCS antibodies themselves explained almost 35% of disease activity. These results suggest that anti-NCS antibodies, besides being potentially useful in SLE diagnosis, could be a biological marker of disease activity.

The presence of anti-NCS antibodies in all SLE patients who were anti-dsDNA-negative and the stronger correlation with disease activity needs to be highlighted, because in this group there is no reliable diagnostic test and no marker of disease activity.

In our study, only anti-NCS antibodies were associated with renal damage, which suggests a possible role in the pathogenesis of lupus nephritis [24, 25]. A positive association of anti-NCS antibodies with renal damage has been demonstrated previously, both in murine models of lupus and in SLE [5, 6, 24, 25]. This association seems to depend on a complex interaction between charges associated with the quaternary structure of the nucleosomes and epitope targets in renal tissue. That is, the histones that constitute part of the nucleosomes have a cationic charge, whereas the glomerular basement membrane has an anionic charge, which permits an interaction between them.

As also reported by Amoura *et al.* [8] and Bruns *et al.* [9], in our study the presence of anti-NCS antibodies was restricted to patients with SLE and MCTD diagnoses. This suggests that anti-NCS antibodies could be useful not only in differentiating individuals with SLE from healthy individuals, but also in differentiating SLE from other forms of SAD. However, the high prevalence of anti-NCS antibodies in MCTD restricted their utility in the differential diagnosis of SLE. Nevertheless, we cannot definitively rule out their usefulness in differentiating SLE and MCTD patients at early stages of disease evolution, because the MCTD patients studied did not have disease of recent onset. However, this result is interesting in view of the fact that MCTD and SLE share many clinical and biological characteristics.

We must acknowledge some limitations of this study. We did not test cross-reaction among anti-chromatin antibodies. However, in two previous reports it was demonstrated that this cross-reaction did not affect the interpretation of the test [8, 9]. Furthermore, we studied a group of SLE patients who were positive only for anti-NCS antibodies and negative for other anti-chromatin antibodies, which is clear evidence of the existence of a group of antibodies that recognize only the quaternary structure of the nucleosomes [26].

Although a strong correlation was found between anti-NCS antibodies and SLEDAI scores, given the cross-sectional nature of the study we were not able to determine whether the titres of

anti-NCS antibodies are sensitive to change with disease activity over time.

Despite these limitations, we may conclude that anti-NCS antibodies could be a useful tool for the diagnosis of SLE, especially at early stages of the disease, and a useful marker of disease activity, particularly in patients who are negative for anti-dsDNA antibodies.

Acknowledgements

The authors thank Drs Zahir Amoura, Jean-Charles Piette and Henry Chabre for sharing their expertise in the field of nucleosomes and anti-nucleosome antibodies; and Dr Luis Felipe Flores-Suárez for his collaboration regarding the sera of patients with primary vasculitis. This study was supported by grant 34750-N from Consejo Nacional de Ciencia y Tecnología.

The authors have declared no conflicts of interest.

References

1. Hannahs B. Antibodies to DNA. N Engl J Med 1998;19:1359–68.
2. Pisetsky DS. Anti-DNA and autoantibodies. Curr Opin Rheumatol 2000;12:364–8.
3. Villarreal GM, Drenkard C, Villa AR *et al*. Prevalence of 13 autoantibodies and of the 16/6 and related pathogenic idiotypes in 465 patients with systemic lupus erythematosus and their relationship with disease activity. Lupus 1997;6:425–35.
4. Fritzler MJ, Salazar M. Diversity and origin of rheumatologic autoantibodies. Clin Microbiol Rev 1991;4:256–69.
5. Amoura Z, Piette JC, Bach JF, Koutouzov S. The key role of nucleosomes in lupus. Arthritis Rheum 1999;42:833–43.
6. Amoura Z, Koutouzov S, Piette JC. The key role of nucleosomes in lupus. Curr Opin Rheumatol 2000;12:369–73.
7. Luger K. Crystal structure of the nucleosome core particle at 2.8 Å resolution. Nature 1997;389:251–60.
8. Amoura Z, Koutouzov S, Chabre H *et al*. Presence of antinucleosome autoantibodies in a restricted set of connective tissue diseases. Arthritis Rheum 2000;43:76–84.
9. Bruns A, Blass S, Hausdorf G, Burmester GR, Hiepe F. Nucleosomes are major T and B cell autoantigens in systemic lupus erythematosus. Arthritis Rheum 2000;43:2307–15.
10. Amoura Z, Chabre H, Koutouzov S *et al*. Nucleosome-restricted antibodies are detected before anti-dsDNA and/or antihistone antibodies in serum of MRL-Mp lpr/lpr and +/+ mice, and are present in kidney eluates of lupus mice with proteinuria. Arthritis Rheum 1994;37:1684–8.
11. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1997;40:1725.
12. Bombardier C, Gladman D, Urowitz MB, Caron D, Chang CH. Derivation of the SLEDAI. A disease activity index for lupus patients. Arthritis Rheum 1992;35:630–40.
13. Alarcón-Segovia D, Cardiel M. Comparison between 3 diagnostic criteria for mixed connective tissue disease. J Rheumatol 1989;16: 328–34.
14. Alarcón-Segovia D, Sanchez-Guerrero J. Primary antiphospholipid syndrome. J Rheumatol 1989;16:482–8.
15. American College of Rheumatology Subcommittee on Classification of Vasculitis. The American College of Rheumatology Criteria for Classification of Vasculitis. Arthritis Rheum 1990;33:1065–144.
16. International Study Group for Behcet's Disease. Criteria for diagnosis of Behcet's disease. Lancet 1990;335:1080–90.
17. Jennete JC, Falk RJ, Andrassy K *et al*. Nomenclature of systemic vasculitis: proposal of an international consensus conference. Arthritis Rheum 1994;37:187–92.
18. Fox RI, Saito I. Criteria for diagnosis of primary Sjögren syndrome. Rheum Dis Clin North Am 1994;20:391–407.
19. Bohan A, Peter JB. Polymyositis and dermatomyositis. N Engl J Med 1975;292:344–7.
20. Subcommittee for scleroderma criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. Preliminary criteria for the classification of systemic sclerosis. Arthritis Rheum 1980;23:581–90.
21. Amoura Z, Piette JC, Chabre H *et al*. Circulating plasma levels of nucleosomes in patients with systemic lupus erythematosus: correlation with serum antinucleosome antibody titers and absence of clear association with disease activity. Arthritis Rheum 1997;40: 2217–25.
22. Burlingame RW, Rubin RL, Balderas RS, Theofilopoulos AN. Genesis and evolution of antichromatin autoantibodies in murine lupus implicates T-dependent immunization with self-antigen. J Clin Invest 1993;91:1687–96.
23. Emlen W, Niebur J, Kadera R. Accelerated *in vitro* apoptosis of lymphocytes from patients with systemic lupus erythematosus. J Immunol 1994;152:3685–92.
24. Mohan C, Adams S, Stanik V, Datta SK. Nucleosome: a major immunogen for pathogenic autoantibody-inducing T cells of lupus. J Exp Med 1993;177:1367–81.
25. Berden JH, Licht R, van Bruggen MC, Tax WJ. Role of nucleosomes for induction and glomerular binding of autoantibodies in lupus nephritis. Curr Opin Nephrol Hypertens 1999;8:299–306.
26. Rahman A, Hiepe F. Anti-DNA antibodies—overview of assays and clinical correlations. Lupus 2002;11:770–3.