# Diagnostic Value of Anti-Nucleosome Antibodies in the Assessment of Disease Activity of Systemic Lupus Erythematosus: A Prospective Study Comparing Anti-Nucleosome with Anti-dsDNA Antibodies

OSWALDO AUGUSTO GUTIÉRREZ-ADRIANZÉN, SOPHIE KOUTOUZOV, ROSA MARIA SALANI MOTA, MARTA MARIA das CHAGAS MEDEIROS, JEAN-FRANÇOIS BACH, and HENRY de HOLANDA CAMPOS

*ABSTRACT.* ***Objective.*** To determine the diagnostic value of anti-nucleosome antibodies in the assessment of clinically active systemic lupus erythematosus (SLE) and active nephritis.

***Methods.*** A 12 month prospective study of 87 patients diagnosed with SLE. At each evaluation, disease activity was scored by SLE Disease Activity Index and Lupus Activity Criteria Count, and blood samples were collected for laboratory tests. Autoantibodies were detected by ELISA.

***Results.*** Nearly all patients were female (96.6%). The mean age was 33 years and the mean disease duration was 60.7 months. About half the patients presented with nephritis (49.4%) and active SLE (50.6%) at the first clinical examination. During the study period, the prevalence of active SLE decreased from 50.6% to 29.1%. The prevalence of anti-nucleosome and anti-dsDNA antibodies was 40.0%–58.6% and 10.9%–21.8%, respectively, throughout the study period. The sensitivity of anti-nucleosome and anti-dsDNA antibodies for active SLE was 72.7%–100% and 31.3%–54.8%, respectively. The specificity of anti-nucleosome and anti-dsDNA antibodies for active SLE was 66.7%–83.7% and 88.7%–100%, respectively. The sensitivity and specificity of anti-nucleosome antibodies for active nephritis were 32.0%–67.5% and 46.2%–67.3%, respectively. The sensitivity and specificity for anti-dsDNA antibodies for active nephritis were 16.0%–35.4% and 85.1-97.5%, respectively.

***Conclusion.*** Anti-nucleosome antibodies are more sensitive than anti-dsDNA antibodies to active SLE and active nephritis. Thus, anti-nucleosome antibody reactivity may be a useful marker in the diagnosis and assessment of active SLE. (First Release June 15 2006; J Rheumatol 2006;33:1538–44)

*Key Indexing Terms:*
ANTI-NUCLEOSOME ANTIBODIES ANTI-dsDNA ANTIBODIES LUPUS NEPHRITIS
SYSTEMIC LUPUS ERYTHEMATOSUS DISEASE ACTIVITY

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the production of a large array of autoantibodies against antigens located in the cytoplasm, nucleus, and surface of the cell. There is abundant evidence for triggering of the immune response in SLE by autoantigens and for its dependence on T cells[1-3]. Double-stranded DNA (dsDNA), although previously believed to be the most important autoantigen in the pathogenesis of SLE and lupus nephritis[4], has been shown to possess relatively few immunological properties[5].

Recent studies have shed new light on the pathogenesis of SLE and lupus nephritis by identifying the nucleosome as the autoantigen responsible for the generation of a number of antinuclear antibodies[6-9]. Studies using murine lupus models have identified nucleosome-specific T cells capable of driving the production of nucleosome-specific autoantibodies along with anti-dsDNA and anti-histone antibodies[10]. Nucleosome-specific antibodies appear earlier than anti-dsDNA and anti-histone antibodies, suggesting that the loss of tolerance to nucleosomal components may be the initial key event of SLE[11,12]. Indeed, nucleosome-bound anti-nucleosome and anti-dsDNA antibodies have been observed in the renal glomerulus of rats. The cationic portion of the histone attached to the nucleosome binds to the anionic portion of heparan sulfate in the glomerular basement membrane[13-15].

Apoptotic defects and impaired apoptotic cell clearance may lead to an overload of autoantigens (DNA, histones, and nucleoprotein complex), particularly of the nucleosomes driving the production of autoantibodies in SLE[16].

The use of the nucleosomes as substrate in immunoenzy-

*From the Department of Internal Medicine, Faculty of Medicine, and Department of Statistics, Federal University of Ceará, Fortaleza, Brazil; Institut Paris-Sud sur les Cytokines, INSERM U131, Clamart, France; and Institut Nationale de la Santé et de la Recherche Médicale, Hôpital Necker, Paris, France.*

*O.A. Gutiérrez-Adrianzén, MSc; M.M. das Chagas Medeiros, PhD; H. de Holanda Campos, PhD, Department of Internal Medicine, Faculty of Medicine, Federal University of Ceará; R.M. Salani Mota, MSc, Department of Statistics, Federal University of Ceará; S. Koutouzov, PhD, Institut Paris-Sud sur les Cytokines, INSERM U131; J-F. Bach, MD, Unité 25, Institut Nationale de la Santé et de la Recherche Médicale.*

*Address reprint requests to Dr. O.A. Gutiérrez-Adrianzén, Rua Deputado Moreira da Rocha 865, Meireles, Fortaleza, Ceará, CEP 60160-060, Brazil. E-mail: oswaldogutierrez@uol.com.br*

*Accepted for publication March 23, 2006.*

matic tests (ELISA) has made detection of anti-nucleosome antibodies possible and is likely to shed more light on the pathogenesis of SLE and on the relevance of anti-nucleosome antibodies to clinical practice. To date few cross-sectional studies have been published surveying the prevalence of anti-nucleosome antibodies in human SLE[17-20].

We prospectively evaluated the diagnostic value of anti-nucleosome antibodies as markers of active SLE and active nephritis, compared to anti-dsDNA antibodies, during a 12 month period.

## MATERIALS AND METHODS

The study population consisted of Brazilian patients referred to a university hospital (HUWC, Fortaleza, Ceará) between April 1999 and February 2001 and diagnosed with SLE according to the criteria of the American College of Rheumatology[21,22]. All patients gave their written informed consent before enrolling in the study.

Patients were followed at the outpatient facility for 12 months and evaluated every 4–6 weeks. Blood and urine samples were collected at each encounter and submitted for blood cell count, dosage of serum urea, creatinine, albumin, C3 and C4, urinary sediment, and 24-hour proteinuria at the university hospital laboratory. After centrifugation, sera were stored in Eppendorf tubes at –80°C for assessment of anti-nucleosome and anti-dsDNA antibodies at the laboratory of the Institut National de la Santé et de la Recherche Médicale (INSERM), Hôpital Necker, Paris, France. Nephritis was diagnosed for samples with a 24-hour proteinuria > 500 mg/dl associated with hematuria (≥ 5 erythrocytes/high power field), leukocyturia (≥ 5 leukocytes/high power field), and/or cylindruria.

SLE activity was assessed at each encounter using the Lupus Activity Criteria Count (LACC)[23] and the SLE Disease Activity Index (SLEDAI)[24]. The former was used to calculate the diagnostic properties of autoantibodies in active SLE. Patients were classified in 4 groups according to the number of episodes of disease activity during the study period: e.g., no activity, mild activity (1–2 episodes), moderate activity (3–5 episodes), and severe activity (≥ 6 episodes).

*Antigens*. Nucleosome core particles (H1-stripped) were prepared as described, with slight modifications[25]. Briefly, nucleosomes were isolated by digesting chromatin from mouse erythroleukemia L 1210 cell nuclei with *Staphylococcus aureus* nuclease. H1 and nonhistone proteins were stripped from the soluble chromatin by adding 5 M NaCl dropwise (0.55 M final concentration), at 0°C. Samples of the stripped chromatin [a maximum of 200 optical density units at 260 nm ($OD_{260}$), corresponding to 50 mg DNA) were fractionated on a Sepharose 6B gel filtration column equilibrated in 10 mM Tris, 1 mM EDTA, pH 7.4 (TE), 0.55 M NaCl, and 0.2 mM phenylmethylsulfonyl fluoride (PMSF), at a flow rate of 7 ml/h. Nucleosome fractions were collected by spectrophotometry after determination of the $OD_{260}$. Polyacrylamide gel electrophoresis was used to examine core particle samples for the presence of contaminant nucleoprotein species and for free DNA[26]. Fractions corresponding to pure mononucleosomes (that eluted at ~270–300 ml) were dialyzed extensively against TE and 0.2 mM PMSF, concentrated on PM-30 filters (Amicon, Lexington, MA, USA), and stored on ice at 1–1.5 mg/ml. Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis showed the presence of the core made up of the 4 histones in equimolar amounts, indicating that this preparation yielded "intact" mononucleosome core particles[26,27].

Lambda phage dsDNA was purchased from Boehringer (Mannheim, Germany).

*Determination of anti-nucleosome and anti-dsDNA antibody reactivities*. Antibody determination was conducted by one of the authors (SK), who was blinded to all clinical information. Autoantibody reactivity was assessed by ELISA, based on the method developed by Burlingame and Rubin[28] and as described[12,25]. Briefly, antigen-coated plates (5 $\mu$g/ml for each antigen) were washed with phosphate buffered saline-0.1% Tween, pH 7.4 (PBST), and postcoated for 2 h with 0.1 ml of PBS-10% fetal calf serum (FCS). Sera to be tested (diluted 1:100 in PBST-10% FCS) were added to the plates and then allowed to react for 2 h. Bound IgG antibodies were detected with peroxidase-conjugated goat anti-$\gamma$ or anti-$\mu$ sera added to plates for 1 h. Binding was measured by adding ABTS substrate solution (Boehringer), and OD was read at 405 nm. Appropriate positive and negative controls were included in each plate. Uncoated plates were used to determine background activity, which was subtracted from the OD obtained with coated plates.

*Cutoff for discriminating anti-nucleosome and anti-dsDNA reactivity*. To determine cutoff values for anti-nucleosome and anti-dsDNA antibodies, antibody reactivity was determined as described above in serum samples obtained from a control group of 26 Brazilian volunteer blood donors. The cutoff value was established as the mean value of the group plus 3 standard deviations. Samples were considered to be positive for anti-nucleosome and anti-dsDNA antibodies at optic density values > 0.167 and > 0.195, respectively.

*Statistical analysis*. Sensitivity and specificity were determined for active SLE and active nephritis. The McNemar test was performed to analyze paired data measured on a nominal scale. Pearson's coefficient was used to investigate the correlation between the 2 criteria of active SLE. The level of statistical significance was set at $p < 0.05$. Microsoft Excel and SPSS 8.0 for Windows were used for data storage and analysis.

## RESULTS

The study cohort included 87 patients examined 8–12 times at an outpatient facility throughout a period of 12 months. The group consisted of 84 women (96.6%) and 3 men (3.4%). The mean age was 33.0 ± 10.9 years, and 65.5% of the patients were non-Caucasian. Mean disease duration was 60.7 ± 66.2 months.

The main clinical manifestations of SLE observed at baseline included arthritis (69%), photosensitivity (63.2%), skin lesions (59.8%), and nephritis (49.4%). When applying the LACC criteria, about half the patients (n = 44) were found to have active SLE at baseline. This figure decreased to 29.1% by the end of the study period (Figure 1A). When disease activity was quantified by SLEDAI, the mean score fell from 8.22 ± 8.28 at baseline to 4.69 ± 5.28 by the end of the study period. The Pearson correlation coefficient between the 2 sets of criteria ranged from 65.5% to 86.5% and was > 70% in 11 of 12 evaluations. Mean findings for hemoglobin, serum albumin, 24-h proteinuria, and prevalence of hypocomplementemia (C3) during the 12 month study period are presented in Figures 1B-1E.

The treatment consisted of an initial endovenous administration of 0.75 g/m$^2$ of cyclophosphamide, followed by monthly doses of 1.0 g/m$^2$ during 6 months, then quarterly doses until completing 2 years. Azathioprine was administered orally to maintain remission, at a dose of 2 mg/day. Mycophenolate mofetil was administered to only one patient, at a dose of 1 g/day. Prednisone was administered at a daily dose of 5–60 mg/day.

The prevalence of anti-nucleosome and anti-dsDNA antibodies ranged from 40.0% to 58.6% and from 10.9% to 21.8%, respectively, throughout the study period (Table 1). Anti-nucleosome antibodies were most prevalent at the fifth evaluation, with a tendency to gradually decrease over time,

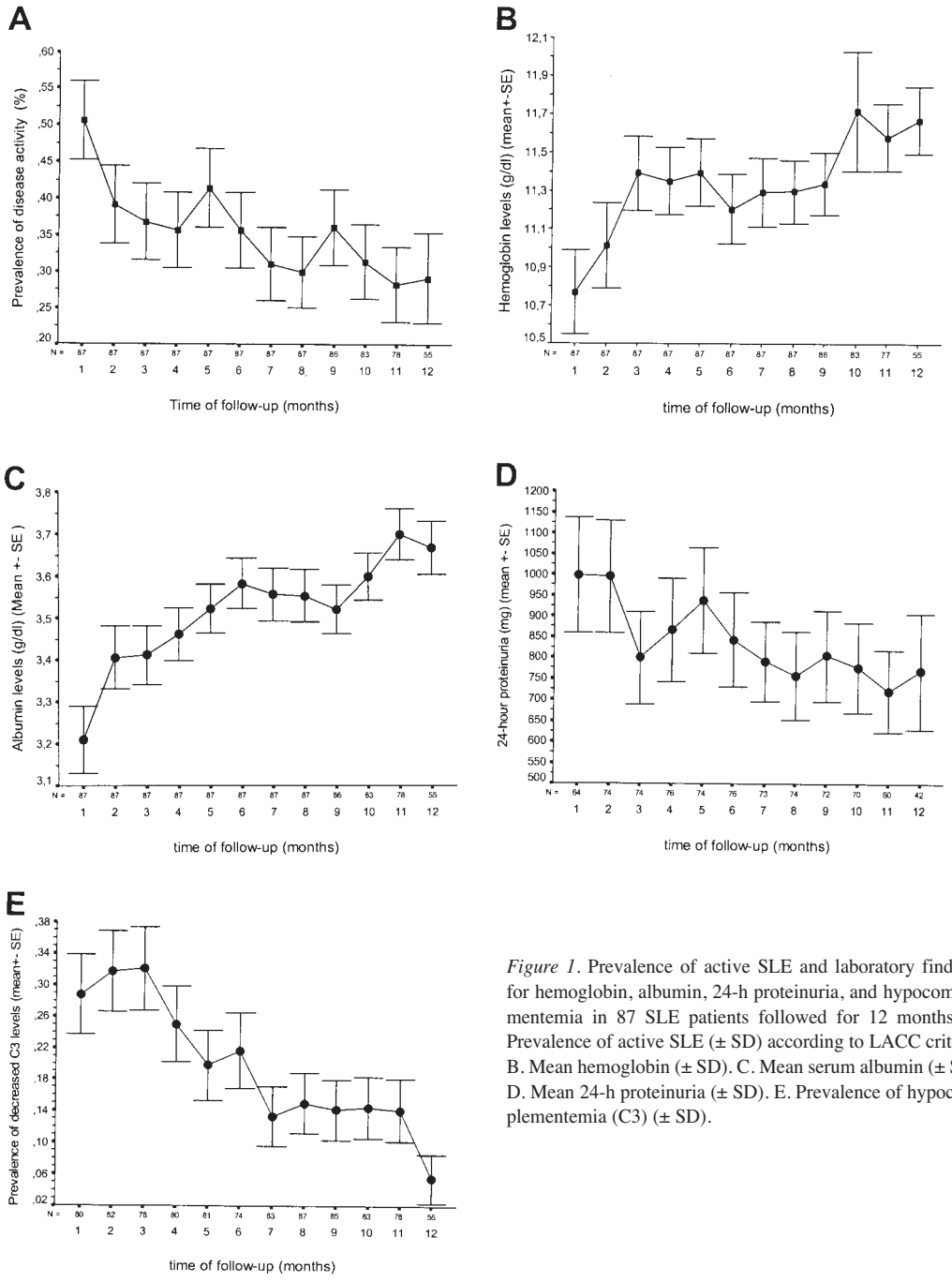


*Figure 1*. Prevalence of active SLE and laboratory findings for hemoglobin, albumin, 24-h proteinuria, and hypocomplementemia in 87 SLE patients followed for 12 months. A. Prevalence of active SLE (± SD) according to LACC criteria. B. Mean hemoglobin (± SD). C. Mean serum albumin (± SD). D. Mean 24-h proteinuria (± SD). E. Prevalence of hypocomplementemia (C3) (± SD).

reaching the lowest levels by the 11th and 12th month. Anti-dsDNA antibodies were most prevalent at the first 2 evaluations (21.8%) and decreased fairly steadily afterwards.

During the 12 month study period, the sensitivity of antibodies to active SLE ranged from 72.7% to 100% for anti-nucleosome antibodies and from 31.3% to 54.8% for anti-dsDNA antibodies. The specificity of antibodies to active SLE ranged from 66.7% to 83.7% for anti-nucleosome antibodies and from 88.7% to 100% for anti-dsDNA antibodies (Table 2). The corresponding values for active nephritis are shown in Table 3. The sensitivity and specificity of anti-nucleosome antibodies to active nephritis ranged from 32.0% to 67.5% and from 46.2% to 67.3%, respectively. The sensitivity and specificity for anti-dsDNA antibodies to active nephritis varied from 16.0% to 35.4% and from 85.1% to 97.5%, respectively.

When patients were classified according to the number of episodes of disease activity throughout the study period, the following distribution was observed: no activity, 22 patients (25.3%); mild activity, 16 patients (18.4%); moderate activity, 23 patients (26.4%); and severe activity, 26 patients (29.9%).

*Table 1*. Prevalence of anti-nucleosome and anti-dsDNA antibodies in patients with SLE during a 12 month observation period.

| Month | No. of Patients | Prevalence of Anti-nucleosome, % (95% CI) | Prevalence of Anti-dsDNA, % (95% CI) |
|---|---|---|---|
| 1 | 87 | 44.8 (34.2–55.5) | 21.8 (13.0–30.7) |
| 2 | 87 | 50.6 (39.9–61.3) | 21.8 (13.0–30.7) |
| 3 | 87 | 44.8 (34.2–55.5) | 18.4 (10.1–26.7) |
| 4 | 87 | 51.7 (41.0–62.4) | 16.1 (8.2–24.0) |
| 5 | 87 | 58.6 (48.1–69.2) | 20.7 (12.0–29.4) |
| 6 | 87 | 52.9 (42.2–63.6) | 20.7 (12.0–29.4) |
| 7 | 87 | 54.0 (43.3–64.7) | 18.4 (10.1–26.7) |
| 8 | 87 | 49.4 (38.7–60.1) | 12.6 (5.5–19.8) |
| 9 | 86 | 51.2 (40.4–61.9) | 16.3 (8.3–24.2) |
| 10 | 83 | 47.0 (36.0–58.0) | 16.9 (8.6–25.1) |
| 11 | 78 | 40.0 (28.6–50.9) | 13.0 (5.2–20.4) |
| 12 | 55 | 40.0 (26.6–53.4) | 10.9 (2.4–19.4) |

*Table 2*. Specificity and sensitivity of anti-nucleosome and anti-dsDNA antibodies for active SLE during a 12 month observation period.

| Evaluation | Antibody | Specificity, % (95% CI) | Sensitivity, % (95% CI) |
|---|---|---|---|
| 1 | Nucleosome | 83.7 (72.2–95.2) | 72.7 (59.0–86.4) |
| | DNA | 100 | 43.2 (27.9–58.4) |
| 2 | Nucleosome | 67.9 (54.9–80.9) | 79.4 (65.1–93.7) |
| | DNA | 88.7 (79.9–97.5) | 38.2 (21.0–55.4) |
| 3 | Nucleosome | 74.5 (62.7–86.4) | 78.1 (63.0–93.3) |
| | DNA | 89.1 (80.6–97.6) | 31.3 (14.3–48.2) |
| 4 | Nucleosome | 67.9 (55.2–80.5) | 87.1 (74.6–99.6) |
| | DNA | 98.2 (94.6–100.0) | 41.9 (23.5–60.3) |
| 5 | Nucleosome | 66.7 (53.3–80.1) | 94.4 (86.6–100) |
| | DNA | 98.0 (94.1–100) | 47.2 (30.1–64.4) |
| 6 | Nucleosome | 71.4 (59.2–83.6) | 96.8 (90.2–100) |
| | DNA | 98.2 (94.6–100) | 54.8 (36.3–73.4) |
| 7 | Nucleosome | 66.7 (54.4–78.9) | 100 |
| | DNA | 96.7 (92.0–100) | 51.9 (31.7–72.0) |
| 8 | Nucleosome | 68.9 (56.9–80.8) | 92.3 (81.3–100) |
| | DNA | 98.4 (95.1–100) | 38.5 (18.4–58.5) |
| 9 | Nucleosome | 72.7 (60.6–84.9) | 93.5 (84.4–100) |
| | DNA | 96.4 (91.3–100) | 38.7 (20.5–56.9) |
| 10 | Nucleosome | 73.7 (61.9–85.5) | 92.3 (81.3–100) |
| | DNA | 94.7 (88.8–100) | 42.3 (22.0–62.7) |
| 11 | Nucleosome | 80.4 (69.6–91.1) | 90.9 (77.9–100) |
| | DNA | 100 | 45.5 (22.9–68.1) |
| 12 | Nucleosome | 79.5 (66.2–92.7) | 87.5 (69.3–100) |
| | DNA | 100 | 37.5 (10.9–64.1) |

The average findings for anti-nucleosome and anti-dsDNA antibodies by level of SLE activity are shown in Figures 2 and 3.

## DISCUSSION

This is the first clinical study to report sequential measurements of anti-nucleosome and anti-dsDNA antibody concentrations in patients with SLE. Patients were followed prospectively for 12 months and tested for anti-nucleosome and anti-dsDNA antibodies by ELISA 8–12 times at an outpatient facility.

Disease activity was observed in 50.6% of patients at baseline, but decreased to 29.1% by the end of the study period, possibly due to improved clinical control of SLE through more frequent medical consultations. Other laboratory findings suggest significant improvements, with a tendency toward increased hematocrit, hemoglobin, and serum albumin levels, and decreased occurrence of hypocomplementemia, reflecting improved disease control.

In our study anti-nucleosome antibodies were more preva-

*Table 3*. Specificity and sensitivity of anti-nucleosome and anti-dsDNA antibodies for active lupus nephritis during a 12 month observation period.

| Evaluation | Antibody | Specificity, % (95% CI) | Sensitivity, % (95% CI) |
|---|---|---|---|
| 1 | Nucleosome | 59.1 (44.4–73.8) | 48.8 (33.7–64.0) |
| | DNA | 90.9 (82.3–99.5) | 34.9 (20.5–49.3) |
| 2 | Nucleosome | 46.2 (30.3–62.0) | 47.9 (33.6–62.2) |
| | DNA | 94.9 (87.9–100.0) | 35.4 (21.7–49.1) |
| 3 | Nucleosome | 52.5 (36.8–68.2) | 42.6 (28.3–56.8) |
| | DNA | 97.5 (92.6–100) | 31.9 (18.4–45.4) |
| 4 | Nucleosome | 48.9 (34.1–63.7) | 52.4 (37.1–67.7) |
| | DNA | 93.3 (86.0–100) | 26.2 (12.7–39.6) |
| 5 | Nucleosome | 48.9 (34.5–63.4) | 67.5 (52.8–82.2) |
| | DNA | 85.1 (74.8–95.4) | 27.5 (13.5–41.5) |
| 6 | Nucleosome | 51.1 (36.6–65.5) | 57.5 (42.0–73.0) |
| | DNA | 87.2 (77.6–96.9) | 30.0 (15.6–44.4) |
| 7 | Nucleosome | 49.0 (35.2–62.9) | 58.3 (42.0–74.7) |
| | DNA | 88.2 (79.3–97.2) | 27.8 (12.9–42.6) |
| 8 | Nucleosome | 48.1 (34.4–61.8) | 45.7 (29.0–62.5) |
| | DNA | 90.4 (82.3–98.5) | 17.1 (4.5–29.8) |
| 9 | Nucleosome | 47.9 (33.6–62.2) | 50.0 (33.9–66.1) |
| | DNA | 87.5 (78.0–97.0) | 21.1 (7.9–34.2) |
| 10 | Nucleosome | 53.1 (38.9–67.2) | 47.1 (30.0–64.1) |
| | DNA | 87.8 (78.5–97.0) | 23.5 (9.1–38.0) |
| 11 | Nucleosome | 67.3 (54.1–80.6) | 51.7 (33.2–70.2) |
| | DNA | 91.8 (84.1–99.6) | 20.7 (5.7–35.7) |
| 12 | Nucleosome | 53.3 (35.2–71.5) | 32.0 (13.3–50.7) |
| | DNA | 93.3 (84.3–100) | 16.0 (1.3–30.7) |

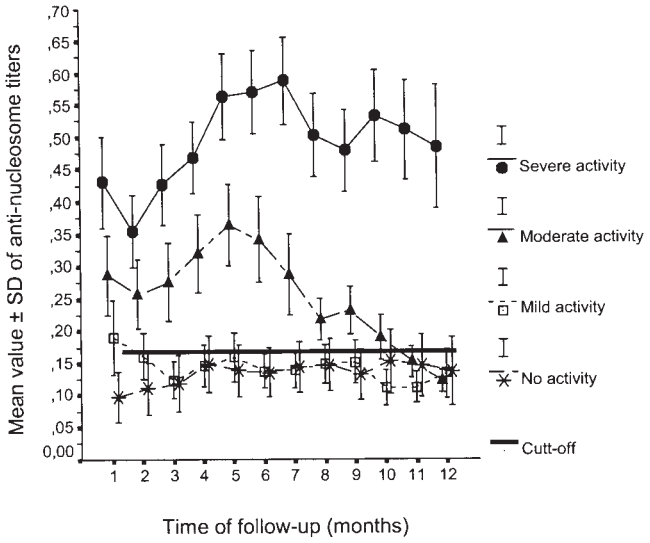


*Figure 2*. Average serum titers (± SD) of anti-nucleosome antibodies according to disease activity (LACC score) in SLE patients during 12 month observation period.

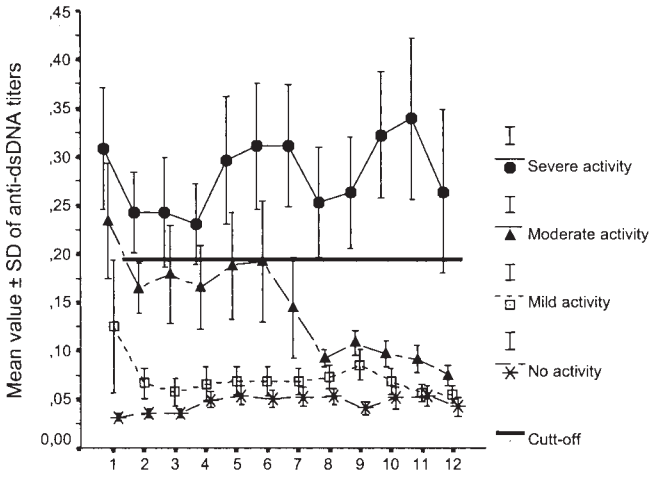


*Figure 3*. Average serum titers (± SD) of anti-dsDNA antibodies according to disease activity (LACC score) in SLE patients during 12 month observation period.

lent than anti-dsDNA antibodies (40.0%–58.8% vs 10.9%–21.8%, respectively). Interestingly, 23.0%–37.8% of patients with negative anti-dsDNA tests were positive for anti-nucleosome antibodies. In other studies dealing with anti-dsDNA-negative sera of SLE patients, 7%–52% of samples were anti-nucleosome antibody-positive[18,19,25,29-32]. Thus, anti-nucleosome antibody reactivity appears to be a useful marker of active SLE in patients who are anti-dsDNA antibody-negative.

In SLE studies reviewed for this report, anti-nucleosome antibodies were found to be more prevalent than anti-dsDNA antibodies (31%–88% vs 21%–82%)[18,19,25,29-32]. However,

differences in prevalence for the same antibody may be caused by the use of different detection techniques[26,33-35].

The association of anti-dsDNA antibodies with active SLE and lupus nephritis is well established. Although nephritis was observed in roughly half the patients at baseline, the prevalence tended to decrease steadily throughout the study period. Similarly, the number of patients with active disease decreased, and were observed in less than one-third of the patients by the end of the study. The highest prevalence of anti-dsDNA antibodies was observed early (first and second evaluations) and halfway through the study period (fifth and sixth evaluations), coinciding with the highest prevalence of active disease. While anti-dsDNA antibodies are mostly present in patients with active SLE, anti-nucleosome antibodies may be detected in active and inactive SLE alike[17,18,25]. Anti-dsDNA antibody levels above the cutoff threshold were almost exclusively observed in patients with severely active disease. In contrast, in most of our patients, anti-nucleosome antibody levels remained elevated at even moderate levels of disease activity.

Our findings draw attention to the fact that anti-nucleosome antibodies are more sensitive than anti-dsDNA antibodies to active SLE (73%–100% vs 31%–55%). Thus, anti-nucleosome antibody reactivity appears to be a potentially valuable tool in the assessment of patients with anti-dsDNA antibody-negative SLE. In a Korean study, 32% of anti-dsDNA antibody-negative patients presented renal disorders and positive anti-nucleosome antibodies[29]. Nephritis was not found in patients who had neither anti-dsDNA nor anti-nucleosome antibodies. Similarly, in our study, anti-nucleosome antibodies were more sensitive than anti-dsDNA antibodies to active nephritis (32.0%–67.5% vs 16.0%–35.4%, respectively). Interestingly, at the first 3 evaluations, when the prevalence of lupus nephritis was higher, no statistically significant difference was observed in the sensitivity rates of the 2 autoantibodies. In this and earlier studies, however, anti-dsDNA antibodies were more specific for active SLE and lupus nephritis. While the specificity of anti-nucleosome antibodies varied from 46.2% to 67.3%, that of anti-dsDNA antibodies ranged from 85.0% to 97.5%.

Thus, considering the high levels of sensitivity observed, anti-nucleosome antibodies may be a useful marker for active SLE and active lupus nephritis, especially in patients who are anti-dsDNA antibody-negative.

## REFERENCES


1. Matsumoto K, Osakabe K, Ohi H, Yoshizawa N, Harada M, Hatano M. Alteration of T-lymphocyte subpopulations in patients with primary renal diseases and systemic lupus erythematosus. Scand J Immunol 1980;11:187-93.
2. Berden JHM. Lupus nephritis. Nephrology Forum. Kidney Int 1997;52:538-58.
3. Spronk PE, ter Borg EJ, Huitema MG, Limburg PC, Kallenberg CG. Changes in levels of soluble T-cell activation markers, sIL-2R, sCD4 and sCD8, in relation to disease exacerbations in patients with systemic lupus erythematosus: a prospective study. Ann Rheum Dis 1994;53:235-59.
4. Ravirajan CT, Rowse L, MacGowan JR, Isenberg DA. An analysis of clinical disease activity and nephritis-associated serum autoantibody profiles in patients with systemic lupus erythematosus: a cross-sectional study. Rheumatology Oxford 2001;40:1405-12.
5. Madaio MP, Hodder S, Schwartz RS, Stollar BD. Responsiveness of autoimune and normal mice to nucleic acid antigens. J Immunol 1984;132:872-6.
6. Amoura Z, Chabre H, Bach JF, Jacob L. The appearance of anti-nucleosome antibodies precedes that of anti-double-stranded DNA and anti-histones in urine lupus. Rev Med Interne 1993;14:980.
7. Amoura Z, Koutousov S, Piette JC. The role of nucleosomes in lupus. Curr Opin Rheumatol 2000;12:369-73.
8. 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.
9. Licht R, van Bruggen MC, Oppers-Walgreen B, Rijke TP, Berden JP. Plasma levels of nucleosomes and nucleosome-autoantibody complexes in murine lupus: effects of disease progression and lipopolysaccharide administration. Arthritis Rheum 2001;44:1320-30.
10. 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.
11. Burlingame RW, Rubin RL, Balderas RS, Theofilopoulos NA. Genesis and evolution of anti-chromatin autoantibodies in murine lupus implicates T-dependent immunization with self-antigen. J Clin Invest 1993;91:1687-96.
12. Amoura Z, Chabre H, Koutouzouv 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.
13. Kramers C, Hylkema MN, van Bruggen MCJ, et al. Anti-nucleosome antibodies complexed to nucleosomal antigens show anti-DNA reactivity and bind to rat glomerular basement membrane in vivo. J Clin Invest 1994;94:568-77.
14. van Bruggen MCJ, Kramers C, Hylkema MN, Smeenk RJ, Berden JH. Significance of anti-nuclear and anti-extra cellular matrix autoantibodies for albuminuria in MRL/l mice. A longitudinal study on plasma and glomerular eluates. Clin Exp Immunol 1996;105:132-9.
15. van Bruggen MCJ, Kramers C, Walgreen B, et al. Nucleosomes and histones are present in glomerular deposits in human lupus nephritis. Nephrol Dial Transplant 1997;12:57-66.
16. Ren Y, Tang J, Mok MY, Chan AW, Wu A, Lau CS. Increased apoptotic neutrophils and macrophages and impaired macrophage phagocytic clearance of apoptotic neutrophils in systemic lupus erythematosus. Arthritis Rheum 2003;48:2888-97.
17. Amoura Z, Piette JC, Chabre H, et al. Circulating plasma levels of nucleosomes in patients with systemic lupus erythematosus: correlation with serum anti-nucleosome antibody titers and absence of clear association with disease activity. Arthritis Rheum 1997;40:2217-25.
18. Amoura Z, Koutouzov S, Chabre H, et al. Presence of anti-nucleosome autoantibodies in a restricted set of connective tissue diseases: anti-nucleosome antibodies of the IgG3 subclass are markers of renal pathogenicity in systemic lupus erythematosus. Arthritis Rheum 2000;43:76-84.
19. Cervera R, Viñas O, Ramos Casals M, et al. Anti-chromatin antibodies in systemic lupus erythematosus: a useful marker for lupus nephropathy. Ann Rheum Dis 2003;62:431-4.
20. Benucci M, Gobbi FL, Del Rosso A, Cessaretti S, Niccoli L,

Cantini F. Disease activity and anti-nucleosome antibodies in systemic lupus erythematosus. Scand J Rheumatol 2003;32:42-5.
21. Tan EM, Cohen AS, Fries JF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271-7.
22. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus [letter]. Arthritis Rheum 1997;40:1725.
23. Urowitz MB, Gladman DD, Tozman EC, Goldsmith CH. The Lupus Activity Criteria Count (LAAC). J Rheumatol 1984;11:783-7.
24. Hawker G, Gabriel S, Bombardier C, Goldsmith C, Caron D, Gladman D. A reliability study of SLEDAI: a disease activity index for systemic lupus erythematosus. J Rheumatol 1993;20:657-60.
25. Chabre H, Amoura Z, Piette JC, Godeau P, Bach JF, Koutouzov S. Presence of nucleosome-restricted antibodies in patients with systemic lupus erythematosus. Arthritis Rheum 1995;38:1485-91.
26. Yager TD, McMurray CT, van Holde KE. Salt-induced release of DNA from nucleosome core particles. Biochemistry 1989;28:2271-81.
27. Koutouzov S, Cabrespines A, Amoura Z, Chabre H, Lotton C, Bach JF. Binding of nucleosome to a cell surface receptor: redistribution and endocytosis in the presence of lupus antibodies. Eur J Immunol 1996;26:472-86.
28. Burlingame RW, Rubin RL. Subnucleosome structures as substrates in enzyme-linked immunosorbent assays. J Immunol Methods 1990;134:187-99.
29. Min DJ, Kim SJ, Park SH, et al. Anti-nucleosome antibody significance in lupus patients lacking anti-double stranded DNA antibody. Clin Exp Rheumatol 2002;20:13-8.
30. Mohan C, Liu F, Xie C, Williams RC. Anti-subnucleosome reactivities in systemic lupus erythematosus patients and their first-degree relatives. Clin Exp Immunol 2001;123:119-26.
31. Ghillani-Dalbin P, Amoura Z, Cacoub P, et al. Testing for anti-nucleosome antibodies in daily practice: a monocentric evaluation in 1696 patients. Lupus 2003;12:833-7.
32. Cairns AP, McMillan SA, Crockard AD, et al. Anti-nucleosome antibodies in the diagnosis of systemic lupus erythematosus. Ann Rheum Dis 2003;62:272-3.
33. Riboldi P, Gerosa M, Moroni G, et al. Anti-DNA antibodies: a diagnostic and prognostic tool for systemic lupus erythematosus? Autoimmunity 2005;38:39-45.
34. Janyapoon K, Jivakanont P, Surbrsing R, Siriprapapan W, Tachawuttiwat T, Korbsrisate S. Detection of anti-dsDNA by ELISA using different sources of antigens. Pathology 2005;37:63-8.
35. Rubin RL, Bell SA, Burlingame RW. Autoantibodies associated with lupus induced by diverse drugs target a similar epitope in the (H2A-H2B)-DNA complex. J Clin Invest 1992;90:165-73.