Detection of anti-nucleosome antibodies in a routine clinical laboratory setting

Sirs,

The Farr assay and the Crithidia luciliae assay are well-established and specific tests for the diagnosis of systemic lupus erythematosus (SLE). The sensitivity of the Farr assay and the Crithidia luciliae assay, however, is limited. Anti-nucleosome antibodies are also reported to be highly specific for SLE (> 95%) (1-5). Moreover, the sensitivity of anti-nucleosome antibodies (56%-86%) is described to be higher than the sensitivity of anti-dsDNA antibodies (1-6). As anti-nucleosome antibodies are specific and more sensitive than anti-dsDNA antibodies, they may have an additional diagnostic value in comparison to anti-dsDNA testing. A number of SLE patients (±20%) lacking anti-dsDNA antibodies display anti-nucleosome antibodies (1, 5, 6).

The objective of the present study was to evaluate the added diagnostic value of determining anti-nucleosome antibodies for SLE. Therefore we studied the medical conditions that are associated with anti-nucleosome antibodies in the absence or presence of anti-dsDNA antibodies in a routine clinical laboratory setting. Such studies are scarce (6), as most studies dealing with the diagnostic characteristics of anti-nucleosome antibodies have been performed on well-defined, selected patient populations, mostly a restricted set of connective tissue diseases.

Consecutive samples submitted to the clinical laboratory for detection of antinuclear antibodies in which indirect immunofluorescence (HEp-2000, Immunoconcepts) revealed a homogeneous pattern with positive staining of the chromosomes (titer 1:640 or higher) were tested for anti-dsDNA antibodies (Farr assay [Trinity Biotech]) and anti-nucleosome antibodies (Euroimmun). The results are shown in Table I.

Table I. Medical conditions associated with samples that displayed a homogenous pattern on indirect immunofluorescence (titer: 1:640 or higher).

Anti-dsDNA negative	Nucleosome positive n=33 (%)	Nucleosome negative n=54 (%)
Systemic lupus erythematosus	10 (30)	4 (7.5)
Rheumatoid arthritis	2 (6)	2 (4)
Rheumatoid arthritis (Infliximab)	8 (24)	11 (20)
Reactive (poly)arthritis	3 (9)	
Juvenile chronic/idiopathic arthritis		5 (9)
Psoriatic arthropathy		1 (2)
Polymyalgia rheumatica		1 (2)
Crohn's disease (infliximab)	3 (9)	5 (9)
Colitis ulcerosa	2 (6)	1 (2)
Indeterminate colitis	1 (3)	
Dermatomyositis (paraneoplastic)	1 (3)	1 (2)
Scleroderma	1 (3)	4 (7)
Wegener's disease	1 (3)	
Erythema nodosum		1 (2)
Arteritis temporalis		1 (2)
Raynaud's phenomenon		1 (2)
Deafness		1 (2)
Pancreatitis		1 (2)
Hypothyroidy		2 (4)
Drug-induced hepatitis		1 (2)
Malignant disease*		6 (11)
No evidence for systemic disease		4 (7)
No clinical information	1 (3)	1 (2)
Anti-dsDNA positive	**Nucleosome positive n=25 (%)**	**Nucleosome negative n=24 (%)**
Systemic lupus erythematosus	21 (84)	5 (21)
Primary Sjögren's syndrome	1 (4)	
Rheumatoid arthritis	1 (4)	
Rheumatoid arthritis (infliximab)	1 (4)	12 (50)
Crohn's disease (infliximab)	1 (4)	5 (21)
Autoimmune hepatitis		2 (8)

*:T-cell large granular lymphoma, nasopharynxcarcinoma, metastased ovarium carcinoma, metastased papillar adenocarcinoma, invasive mamma carcinoma, invasive adenocarcinoma.

In 87 patients (20 males and 67 females; age range 1-92 years [median: 52 year]), no anti-dsDNA antibodies were present (<7 IU/mL). Anti-nucleosome antibodies were found in 33 of these patients. In 10 (30%) of the 33 positive patients, medical records revealed SLE. Ten (30%) patients had rheumatoid arthritis, eight of which received infliximab. Three patients had reactive (poly) arthritis. Six (18%) patients had inflammatory bowel disease, three of which received Infliximab. An additional three patients had paraneoplastic dermatomyositis, scleroderma, and Wegener's disease. Taken together, in the anti-dsDNA negative group, anti-nucleosome antibodies were detected not only in SLE patients, but also in other diseases including patients with rheumatoid arthritis and Crohn's disease receiving anti-TNF-alpha inhibitor (Infliximab).

In 49 patients, anti-dsDNA antibodies were found (Farr assay > 20 IU/mL). Twenty-five (51%) samples were anti-nucleosome positive whereas twenty-four (49%) samples were anti-nucleosome negative. Twenty-one (84%) of the 25 anti-nucleosome positive samples were from patients with SLE, whereas only 5 (21%) of the 24 anti-nucleosome negative samples were from patients with SLE ($p<0.0001$ χ^2). Seventeen (71%) of the 24 anti-dsDNA positive anti-nucleosome negative patients received TNF-alpha inhibitor therapy. The observation that anti-dsDNA antibodies in the absence of anti-nucleosome antibodies were associated with patients receiving anti-TNF-alpha therapy is consistent with the fact that patients receiving anti-TNF therapy produce IgM anti-dsDNA antibodies detected by the Farr assay (7).

Thirty-one of 40 SLE patients had anti-nucleosome antibodies. Proteinuria was present in 16 of the 31 anti-nucleosome positive patients and in none of the nine anti-nucleosome negative patients (χ^2: p=0.02). These data are in line with previous reports (9, 10) suggesting that anti-nucleosome reactivity is associated with lupus nephritis and disease flare.

In our study, anti-dsDNA antibodies had a sensitivity of 65% and a specificity of 75.8% for SLE and anti-nucleosome antibodies had a sensitivity of 77.5% and a specificity of 72.6% for SLE.

In conclusion, our data confirm previous observations that anti-nucleosome antibodies are found in anti-dsDNA negative SLE patients. However, anti-nucleosome antibodies are not exclusively associated with SLE.

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Letters to the Editor

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References

1. SUER W, DÄHRICH C, SCHLUMBERGER W *et al.*: Autoantibodies in SLE but not in scleroderma react with protein-stripped nucleosomes. *J Autoimmunity* 2004; 22: 325-34.
2. AMOURA Z, KOUTOUZOV, CHABRE H *et al.*: Presence of antinucleosome autoantibodies in a restricted set of connective tissue diseases. *Arthritis Rheum* 2000; 43: 76-84.
3. GHIRARDELLO A, DORIA A, ZAMPIERI S *et al.*: Antinucleosome antibodies in SLE: a two-year follow-up study of 101 patients. *J Autoimmunity* 2004; 22: 235-40.
4. CAIRNS AP, MCMILLAN SA, CROCKARD AD *et al.*: Antinucleosome antibodies in the diagnosis of systemic lupus erythematosus. *Ann Rheum Dis* 2003; 62: 272-3.
5. 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.
6. 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.
7. DE RYCKE L, BAETEN D, KRUITHOF E, VAN DEN BOSCH F, VEYS EM, DE KEYSER F: Infliximab, but not etanercept, induces IgM anti-double-stranded DNA autoantibodies as main antinuclear reactivity: biologic and clinical implications in autoimmune arthritis. *Arthritis Rheum* 2005; 52: 2192-201.
8. BENUCCI M, GOBBI FL, DEL ROSSO A, CESARETTI S, NICCOLI L, CANTINI F: Disease activity and antinucleosome antibodies in systemic lupus erythematosus. *Scand J Rheumatol* 2003; 32: 42-5.
9. CERVERA R, VINAS 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.
10. NG KP, MANSON JJ, RAHMAN A, ISENBERG DA: Association of antinucleosome antibodies with disease flare in serologically active clinically quiescent patients with systemic lupus erythematosus. *Arthritis Rheum* 2006; 55: 900-4

Erratum Corrige

The authors of the Imaging article in our previous issue (*Clin Exp Rheumatol 2008; 26: 1-4)* have brought to our attention that they had not included the abstract, which we herewith print as follows:

Ultrasound imaging for the rheumatologist
XIII. New trends. Three-dimensional ultrasonography
E. Filippucci, G. Meenagh, O. Epis, A. Iagnocco, L. Riente, A. Delle Sedie, C. Montecucco, G. Valesini, S. Bombardieri, W. Grassi

ABSTRACT
Despite its indubitable potential, ultrasonography still has limited diffusion in rheumatology related principally to the image acquisition process due to at least five main factors: the steep learning curve, lack of standardisation of the technique, intra- and inter-observer variability, time consumption and the high initial cost of top quality sonographic equipment. Of all these barriers, the first four are undoubtedly the most difficult to overcome. This review discusses the available evidence supporting the potential of three-dimensional ultrasound with high-frequency volumetric probe to overcome the first four barriers. The challenge to three-dimensional ultrasound is to prove itself to be a method that requires no particular skills, that can be mastered in just a few minutes and is not operator-dependant.