

ANTI-NUCLEOSOME ANTIBODIES – A NOVEL TEST IN SYSTEMIC LUPUS ERYTHEMATOSUS

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In this study the serum levels of specific anti-nucleosome autoantibodies (autoAb) were measured in the follow up of the group of SLE patients and compared to the levels of anti-dsDNA, C3, C4 components of complement, to ECLAM- European Lupus Activity Measure and to the levels of sVCAM-1, sICAM-1, neopterin, sIL-2r, IL-10 and fas ligand. Anti-nucleosome antibodies were detected using an enzyme immunoassay for the quantitative determination of autoAb to nucleosomes and their components in the human serum in the group of 52 patients, 50 of them female, who all fulfilled criteria of SLE according to ACR (American College of Rheumatology). Anti-nucleosome antibodies were followed up during 3 subsequent measurements during six months and compared to the development of ECLAM index, the levels of anti ds-DNA antibodies and C3, C4 complement components levels and other activity markers.

The probands with the stable low disease activity had the lowest levels of anti-nucleosome Ab (83 ± 33.0 IU/ml). The patients with the stable high or increasing activity had the mean titers in the first series 200.42 ± 36 IU/ml and 192 ± 65 IU/ml and 188 ± 58.11 IU/ml in the 2. and 3. serial measurement. Subgroup with the high starting activity and with decrease of the activity index had the mean levels of 223 ± 39.0 IU/ml, 185.6 ± 37.6 IU/ml and 207 ± 55.5 IU/ml during three measurements. In the subgroup of patients with lupus nephritis (LN) $n=15$, the anti-nucleosome Ab levels were 176 ± 34.0 IU/ml, 174 ± 30.0 IU/ml and 148 ± 32.0 IU/ml. A strong correlation was found between levels of anti-nucleosome and anti-dsDNA antibodies. All sera with anti-dsDNA antibodies over 40 had at the same time positive titer of anti-nucleosome antibodies, however, 21 sera with negative anti-dsDNA had positive test for anti-nucleosome antibodies. Anti-nucleosome antibodies correlated with ECLAM activity index and reversibly with C4. No correlation was established with levels of C3, sVCAM-1, sICAM-1, IL-10, fas ligand, neopterin and sIL2r. The examination of anti-nucleosome Ab contributes to the early diagnosis and is an instrument in the differential diagnosis of the connective tissue diseases and it seems to be especially valuable in anti-dsDNA negative lupus. The use of anti-nucleosome antibodies measurement as a marker of disease activity deserves further evaluation.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a disease characterized by the B cell hyperactivity associated with production of autoantibodies directed against components of cell and by tissue depositions of immune complexes, leading to organ damage¹.

Antibodies against double-stranded DNA (dsDNA) present in 40–60% of cases of the human lupus as well as in the mouse lupus model are the key marker of SLE and their plasma levels tend to fluctuate according to the disease activity. Anti-dsDNA/DNA immune complexes have been considered responsible for the development of lupus nephritis^{2, 3}. But these complexes are difficult to demonstrate and murine and human lupus sera showed low levels of circulating DNA immune complexes^{4, 5}. Currently, the evidence has accumulated on the important role, which defect apoptosis might

play in SLE pathogenesis^{3, 6, 7}. At the same time the attention is attracted to the nucleosome^{8, 9}, the unit of chromatin and the product of cell apoptosis. The nucleosome consists of the core particle composed of an octamer of histones around which the helical DNA is wrapped and histone H1, which is located at the point, where DNA enters and exits the nucleosome¹⁰. Some studies have demonstrated the ability of anti-dsDNA and antihistone lupus antibodies to react with certain target epitopes of the nucleosome¹¹. Specific anti-nucleosome antibodies which only target the native nucleosome particle, but not the individual components (DNA anti histone), occur early in the disease and precede the formation of anti-dsDNA and antihistone antibodies^{12, 13}. The nucleosome has been identified to be the most important immunogen in SLE pathogenesis, but the clinical significance of specific anti-nucleosome antibodies has not been established yet. The serum reactivity to nucleosome was demonstrated in 70–80% of lupus

patients, which is far more than positivity of anti-dsDNA antibodies and can be demonstrated in the early phase of the disease¹⁴.

In this study we measured in the follow up of the group of SLE patients the serum levels of specific anti-nucleosome antibodies and compared them to the levels of anti-dsDNA, C3, C4 components of complement, to disease activity index (ECLAM- European Lupus Activity Measure)¹⁵ and to the levels of some newer SLE activity markers as molecules sVCAM-1, sICAM-1, neopterin, sIL-2r IL-10, fas ligand and thrombomodulin.

MATERIALS AND METHODS

Patients

Anti-nucleosome antibodies were measured in the group of 52 patients, 50 of them female, mean age 39 years (20–73), mean duration of disease 4 years (0–20), who all fulfilled at least 4 criteria of SLE according to ACR (16). Anti-nucleosome antibodies were followed up during 3 subsequent measurements during six months and compared to the development of ECLAM system, the levels of anti ds-DNA antibodies and the C3, C4 components of complement levels.

The patients received the following treatment: Twelve patients received corticosteroid in low dose (prednisone ≤ 7.5 mg/day), four patients antimalarials, one patient methotrexate and one patient azathioprine as the single treatment. The rest of the group received the combined drug treatment: Five patients antimalarials and low dose corticosteroid, one patient methotrexate and corticosteroid > 7.5 mg/day, ten patients azathioprine and corticosteroid > 7.5 mg/day, ten patients cyclophosphamide with corticosteroid > 7.5 mg/day and eight patients cyclosporin A with corticosteroid > 7.5 mg/day. All patients treated with cyclophosphamide (10) had active lupus nephritis, four of those were classified as III.B, two were IV.B according to WHO classification, the renal biopsy was not performed in four patients. In patients treated with cyclosporin (8) the lupus nephritis was present in 5 of them, and was documented by histology in 3 patients (one III.A, one IV.B, one V.A)

Methods

Anti-nucleosome antibodies were detected with commercial test Medizym anti-Nucleo (Medipan Diagnostica, Selchow, Germany). It is an enzyme immunoassay for the quantitative determination of autoAb to nucleosomes and their components in human serum. AutoAb of diluted patient samples and standard react with nucleosome antigen immobilized on the solid phase of a microtiter plate. Medizym guarantees the specific binding of nucleosome autoAb of the specimen under investigation by employing highly purified nucleosome antigen for coating. The interpretation of quantitative

evaluation of antibodies is the following: ≥ 25 IU/ml positive, < 25 IU/ml negative.

Anti ds-DNA antibodies were detected also by ELISA (set Ubi-Magiwel, Mountain View, CA, USA) with the following evaluation: < 30 IU/ml negative, 30–40 IU/ml borderline positive, > 40 IU/ml positive.

The levels of C3 and C4 components of complement were measured by nephelometry using the diagnostic antiserum Behringer AG, Marburg, Germany, with normal values for C3 0.65–1.45 g/l, for C4 0.18–0.50 g/l.

Commercial ELISA sets were used for measurement of sVCAM-1, sICAM-1 (both R&D Systems, Abingdom, UK), neopterin (Milenia, DPPC, LA, USA), sIL-2r (Immunotech International, Marseille, France), IL-10 (R&D Systems, Abingdom, UK) and fas ligand (MBL Co., Nagoya, Japan). Pearson correlation test and ANOVA test were used for statistical evaluation of results.

RESULTS

According to ECLAM index the group could be divided into 3 subgroups with

1. Stabilized disease activity (ECLAM index ≤ 3 and the difference in ECLAM index during the period of follow up ≤ 1) – subgroup A 22 patients
2. High and/or rising activity (ECLAM > 3 and/or the difference $> +1$ in ECLAM score) – subgroup B 12 patients
3. Declining activity (ECLAM > 3 and the difference between ECLAM score during the follow up ≤ -1) subgroup C 18 patients.

Anti-nucleosome antibodies: The mean level of anti-nucleosome antibodies during the first series of measurement were 159.0 ± 33 IU/ml, during the second 157 ± 22.0 IU/ml and during the third 165 ± 33.0 IU/ml. A strong correlation was found between the levels of anti-nucleosome and anti-dsDNA antibodies ($r = 0.58$, $p = 0.000006$). All sera with anti-dsDNA antibodies over 40 had at the same time positive titer of antinucleosome antibodies, however, 21 sera with negative anti-dsDNA had positive test for anti-nucleosome antibodies. Weaker but still significantly anti-nucleosome antibodies correlated with ECLAM activity index ($r = 0.32$, $p = 0.01$) and reversibly with C4 ($r = -0.30$, $p = 0.02$) See fig. 1. There was some trend toward relation with C3 ($r = -0.23$, $p = 0.09$) and sICAM ($r = 0.23$, $p = 0.09$), which did not reach the level of 5% significance. No correlation was established with levels of sVCAM, IL-10, fas ligand, neopterin and sIL2r.

In three subgroups of patients divided according to ECLAM activity the following results were obtained: the subgroup A with 22 patients with the stable low disease activity had the mean levels of anti nucleosome antibodies 83 ± 33.0 IU/ml in 1. series of measurement, followed by 55.62 ± 22.50 IU/ml and 35.71 ± 13.45 IU/ml in the second respectively third series. Subgroup B with 12 patients characterized by the stable high or increa-

sing activity the mean anti-nucleosome Ab titers were during the first series 200.42 ± 36.0 IU/ml and 192 ± 65 IU/ml and 188 ± 58.11 IU/ml in the 2. and 3. serial measurement. Finally, subgroup C (18 patients) with the high starting activity and with decreasing trend of ECLAM activity index during the follow up, as defined above, had the mean levels of anti-nucleosome plasma activity of 223 ± 39.0 IU/ml, 185.6 ± 37.6 IU/ml and 207 ± 55.5 IU/ml during three measurements. In subgroup of patients with lupus nephritis (LN) $n = 15$, the anti-nucleosome levels were 176 ± 34.0 IU/ml, 174 ± 30.0 IU/ml and 148 ± 32.0 IU/ml.

A significant differences in anti-nucleosome Ab levels were found between the subgroups A and B ($p = 0.007$), A and C ($p = 0.001$) and A and LN ($p = 0.002$), but not between B and C and LN, and either series of measurement showed significant changes in antibody levels. For comparison of the characteristics of subgroups and results see Table 1 and 2 and Fig. 2.

In seven patients the anti-nucleosome antibodies could be obtained at five or six occasions. The comparison of anti-nucleosome and anti-dsDNA antibody profiles is in the Fig. 3.

Table 1. Anti-nucleosome levels in subgroup A, B, C, LN in the serial measurement

subgroup and series	anti nucleosome Ab/ \bar{x}	Min-max	Median M	Stand. dev. δ
A I.	81.0	12–644	15.0	149.6
A II.	55.6	12–144	17.0	62.6
A III.	35.7	11–109	25.5	35.5
B I. *	200.4	12–625	184.5	184.5
B II. *	192.0	12–527	180	207.7
B III. *	188	12–400	185	174.0
C I. *	223.7	12–539	255	164.5
C II. *	185.6	12–518	190	151.2
C III. *	207	15–478	190	166.5
LN I. *	176.8	12–414	190.0	162.3
LN II. *	174.4	12–471	185	148.2
LN III. *	148	12–478	140	142

min–max – minimal and maximal values

stand. dev. – standard deviation

ANOVA statistic, <0.05

* statistically significant difference between subgroups B,C and LN on one and subgroup A on the other side during three series of measurements.

Table 2. Characteristics of subgroups A, B, C LN (A – stabilized, B high and/or increasing activity, C – decreasing activity according to ECLAM system; LN patients with lupus nephritis) I, II, III. serial measurement

Subgroup and series	ECLAM / \bar{x}	dsDNA Ab IU/ml, / \bar{x}	C3 / \bar{x} g/l	C4 / \bar{x} g/l
A I.	1.7	22.29 (± 20.0)	0.77	0.19
A II	1.7	10.5 (± 14.2)	0.76	0.18
A III.	1.8	13.25 (± 10.0)	0.75	0.19
B I.	3.7	85.12 (± 79.1)	0.60	0.15
B II.	4.2	109.9 (± 62.1)	0.60	0.14
B III.	4.4	90.37 (± 62.9)	0.65	0.14
C I.	4.0	77.83 (± 77.8)	0.56	0.15
C II.	3.0	57.81 (± 54.6)	0.61	0.16
C III.	2.4	39.91 (± 21.0)	0.68	0.17
LN I.	5.0	90.07 (± 76.5)	0.50	0.14
LN II.	3.3	73.53 (± 60.97)	0.59	0.12
LN III.	3.4	75.44 (± 55.0)	0.58	0.15

DISCUSSION

The results of the measurement and the statistical analysis are encouraging in respect of the practical use of anti-nucleosome antibodies as a potential marker of SLE. Serum levels of anti-nucleosome antibodies correlate with anti ds-DNA antibodies, which is a recognized test for SLE and also one of the ACR criteria for the disease. Nevertheless, there is not a complete overlap of positivity for these two tests. High levels of anti-nucleosome antibodies have been found in 21 sera with no anti-ds-DNA reactivity, however, not *vice versa*. Anti-nucleosome specific antibodies represent the early marker of SLE recognizing conformational epitopes shared by the native nucleosome molecule¹³. Later, the autoimmune response can diversify to the components of the nucleosomes, DNA and histones as a part of the intermolecular spreading. Anti nucleosome antibodies are also relatively specific for SLE, besides SLE they were detected just in the mixed connective tissue disease (MCTD) and systemic scleroderma¹⁴. They were absent in patients with inflammatory myopathies, primary Sjögren's syndrome, RA, primary antiphospholipid syndrome, Wegener's granulomatosis, giant cell arteritis, Takayasu arteritis, relapsing polychondritis, sarcoidosis, Behcet disease and hepatitis C virus infection¹⁴.

The significant correlation with ECLAM index and C4 demonstrates anti-nucleosome Ab as a possible marker of the disease activity, although according to the results of this study anti-dsDNA antibodies seem to response to the changing disease activity better. In the subgroup of patients with stabile disease with low activity the trend to low levels of anti-nucleosome antibodies was observed, thus this subgroup significantly differed

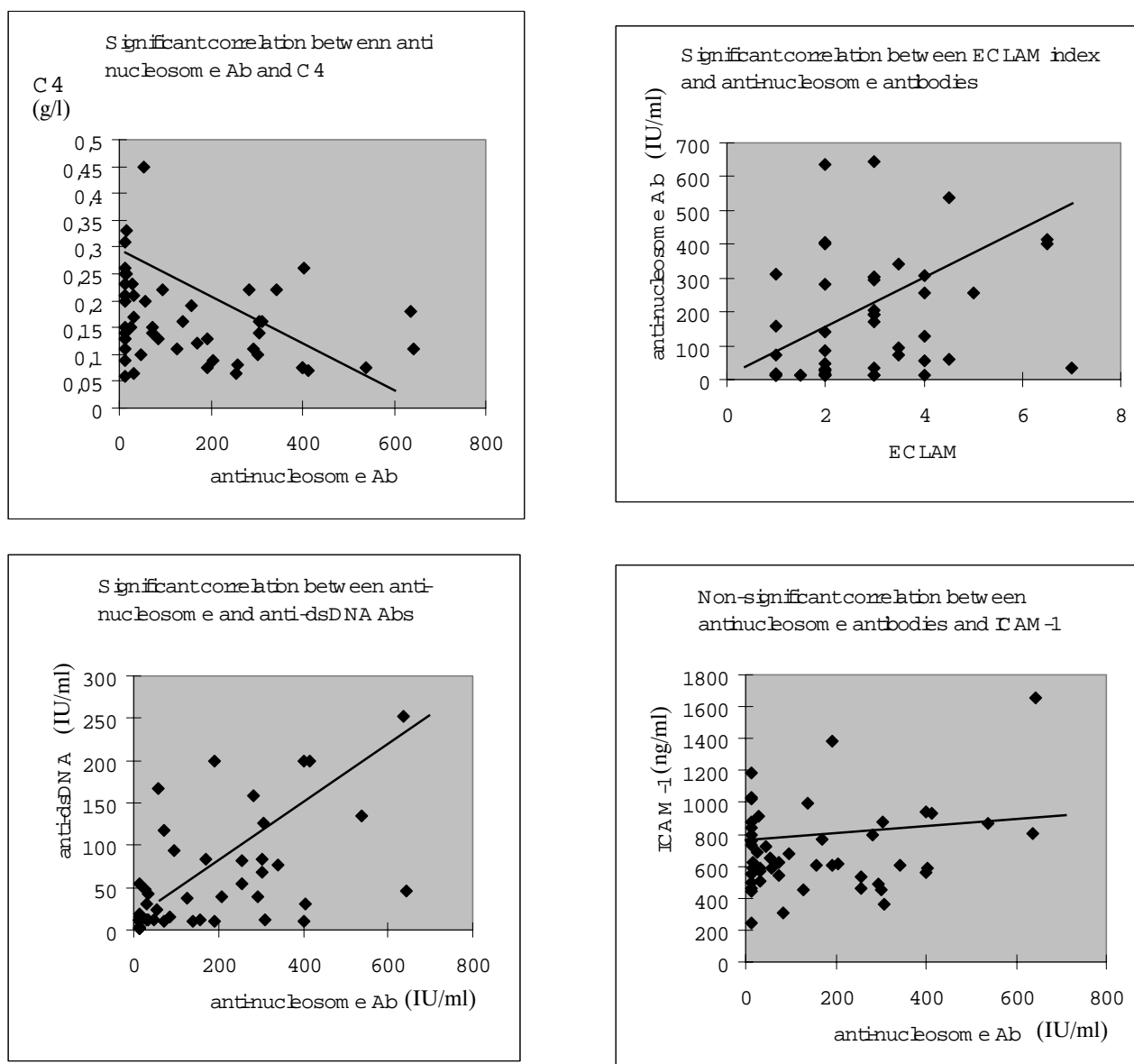


Fig. 1 Correlations associated with anti-nucleosome antibodies in the group of 52 lupus patients

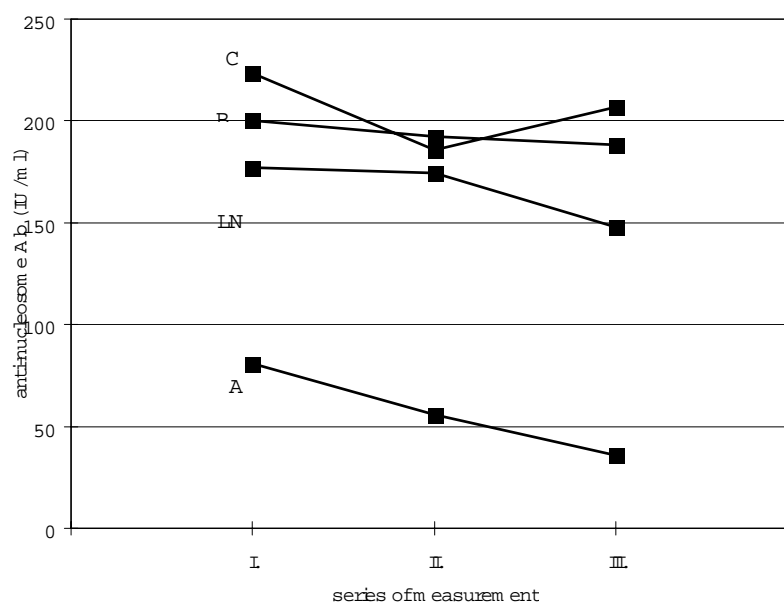


Fig. 2 Development of anti-nucleosome antibodies mean levels in patients with stable (A), rising (B) and decreasing (C) disease activity index and in patients with lupus nephritis (LN)

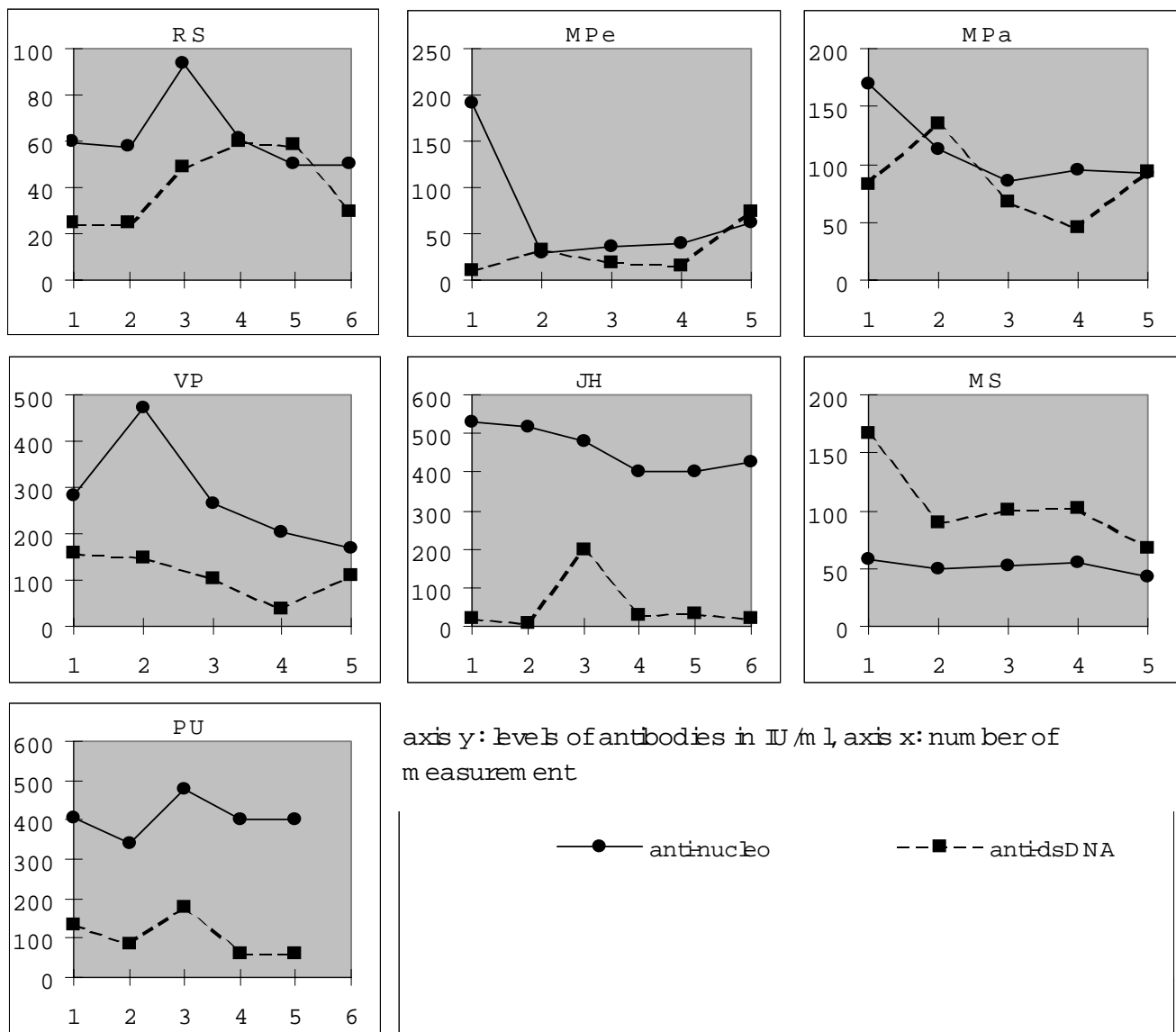


Fig. 3 Development of anti-nucleosome and anti-dsDNA antibodies in seven patients during the five of six measurements

from the others (active disease, lupus nephritis). In the subgroup of probands with decreasing disease activity according to ECLAM system and with response seen also in anti-dsDNA levels, no significant decline in anti-nucleosome antibodies was observed.

The high levels of anti-nucleosome antibodies were also found in patients with lupus nephritis regardless of their overall disease activity. It supports the concept of nucleosome and anti-nucleosome complexes as the major pathogenic agent in lupus nephritis^{2, 9, 17, 18}.

One of the most striking features of SLE is the production of auto-antibodies targeting the chromatin components as deoxyribonucleic acid, ribonucleic acid and histones. Mammalian DNA was proved to be not immunogenic, immunization with mammalian DNA does not induce pathogenic anti DNA antibodies¹⁹. Burlingame et al. were the first to show that the production of anti-chromatin antibodies is induced by T cell

dependent immune response with selfantigen²⁰. Recent findings in murine and human lupus strongly suggest that the initial autoimmune response is directed against nucleosomes^{17, 18}.

There are many unanswered question remaining, such as the timing of anti dsDNA production after the immune answer to nucleosome, the predictive value of anti-nucleosome antibodies for lupus flare and the mechanisms of contributions of various antibodies to the development of tissues damage. Understanding to the last could have in the future a major impact not only on our understanding the lupus pathogenesis, but can help to improve the treatment of SLE.

In conclusion it should be emphasized that the determination of anti-nucleosome as the earliest antibody in SLE contributes to the diagnosis and is an instrument in differential diagnoses of connective tissue diseases and it seems to be especially valuable in anti-dsDNA

negative lupus. Use of anti-nucleosome antibody measurement as a marker of disease activity deserves further evaluation.

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