Asian Pacific Journal of Allergy and Immunology (1989) 7 : 107-112

Antibodies to Extractable Nuclear Antigens in Connective Tissue Disorders in India : Prevalence and Clinical Correlations

Ram R. Singh*, Anand N. Malaviya**, S. Kailash+, T. Varghese**, H. Singh** and K.R. Sundaram‡

SUMMARY **Antibodies to Extractable Nuclear Antigens (ENAs) namely Sm, nRNP, SS-A and SS-B were studied in 397 patients with various connective tissue diseases (CTD), 146 patients with inflammatory polyarthropathies, 16 cases of systemic vasculitides, and 39 normal subjects using counterimmunoelectrophoresis and double immunodiffusion methods. Anti-ENA antibodies were positive in 40.8 percent cases of Systematic lupus erythematosus (SLE) (n = 191), 36.4 percent of overlap CTD (OCTD, n = 44), 27.8 percent of Sjogren's syndrome (n = 18), 10.6 percent of progressive systemic sclerosis (PSS, n = 66) and 2.7 percent of rheumatoid arthritis (n = 111) patients. The correlation of these antibodies with disease features was done. The significant finding was negative association of anti-nRNP antibodies (when present alone) with renal involvement. Anti-Sm antibodies did not correlate with any disease feature. The other associations included correlation of anti-nRNP with pulmonary parenchymal lesions, anti-SS-A with serositis and pulmonary hypertension, and anti-SS-B with myocarditis and recurrent diarrhoea.**

We conclude that Anti-ENAs may correlate with certain subsets of these diseases but the subject is controversial.

Autoantibodies to nuclear antigens (ANAs) are considered a hallmark of systemic lupus erythematosus (SLE) and related connective tissue diseases (CTDs).[1,2] Since the original observation of Hollman and Deicher regarding antibodies to extractable nuclear antigens (ENAs),[3] several laboratories around the world have shown wide heterogeneity of ANAs reactive with different nuclear components and nuclear proteins.[1-8] Considering that SLE and related CTDs show marked clinical variability and severity from patient to patient, it has been tempting to correlate the specific anti-ENA with particular disease subjects. Several such studies have appeared recently describing conflicting results.[2,4,9,12]

Recently we have reported certain differences in the clinical profile of SLE in Indian patients especially the severity and short survival.[13] In the present work an attempt was made (1) to study the anti-ENAs with different reactivity, and (2) to find the clinical correlation of anti-ENA antibodies in Indian patients with SLE.

MATERIAL AND METHODS

Subjects:

Patients were selected from the Immunology Clinic of Department of Medicine, All India Institute of Medical Sciences. One hundred and ninety one consecutive SLE patients diagnosed at this clinic were included in this work. Controls were patients with other connective tissue diseases, inflammatory polyarthritides, systemic vasculitides and other diseases, and normal healthy volunteers from among the laboratory staff (Table 1).

The diagnosis of SLE and other conditions was based on standard criteria (American Rheumatism Asso-

From the *Department of Immunology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Post Box No. 375, Lucknow-226 001; **Clinical Immunology Section, Department of Medicine, +Department of Biophysics and ‡Department of Biostatistics, All India Institute of Medical Sciences, New Delhi-110029, India.

Correspondence : Dr. A.N. Malaviya, Clinical Immunology Section, Department of Medicine, All India Institute of Medical Sciences, New Delhi-110029, India.

ciation criteria where available; and criteria mentioned in Text book of Rheumatology by Kelly *et al* for other diseases).[14] The work up included a detailed clinical evaluation, marker antibodies (antinuclear antibody by standard immunofluorescence, anti-double stranded DNA (dsDNA) by ELISA and rheumatoid factor etc.) wherever available, biopsies whenever required and other invasive and noninvasive procedures for unequivocal establishment of the diagnosis. The disease features of most of the patients included in present study have been published earlier.[15]

Screening for antibodies to extractable nuclear antigens was performed by counterimmunoelectrophoresis (CIE) as previously described by Venables, *et al.*[16] In short, wells of 4 mm diameter were set in rows, 5 mm apart, in 13 ml of 1 percent agarose in barbitone buffer (0.025M, pH 8.4) poured onto 80 mm photographic plates. Twenty microliters of sera were placed in the anodal well and twenty microliters of antigen in the cathodal well. A current of 13 mA per plate at 110 V was applied for 45 min. The plates were examined for precipitin lines immediately after electrophoresis and again after incubation at 4° C for 18 hours. Antigen sources used were a saline extract of rabbit thymus powder (Pelfreeze Biologicals, Rogers, Arkansas, USA), native or digested with RNase or trypsin, and a saline extract of human spleen.

Double immunodiffusion was performed on all ANA or CIE positive sera to confirm the specificity of antibodies to soluble antigens. Wells 5 mm in diameter (3 mm apart) were cut in 0.5 percent agarose in PBS. Six wells were grouped around a central well containing the antigen. Reference sera were placed in two of the wells and test sera were placed into the remaining four wells. Rabbit thymus extract (RTF) was used as a source of nuclear ribonucleoprotein (nRNP), Smith (Sm) and Sjogren's syndrome B (SS-B) antigens. Human spleen extract (HSE) was used as a source of Sjogren's syndrome A (SS-A) antigen.

Analysis for clinical correlations was carried out with different anti-ENA antibodies using Chi square test with Yates' correction.

Table 1. Frequency of various anti–ENA antibodies in connective tissue disorders and controls (results expressed in percentage)

Subject groups	No. of subjects (n)	Subjects having Anti–ENA	Sm	nRNP	SS–A	SS–B
A) CTDs						
1. SLE	191	40.8	14.1	19.9	19.9	7.8
2. OCTD/MCTD	44	36.4	9.1	27.3	6.8	2.3
3. SS	18	27.8	11.1	5.6	5.6	5.6
4. PSS	66	10.6	0+**	4.5++ **	4.5 **	1.5
5. DM–FM	50	4.0	0	2.0++ **	0 **	2.0
6. UCTD	14	7.1	0	7.1	0	0
7. Other CTD (BS, DLE)	14	0	0	0	0	0
B) Inflammatory arthropathies						
1. RA	111	2.8	0++***	1.9+++***	0.9***	0**
2. Others (JCA, SSA)	35	0	0*	0++**	0**	0
C) Systemic vasculitides	16	0	0	0+	0	0
D) Other diseases						
1. OSMF	22	4.5	4.5	0	0	0
2. Others #	91	0	0+***	0+++***	0***	0*
E) Normal controls	39	0	0*	0++**	0**	0

BS=Behcet's syndrome, DLE=discoid lupus erythematosus, DM–PM=dermato/-polymyositis, JCA=juvenile chronic arthritis, MCTD=mixed connective tissue disease, OCTD=overlap connective tissue disease, OSMF=oral submucosal fibrosis, PSS=progressive systemic sclerosis, RA=rheumatoid arthritis, SS=Sjogren's syndrome, SSA=seronegative spondarthritis, UCTD=undifferentiated connective tissue disease.

This group included patients with rheumatic fever, arthritis associated with infections and nonspecific musculoskeletal disorders.

(* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (Significantly lower proportion than in SLE)

+ $p < 0.05$, ++ $p < 0.01$, +++ $p < 0.001$ (Significantly lower proportion as compared to OCTD).

RESULTS

Table 1 shows the frequency of occurrence of various anti-ENA antibodies and their different reactivities in cases of CTD and controls. Antibodies were found most often in SLE (40.84%), overlap syndrome (OCTD; 36.36%) and Sjogren's syndrome (SS, 27.8%). Other disorders with autoantibodies to ENA in low frequency included progressive systemic sclerosis (PSS, 10.61%), undifferentiated connective tissue disease (UCTD; 7.1%), dermatopolymyositis (DM-PM, 4%), rheumatoid arthritis (RA, 2.7%), and oral submucosal fibrosis (OSMF, 4.5%). None of the other groups including normal subjects showed ENA auto-antibodies.

In group A (patients with CTD), there were 353 female and 44 male patients. Their age range was 4 to 73 years (median 26 yrs). All patients were in the follow up for 2 months to 11 years (median 27 months). Twenty nine (7.3%) patients died during this period.

As shown in Table 1, anti-nRNP and anti-SS-A antibodies occurred with equal frequencies in patients with SLE (38 each), followed by anti-Sm (27) and anti-SS-B (15) antibodies. In patients with OCTD, anti-nRNP was the commonest anti-ENA. Anti-ENAs in combinations were seen only in SLE (17.3%) and OCTD (6.8%) patients.

Anti-Sm was found almost exclusively in SLE, OCTD and SS. One patient of OSMF was also found to have anti-Sm antibody. Using Chi-square test, anti-Sm was more frequently observed in SLE patients than PSS, DM-PM, RA, other inflammatory arthropathies (group B-2), other diseases (group D-2) and normal controls. It was significantly more frequently present in patients with OCTD/MCTD as compared to PSS, RA and other diseases (group D-2).

Anti-nRNP was seen in various disorders but significantly more often in OCTD and SLE as compared to PSS, DM-PM, RA, other inflammatory arthropathies (group B-2), systemic vasculitides, other diseases (group D-2) and normal controls.

Anti-SS-A antibodies were seen mainly in SLE. Statistically it was more frequently observed in SLE as compared to PSS, DM-PM, RA, other inflammatory arthropathies (group B-2), other diseases (group D-2) and normal controls.

Anti-SS-B was also seen mainly in SLE and SS. Statistically it was more frequently present in SLE as compared to RA and other diseases (group D-2).

Table 2. Correlation of various anti–ENA antibodies in SLE (n=191) with major clinical features (results expressed in percentage)

Clinical feature	Anti–Sm		Anti–nRNP		Anti–SS–A		Anti–SS–B	
	+	–	+	–	+	–	+	–
	(27)	(164)	(38)	(153)	(38)	(153)	(15)	(176)
1. Renal	81.5	79.3	65.8	83	89.5	77.1	86.7	78.9
2. Neuro–psychiatric	48.2	44.5	42.1	45.8	47.4	44.4	60	43.8
3. Joint	96.3	92.7	97.4	92.2	97.4	92.2	93.3	93.2
4. Skin	96.3	92.1	92.1	92.8	87.5	92.8	93.3	92.6
5. Mucous membrane lesions	77.8	75.6	76.3	75.8	84.2	73.9	93.3	74.4
6. Serositis	51.9	44.5	55.3	43.1	71.1	39.2***	53.3	44.9
7. Myocarditis	37.0	23.8	39.5	22.2	36.8	22.9	53.3	23.3*
8. Pulmonary parenchymal lesions	33.3	30.5	47.4	26.8*	39.5	28.8	53.3	28.9
9. Raynauds phenomenon	40.7	26.8	39.5	26.1	31.6	28.1	13.3	30.1
10. Vasculitis	55.6	37.2	47.4	37.9	52.6	36.6	33.3	40.3
11. Hemolytic anemia	11.1	11.6	10.5	11.8	18.4	11.2	13.3	11.4
12. Thrombo–cytopenia	14.8	17.1	10.5	18.3	13.2	17.7	20.0	16.5
13. Leukopenia	29.6	20.7	18.4	22.9	28.9	20.3	26.7	21.6
14. Myositis	37.0	21.3	34.2	20.9	23.7	23.5	26.7	23.3
15. Sicca complex	3.7	3.1	5.3	2.6	2.6	3.3	6.7	2.8
16. Arterial /venous thrombosis	3.7	9.8	12.6	10.5	5.3	9.8	0	9.7
17. Pulmonary hypertension	3.7	2.4	2.6	2.6	10.5	0.65**	13.3	1.7

p * < 0.05, ** < 0.005, *** < 0.001
The number of subjects is in parenthesis.

Table 3. Correlation of anti–ENA antibodies with histopathological types of Renal SLE (results expressed in %)

Histological types	Sm		nRNP		SS–A		SS–B	
	+	–	+	–	+	–	+	–
	(10)	(73)	(14)	(69)	(19)	(64)	(6)	(77)
1. Diffuse proliferative	50.0	41.1	42.9	42.0	57.9	37.5	66.7	41.6
2. Mesangio–proliferative	30.0	16.4	21.4	17.4	26.3	15.6	16.7	16.9
3. Focal proliferative	0.0	9.6	0.0	10.1	10.5	7.8	0.0	9.1
4. Membranous	10.0	23.3	14.3	23.2	5.3	26.6	16.7	22.1
5. Minimal change	10.0	8.2	14.3	7.3	0.0	10.9	0.0	9.1
6. Normal histology	0.0	1.4	7.1	0.0	0.0	1.6	0.0	1.3

The number of subjects is in parenthesis.

Clinical correlation of various anti-ENA antibodies in SLE

Table 2 shows the comparison of clinical features between patients having a particular ENA antibody and those without it using Chi square test. Anti-Sm did not correlate with any of the clinical features. Anti-nRNP antibody correlated with the presence of pulmonary parenchymal lesions. Anti-SS-A antibody correlated with the presence of serositis and pulmonary hypertension, while anti-SS-B correlated with the presence of myocarditis and recurrent diarrhoea.

The patients having anti-nRNP alone (n = 17) had a lesser frequency of renal involvement (52.9%) as compared to that in rest of the cases (83.1%) ($p < 0.005$; not shown in tables).

Renal Histology:

Renal biopsy was performed in 83 cases. As shown in Table 3, there was no association between any of the histopathological types and antibodies.

Neuro-psychiatric features

When similar comparison was done for various neuropsychiatric features (Table 4), the only statistically significant association was between anti-SS-A and focal neurological deficit.

Cutaneous Features

None of the cutaneous manifestations correlated with any of the antibodies (Table 5).

Immunological parameters

The correlation of these ENA antibodies was also sought with other immunological parameters (Table 6). There was statistically significant association with speckled pattern of ANA and negative association with homogeneous pattern of ANA with both anti-Sm and anti-nRNP. Also the number of cases having low serum C3 (Normal = 70-120 mg/dl) levels was significantly more in those having anti-SS-A as compared to those without it.

Clinical Association in patients with OCTD

No significant difference in various clinical manifestations was found between patients with anti-nRNP(n = 12) and those without it (n = 32) (data not shown). The number of patients having other antibodies were small.

Anti-ENA in other disorders, clinical correlation

The number of patients with positive anti-ENA antibodies were small in other disorders. In SS, it was positive in 2 out of 4 cases of primary SS and 3 out of 14 patients with secondary SS. There were 7, 2, 3 and 1 patients having anti-ENA antibodies in PSS, DM-PM, RA and OSMF group respectively. Apparently, these cases (having anti-ENA

Table 4. Correlation of anti–ENA antibodies with different neuropsychiatric features#

Feature	Sm		nRNP		SS–A		SS–B	
	+	–	+	–	+	–	+	–
	(27)	(164)	(38)	(153)	(38)	(153)	(15)	(176)
1. Seizures	11.1	7.9	5.3	9.2	7.9	8.6	20.0	7.4
2. Focal neurological deficit	18.5	9.2	13.2	9.9	21.1	7.9*	26.7	9.1
3. Neuropathy (peripheral/ cranial)	7.4	14.1	13.2	13.2	13.2	13.2	0	14.3
4. Spinal cord lesions	7.4	1.8	7.9	1.3	5.3	2.0	0	2.9
5. Organic brain syndrome	25.9	13.5	18.4	14.5	18.4	14.5	13.3	15.4
6. Psychiatric disorders	25.9	29.0	29.0	29.0	26.3	29.6	33.3	28.6

* $p < 0.05$
# Results expressed in %
The number of subjects is in parenthesis.

Table 5. Various anti–ENA Antibodies: correlation with different cutaneous features of SLE (results expressed in %)

Feature	Anti–Sm		Anti–nRNP		Anti–SS–A		Anti–SS–B	
	+	–	+	–	+	–	+	–
	(27)	(164)	(38)	(153)	(38)	(153)	(15)	(176)
1. Photosensi–tivity	77.8	66.1	68.4	67.6	65.8	68.2	66.7	67.8
2. Malar rash	81.5	76.5	76.3	77.5	76.3	77.5	73.3	77.6
3. SCLE	11.1	6.2	7.9	6.6	7.9	6.6	13.3	6.3
4. Discoid lesions	11.1	8.6	5.3	9.9	10.5	8.6	0	9.8
5. Alopecia	92.6	87.7	92.1	87.4	89.5	88.1	100	87.4

SCLE=Subacute cutaneous lupus erythematosus
The number of subjects is in parenthesis.

Table 6. Correlation of anti–ENA antibodies with other immunological parameters

Feature	Sm		nRNP		SS–A		SS–B	
	+	–	+	–	+	–	+	–
	(27)	(164)	(38)	(153)	(38)	(153)	(15)	(176)
1. ANA–patterns								
(i) Homogeneous	48.2	73.8*	55.3	73.9*	68.4	70.6	60.0	71.0
(ii) Speckled	66.7	34.8**	65.8	32.6#	34.2	40.5	53.3	38.1
(iii) Nucleolar	3.7	1.2	0	2.0	0	2.0	0	1.7
(iv) Peripheral	3.7	14.0	13.2	12.4	15.8	11.8	20.0	11.9
2. Anti–dsDNA	71.4	52.9	52.0	56.0	64.0	52.8	60.0	54.7
3. Anti–ssDNA	83.3	70.4	77.8	70.8	81.3	70.0	87.5	70.7
4. Lupus anticoagulant	0	18.0	0	18.8	11.1	15.6	0	15.8
5. Low C3 levels	92.0	68.7	80.6	74.7	91.7	71.8*	92.9	74.4
6. Low C4 levels	85.7	67.4	82.8	67.0	80.7	73.2	84.6	68.6

p * < 0.05, ** < 0.005, # < 0.0001
The number of subjects is in parenthesis.

antibodies) were no different from other cases in their respective groups.

DISCUSSION

In the present study, the frequency of most anti-ENA antibodies using RTE and HSE as sources of nuclear antigens was less in many connective tissue disorders as compared to many other studies. [1,2,4,9] In an earlier study from this centre, where 89 cases of SLE from northern India were tested in the laboratory of Professor R.N.Maini (UK), the frequency of anti-ENA antibodies was found to be 57 percent. [17] This difference from the present study may be explained on the basis of patient selection. [4] While in earlier study, fresh (majority having active disease) patients were included, the present study was routine screening in all patients attending the immunology clinic.

None of the antibody was exclusively specific for any disorder. Anti-Sm was detected in SLE, OCTD and SS cases. This is in disagreement with other reports showing anti-Sm to be specific for SLE. [4,18] Similar to our findings, anti-Sm antibodies have been detected in RA (5%), MCTD (8%) and primary SS (12%) in earlier studies also (reviewed by Smeenk *et al.* [2]) Similarly, other anti-ENAs were also not exclusively specific for any CTD. However, they were seen significantly more often in certain disorders (as shown in results).

Overall agreement on the association between various anti-ENA antibodies and connective tissue disease features does not exist in the literature. [2] In the present study, anti-Sm did not correlate with any disease feature in patients with SLE, as also observed by Synkowski, *et al.* [19] Many clinical associations of anti-Sm have been reported in literature. Some of them are conflicting, for example benign form of SLE [20,21], increased disease activity or flare of disease [22], more renal disease [23,24], milder renal disease [11,20,21,25], three-fold increase in CNS disease [22,26] and milder CNS disease. [20]

In a review on clinical significance of various ANAs, Smeenk, *et al.* mentioned absence of any clinical association with patients having anti-nRNP. [2] Many authors did not find a good correlation between high titer anti-nRNP and MCTD which is contradictory to earlier obsevations by Sharp *et al.* [10, 23,25,27-29] In the present study, myocarditis and pulmonary lesions were significantly more frequent in patients with SLE having anti-nRNP antibodies. No statistically significant clinical association of anti-nRNP was observed in patients with OCTD. Anti-nRNP, when present alone correlated significantly with lower frequency of renal disease, which is consistent with observations made by other workers. [10]

Anti-SS-A and anti-SS-B have been correlated with a benign form of SLE, [30] widespread non-scarring cutaneous lesions of histologically proven SLE, [31] vasculitis, [32] thrombocytopenia [33] and sicca complex. [12] No such associations were found in the present study. Instead, a statistically significant association of anti-SS-A was found with serositis, lymphadenopathy, hepatomegaly, focal neurological deficit and pulmonary hypertension in patients with SLE. These associations of anti-SS-A and anti-SS-B have not been described earlier.

Thus the review of literature and results of the present study raise more conflicts, as far as the clinical relevance of these antibodies is concerned. We agree with the comment of Smeenk *et al.* [2] that anti-ENAs may not have any meaning in the diagnosis of (subsets of) connective tissue diseases, until further characterization of these antigens is carried out.

ACKNOWLEDGEMENTS

This work has been supported by a grant from Indian Council of Medical Research, New Delhi No. 5/12(4)/85 - EMS (8502310). The help extended by Dr. Ashok Kumar, Dr. Sadhna Mian, Miss Veena Taneja and Mr. R.L.Taneja is gratefully acknowledged.

REFERENCES

1. Nakamura RM, peebles CL, Rubin RL, Molden DP, Tan EM. Autoantibodies to nuclear antigens. Advances in laboratory tests and significance in systemic rheumatic diseases. Chicago American Society of Clinical Pathologists press. 1985 : 1-40.

2. Smeenk R,Westgeest T, Swaak T. Antinuclear antibody determination : the present state of diagnostic and clinical relevance. Scand J Rheumatol 1985; Suppl. 56 : 78-92.

3. Hollman HR, Deicher R. The reaction of the lupus erythematosus (LE) cell factor with deoxyribonucleoprotein of the cell nucleus. J Clin Invest 1959; 38 : 2059-72.

4. Tan EM, Chan EKL, Sullivan KF, Rubin RL. Antinuclear antibodies (ANAs) : Diagnostically specific immune markers and clues toward the understanding of systemic autoimmunity. Clin Immunol Immunopathol 1988; 47 : 121-41.

5. Mattioli M, Reichlin M. Characterization of a soluble nuclear ribonucleoprotein antigen reactive with SLE sera. J Immunol 1971; 107 : 1281-90.

6. Lerner MR, Steitz JA. Antibodies to small nuclear RNAs complexed with protein are produced by patients with systemic lupus erythematosus. Proc Natl Acad Sci USA 1979; 76 : 5495-7.

7. Nishikai M, Reinchlin M. Heterogeneity of precipitating antibodies in polymyositis and dermatomyositis. Arthritis Rheum 1980; 23 : 881-8.

8. Douvas AS, Achten M, Tan EM. Identification of a nuclear protein (Sc1-70) as a unique target of human antinuclear antibodies in scleroderma. J Biol Chem 1979; 254 : 10514-22.

9. Barland P, Hamburger M, Hodes S. The clinical significance of anti-Sm and anti-RNP antibodies (abstr). Arthritis Rheum 1975; 18 : 384.

10. Sharp GC, Irvin WS, Tan EM, Gould RG, Holman HR. Mixed connective tissue disease: an apparently distinct rheumatic disease syndrome associated with a specific antibody to an ENA. Am J Med 1972; 52 : 148-59.

11. Beaufuls M, Kouki F, Mignon F, Camus J, Morel-Maroger L, Richet G. Clinical significance of anti-Sm antibodies in systemic lupus erythematosus. Am J Med 1983; 74 : 201-5.

12. Alspaugh MA, Talal N, Tan EM. Differentiation and characterization of autoantibodies and their antigens in Sjogren's syndrome. Arthritis Rheum 1976; 19 : 216-22.

13. Malaviya AN, Mishra R, Banerjee S, *et al.* Systemic lupus erythematosus in North Indian Asians. A prospective analysis of clinical and immunological features. Rheumatol Int 1986; 6 : 97-101.

14. Kelly WN, Harris ED, Ruddy S, Sledge CB (Editors). Text book of Rheumatology. W.B. Saunders, Philadelphia, 1985.

15. Malaviya AN, Kumar A, Singh RR, Sehgal GS, Mehra NK, Tiwari SC. The spectrum of rheumatic diseases as seen by a tertiary referral centre in northern India. SEAPAL Bull 1987; 5 : 11-6.

16. Venables PJW, Smith PR, Maini RN. Purification and characterization of the Sjogren's syndrome A and B antigens. Clin Exp Immunol 1983; 54 : 731-8.

17. Mishra R, Malaviya AN, Charales P, Venables PJW, Maini RN. Antinuclear antibody profile in systemic lupus erythematosus from north India. Indian J Allergy Appl Immunol 1987; 1 : 41-4.

18. Notman DD, Kurata N, Tan EM. Profiles of antinuclear antibodies in systemic rheumatic diseases. Ann Intern Med 1975; 83 : 464-9.

19. Synkowski DR, Reichlin M, Provost T. Serum autoantibodies in SLE and correlation with cutaneous features. J Rheumatol 1982; 9 : 380.

20. Winn DM, Wolfe JF, Lindberg DA, Fristoe RH, Kingsland L, Sharp GC. Identification of a clinical subset of systemic lupus erythematosus by antibodies to the Sm antigen. Arthritis Rheum 1979; 22 : 1334-7.

21. Powers R, Akizubi M, Boechm-Truitt MJ, Holman HR, Substantial purification of the Sm antigen and association of high titer antibody to Sm with a clinical subset of systemic lupus erythematosus (abst). Arthritis Rheum 1977; 20 : 131.

22. Barada FA, Andrews BS, Davis JS, Taylor RP. Antibodies to Sm in patients with SLE: correlation of Sm antibody titers with disease activity and other laboratory parameters. Arthritis Rheum 1981; 24 : 1236.

23. Sharp GC, Irvin WS, May CM, *et al.* Association of antibodies to ribonucleoprotein and Sm antigens with mixed connective tissue disease, SLE and other rheumatic diseases. N Engl J Med 1976; 295 : 1149-54.

24. Peltier AP, Gaudreau A, Amor B, *et al.* Signification clinique des anticorps specifiques d' antigens nucleaires solubles. Nouv Press Med 1978; 7 : 1813-8.

25. Parker MD. Ribonucleoprotein antibodies: frequency and clinical significance in SLE, scleroderma and mixed connective tissue diseases. J Lab Clin Med 1973; 82 : 769-75.

26. Winfield JB, Brunner CM, Koffler D. Serologic studies in patients with SLE and central nervous system dysfunction. Arthritis Rheum 1978; 21 : 289-94.

27. Leibfarth JH, Persellin RH. Characteristics of patients with serum antibodies to extractable nuclear antigens. Arthritis Rheum 1976; 19 : 851-6.

28. Gaudreau A, Amor B, Kahn MF, Ryckewart A, Sany J, Peltier AP. Clinical significance of antibodies to soluble extractable nuclear antigens (anti-ENA). Ann Rheum Dis 1978; 37 : 321-7.

29. Farber SJ, Bole GG. Antibodies to components of extractable nuclear antigen. Clinical characteristics of patients. Arch Intern Med 1978; 136 : 425-31.

30. Maddison P, Mogavero H, Reichlin M. The clinical significance of autoantibodies to a soluble cytoplasmic antigen in systemic lupus erythematosus and other connective tissue diseases. J Rheumatol 1979; 6 : 189-95.

31. Sonthemer RD, Stastany P, maddison P, Reichlin M, Gilliam JN. Anti-Ro and La antibodies and further HLA associations in subacute cutaneous lupus erythematosus. Arthritis Rheum 1980; 23 : 750.

32. Alexander EL, Hirsch TJ, Arnett FC, provost TT, Stevens M. Ro (SS-A) and La (SS-B) antibodies in the clinical spectrum of Sjogrens syndrome. J Rheumatol 1982; 9 : 239-46.

33. Morley KD, Bernstein RM, Bunn CC, *et al.* Thrombocytopenia and anti-Ro (letter). Lancet 1981; 2 : 940.