

Antibodies to the Extractable Nuclear Antigens (ENA) in Pakistani Patients with Systemic Rheumatic Disease

Pages with reference to book, From 12 To 14

Tahir A. Ahmed, Iftikhar A. Malik, Muhammad Saleem (Armed Forces Institute of Pathology, Rawalpindi.)

Ashfaq A. Khan, Ahsan Hameed, Syed TA. Shah (Military Hospital, Rawalpindi.)

Safia J. Amin, Abid Farooqi (Pakistan Institute of Medical Sciences, Islamabad.)

Abstract

Autoantibodies to the saline extracts of cells are being used as diagnostic and prognostic markers in the investigation of patients with suspected systemic rheumatic disease. These are also called antibodies to the extractable nuclear antigens or anti ENA antibodies. We used saline extracts of human spleen cells and rabbit thymus extract to detect six subtypes of anti ENA antibodies, namely anti SSA (anti Sjogren's syndrome A), anti SSB (anti Sjogren's syndrome B), anti RNP (anti Ribonucleoprotein), anti Sm (anti Smith), anti Jo-1 (anti Jo-i) and anti Scl 70 (anti Systemic sclerosis 70) antibodies. Two hundred and thirty-seven patients' sera were screened in the department of Immunology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, over a period of 16 months. Fifty-seven were positive for auto antibodies, of which 26 had anti SSA antibodies, 12 anti SSA÷ anti RNP+ anti Sm antibodies, 7 anti RNP+ anti Sm antibodies, 6 anti SSA+ anti SSB antibodies, 5 anti SSA+ anti RNP antibodies while one patient showed only anti RNP antibody. The detection of the anti ENA antibodies was specially helpful in diagnosing patients with systemic lupus erythematosus presenting with atypical features and with mixed connective tissue disease (JPMA 45:12, 1995).

Introduction

Systemic rheumatic diseases are a heterogeneous group, characterised by the presence of one or more autoantibodies against various cellular components. The commonly employed antinuclear antibody test, is a good method of screening. Auto antibodies to the double stranded DNA (anti ds DNA) are good diagnostic markers for systemic lupus erythematosus (SLE). Though there is little in terms of laboratory investigations for the anti DNA negative SLE and other rheumatic diseases such as Sjogren's syndrome (SS), mixed connective tissue disease (MCTD), progressive systemic sclerosis (PSS), dermatomyositis (DM) and polymyositis (PM), the picture is further complicated when the patient presents with overlapping features of two or three diseases. The use of the anti ENA antibodies has significantly improved the diagnostic ability in recent years¹. These autoantibodies are relatively few in number, mostly directed against the enzymes or proteins engaged in DNA replication and show a remarkable disease and subset association². These properties make them not only useful diagnostic markers, but they are also utilised as tools to investigate the biological mechanisms at molecular level.

A number of the anti ENA antibodies have been discovered. Of these six namely anti Sjogren's syndrome A (SSA), anti Sjogren's syndrome B (SSB), anti Ribonucleoprotein (RNP), anti Smith (Sm), anti Jo-i (Jo-1) and anti Systemic sclerosis 70 (Scl 70) antibodies are most commonly detected in patient's sera. The diagnostic utility of these autoantibodies has been demonstrated and some of them are established as marker antibodies for particular diseases^{2,3} (Table I).

Table I. Anti ENA antibody profiles in patients with systemic rheumatic diseases.

Anti ENA antibody	SLE %	Sjogren's Syd./ %	MCTD %	DM/PM %	Systemic Scl. %
Anti SSA	24-40	60-75	17	8	4
Anti SSB	9-15	40-60	0	0	0
Anti Sm	30-40	0	3	0	0
Anti RNP	23	4	100	14	3
Anti Jo-1	<1	0	3	25-31	0
Anti Scl 70	0	0	0	0	15-20

SLE = Systemic lupus erythematosus, Syd. = Syndrome

MDTC= Mixed connective tissue disease, DM = Dermatomyositis

PM = Polymyositis, Scl = Sclerosis

We studied these autoantibodies mainly with a view to determine their clinical utility in the management of our patients with systemic rheumatic diseases.

Material and Methods

Patients with a clinical diagnosis of systemic rheumatic disease were selected by the attending physicians in the departments of medicine, dermatology and nephrology of the army and civil hospitals in Rawalpindi and Islamabad. Patients with positive sera were called to AFIP, Rawalpindi to document the clinical data.

Five ml of blood was collected in plain containers. Serum was separated and the complement was inactivated by heating at 56°C for 30 minutes. Specimens were then stored at -30°C. The procedure was based on protocol provided by the Immunology Department of St. Thomas' Hospital, London, using countercurrent immunoelectrophoresis. Agarose gel (1%) was prepared using type IIIA, HEEO agarose (Sigma) in barbitone buffer at pH 8.4. V/V 1% polyethylene glycol 600 (PEG 600 BDH) was added to promote immunoprecipitation. Moltengel was poured over Gelbond sheets (Sigma) (13-14ml of molten gel and over 127mmx60mm sheets of gel bond). The gel was allowed to set for 30 minutes. It was then kept at 4°C for at least 60 minutes in a moist box. Troughs were cut (2 mm wide) and holes punched (3 mm diameter) using locally fabricated instruments (Figure 1).

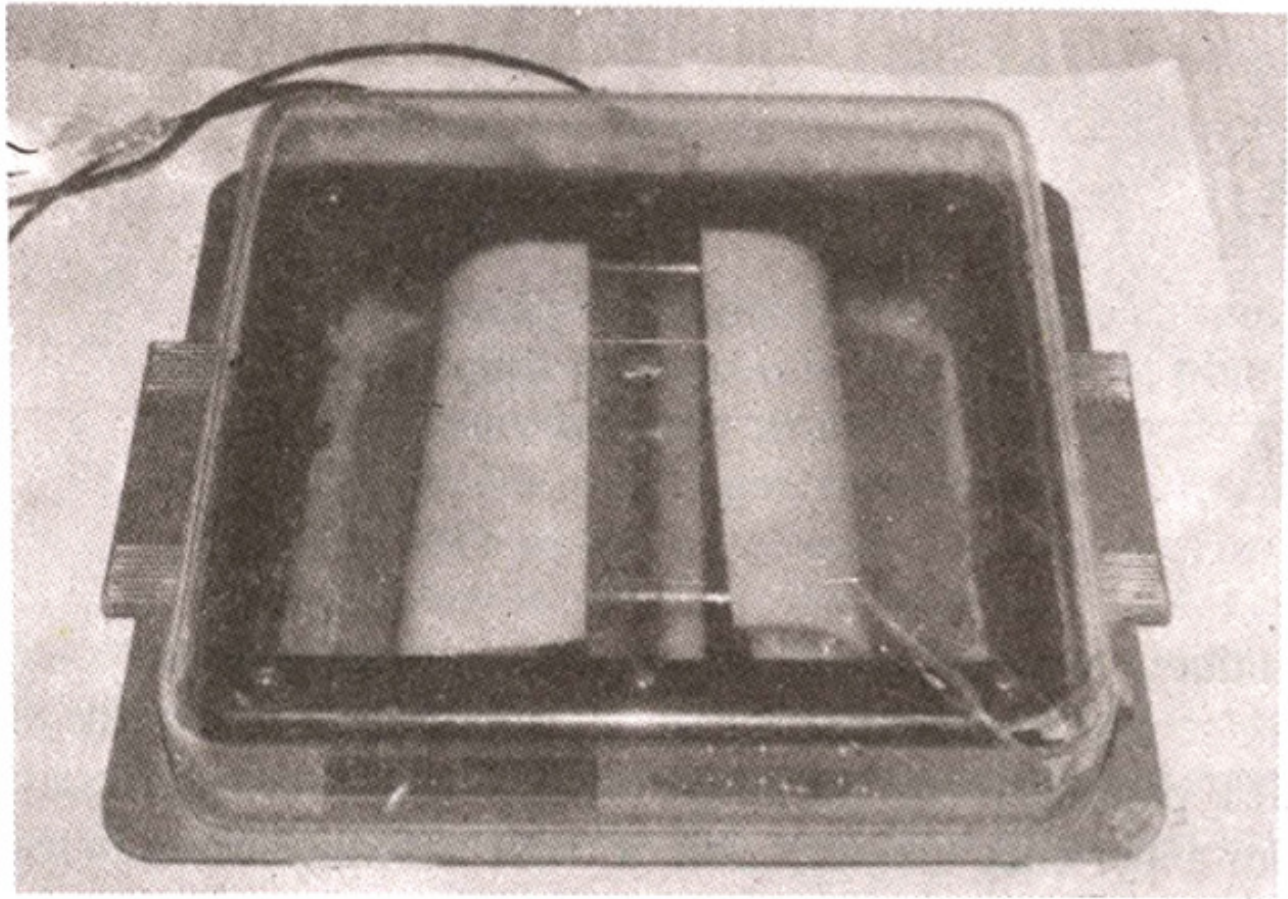


Figure 1. Tank used for countercurrent immunoelectrophoresis.

The test and control sera were put in the holes and saline extracts used as antigens, were placed in the troughs. We used human spleen extract (Postmortem specimen) as the source for the SSA antigen while saline extract of the rabbit thymus (Pel Freeze) was used as the source for the SSB, RNP, Sm, Scl 70 and the Jo-1 antigens. The test was in two stages. In the first stage all sera were screened for the presence of any anti ENA antibody and later all the positive sera were tested again in the presence of positive control sera (provided by Prof. Dumonde and Mr. G.T. Swana from St Thomas's Hospital). The reactions of identity, partial identity and non-identity were used to identify the individual anti ENA antibodies. Electrophoresis was done using barbitone buffer at pH 8.4 with the help of a "Gelman" power pack. A constant voltage of 120mV was applied for 45 minutes, the gel was then carefully picked up and incubated overnight at 4°C in a moist box. Washing for 4-5 days was done with 2 changes of 300 ml each, of normal saline to wash off the non precipitated proteins. The gel was then dried at 37°C in an incubator and stained with 3% Ponceu S stain (Sigma) in 5% glacial acetic acid (Merck) (Figure 2).

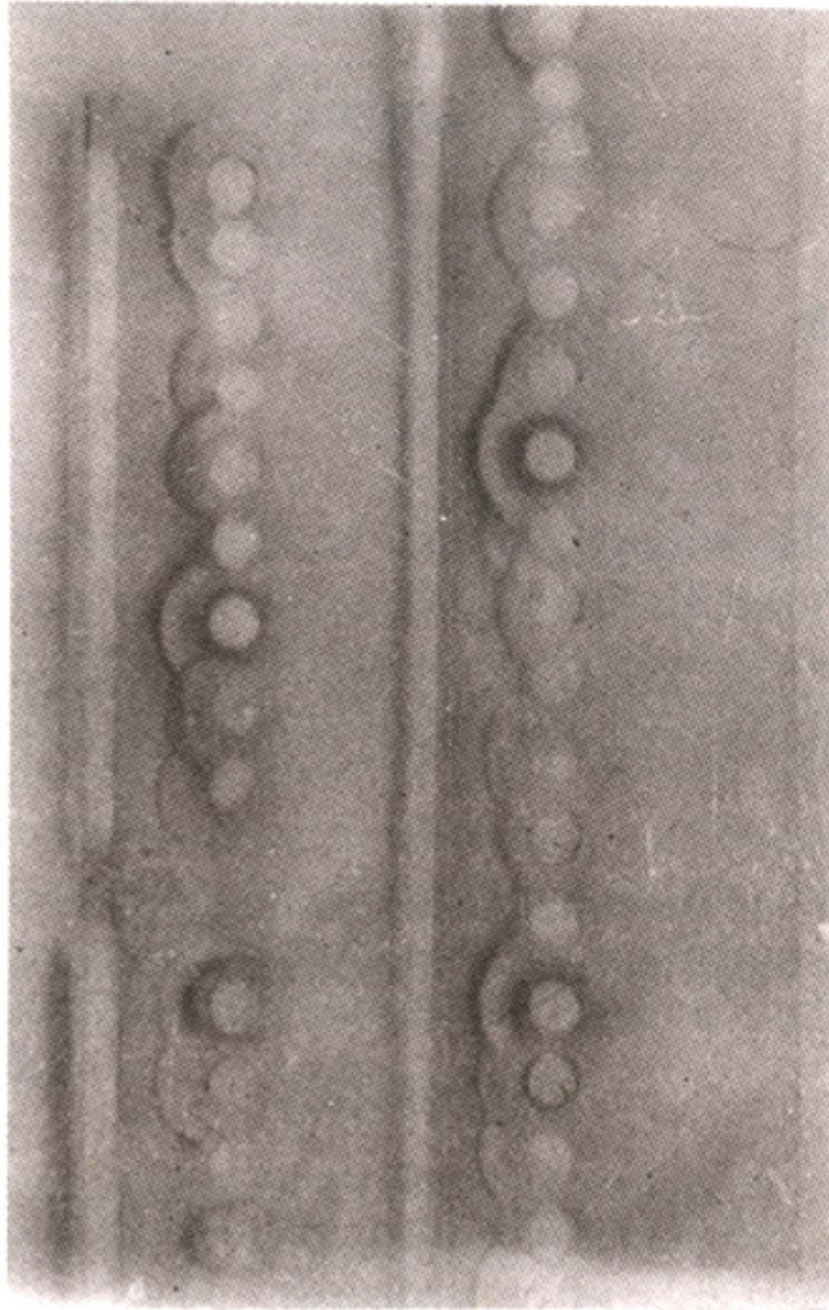


Figure 2. Dried gel stained with 3% ponceous stain showing precipitin lines with reactions of identity/partial identity and non-identity.

Destaining was carried out in 5% acetic acid. Quality control was ensured with the use of the control specimens and by regular participation in the UK, NEQAS (national external quality assurance scheme) for autoimmune serology and special immunochemistry.

Results

Of 237 sera screened, 57 were positive for anti ENA antibodies (Table II).

Table II. Anti ENA antibody specificities in Pakistani patients.

Anti ENA antibodies	Anti SSA	Anti SSA+ RNP+Sm	Anti RNP +Sm	Anti SSA +SSB	Anti SSA +RNP	Anti RNP
Number of patients	26	12	7	6	5	1

Clinical details were available in 45 patients. Thirty-two of these were diagnosed as SLE (30 females age range 18-61 years, 2 males aged 3 and 36 years), 2 had subacute cutaneous lupus erythematosus (SCLE, 1 female aged 24 years and 1 male aged 7 years), 5 had Sjogren's syndrome (4 females age range 28-50 years, 1 male aged 30 years), 2 had systemic sclerosis (2 females aged 45 and 55 years), while 4 were diagnosed as mixed connective tissue disease (3 females age range 30-60 years, 1 male) (Table III).

Table III. Anti ENA antibody profiles as detected in patients with systemic rheumatic disease (n=45).

Anti ENA	SLE	SCLE	Sjogren's Syd.	Systemic Scl.	MCTD
Antibody	(32)	(2)	(5)	(2)	(4)
Anti SSA	10	2	-	2	-
Anti SSA+SSB	-	-	5	-	-
Anti SSA+ RNP+Sm	12	-	-	-	-
Anti RNP+Sm	7	-	-	-	-
Anti SSA+RNP	3	-	-	-	3
Anti RNP	-	-	-	-	1

SLE = Systemic lupus erythematosus, SCLE = Subacute cutaneous lupus erythematosus
Syd. = Syndrome, Scl = Sclerosis, MCTD = Mixed connective tissue disease

Discussion

Anti ENA antibodies have been established as useful diagnostic markers with close disease and subset association. Anti SSA and anti SSB antibodies are indicators for Sjogren's syndrome⁴. Patients with SLE or overlap syndromes found positive for anti ENA antibodies are likely to develop sicca manifestations⁵. Anti SSA antibody has been associated with complete congenital heart block⁶, a marker for the anti nuclear antibody negative SLE, SCLE and SLE associated with complement C2 deficiency⁷⁻⁹. Anti Sm antibody has been established as a marker antibody for SLE¹⁰. This is usually found in association with anti RNP antibodies. But studies looking into the differential expression of the isotype of these auto antibodies have shown that anti Sm antibody is more often IgM in nature which may be missed and under reported if counter current immunoelectrophoresis is employed for its detection¹¹. Anti RNP antibodies are used as part of the diagnostic criteria for the mixed connective tissue disease. A subtype of these antibodies has been associated with the presence of the active disease¹². Anti Scl 70 antibody is a marker for systemic sclerosis. A Thai study has linked it to an aggressive disease in local patient population^{13,14}. Anti Jo-1 antibody is present in at least 50% of patients with polymyositis and adult dermatomyositis associated with interstitial lung disease¹⁵. In our study, the detection of anti ENA antibodies helped in establishing an early and definite diagnosis in patients with a suspicion of systemic rheumatic disease. They were of particular help in cases with atypical manifestations. Thirty-two (71%) of 45 patients, where the clinical data was available were

suffering from SLE. Nineteen (42%) of them had anti Sm antibody. The presence of this antibody was of particular value in establishing a clearcut diagnosis in these patients. Anti SSA antibody was also found to be useful marker. It was detected in the sera of 25 (78%) SLE patients and in 10(31%) of the total SLE patients it was the single anti ENA antibody detected. In our experience the detection of these autoantibodies helps to reach the diagnosis at a comparatively early stage particularly in patients with atypical manifestation.

References

1. Moutsopoulos, H.M., Giotaki, H., Maddison, P.J. et al. Antibodies to cellular antigens in Greek patients with autoimmune diseases: anti-Ro (SSA) a possible marker of penicillamine-D intolerance. *Am.Rheum.Dis.*, 1984;43:285-87.
2. Bernstein, R.M. Humoral immunity in systemic rheumatic disease. A review. *J.R.Coll.Physicians Lond.*, 1990;24: 18-25
3. Tan, EM. Autoantibodies to nuclear antigens (ANA): their immunobiology and medicine. *Adv. Immunol.*, 1982;33: 167-240.
4. Elkon, K.B., Gharavi, A., Hughes, et al. Autoantibodies and sicca syndrome (Primary Sjogren's syndrome). *Ann. Rheum. Dis.*, 1984;43 :243-45.
5. Maddison, P.J. Overlap syndrome, mixed connective tissue disease and eosinophilic fascitis. *Curr.Opinion Rheum.*, 1989; 1:523-28.
6. Reichlin, M., Friday, K. and Herley, J.B. Complete congenital heart block followed by anti-Ro(SSA) in adult life. *Am.J.Med.*, 1988;84:339-44.
7. Smiley, J.D. and Moore, SE. Southwestern internal medicine conference: molecular mechanisms of autoimmunity. *Am. J.Med.Sci.*, 1988;295 :478-96.
8. Southeimer, RD. Immunological significance of the Ro/SSA antigen antibody system. Editorial. *Arch.Dermatol.*, 1985; 121:327-30.
9. Vanderstein, P.R., Provost, T.T., Jordan, RE. et al. C2 deficient systemic lupus erythematosus. Its association with anti Ro-(SSA) antibodies. *Arch. Dermatol.*, 1982;118:584-87.
10. Tan, EM. Antinuclear antibodies: diagnostic markers for autoimmune disease and probes for cell biology. *Adv. Immunol.*, 1989;44:93-151.
11. Gouldner, H.H., Lakomek, H.J. and Bantz, F.A. Anti.(UI) RNP antibody profiles in patients with systemic rheumatic diseases. Differential detection of immunoglobulin G and M by immunoblotting. *Clin. Immunol. Immunopathol.*, 1986;40:532-38.
12. Peterson, I., Wang, G., Smith, EL. et al. The use of immunoblotting and immunoprecipitation of (U) small nuclear Ribonucleoproteins in the analysis of the sera of patients with mixed connective tissue disease and systemic lupus erythematosus. *Arthritis Rheum.*, 1986;29:986-96.
13. Kom, J.H. Immunologic aspects of scleroderma. *Curr.Opinion Rheum.*, 1989;1 :479-86.
14. McNeilage, L.J., Yougchaiyud, U. and Whittigham, S. Racial differences in antinuclear antibody patterns and clinical manifestations of Scleroderma. *Arthritis Rheum.*, 1989;32:54-60.
15. Dalakas, MC. Polymyositis, dermatomyositis and inclusion-body myositis. *N.Engl.J.Med.*, 1991 ; 325: 1487-98.