

Detection of Antibody against Extractable Nuclear Antigen by an Enzyme-Linked Immuno-Sorbent Assay

Results from Patients with Rheumatic and Internal Medical Diseases

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Anti-ENA antibody determination by ELISA technique may offer a valuable diagnostic help in the discrimination of patients with mixed connective tissue disease (MCTD) from those with other chronic inflammatory connective tissue diseases. Determination of this antibody was performed in a prospective designed investigation among 101 blood donors, 154 patients with various non-rheumatic internal medical diseases, and 229 patients with chronic inflammatory connective tissue diseases, including five patients with MCTD. A positive titre of anti-ENA antibody was found in approximately 10 % of blood donors and patients with various internal medical disorders. A highly positive anti-ribonucleoprotein (RNP) titre was found in the patients with MCTD, but was also observed in patients with other chronic inflammatory connective tissue diseases, giving a predictive value of 56 % for MCTD. We conclude that highly positive anti-RNP antibody values do not automatically indicate the diagnosis MCTD. Other diagnostic possibilities should still be considered.

Key words: ELISA technique; ENA-antibody; extractable nuclear antigen; mixed connective tissue disease.

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CLINICAL ASPECTS

High serum titres of antibody against ribonucleoprotein (RNP), the RNase sensitive part of extractable nuclear antigen (ENA), is a characteristic finding in mixed connective tissue disease (MCTD). This disease entity is rare even in rheumatological centres. The paper describes the diagnostic value of anti-ENA and anti-RNP antibody determination in a prospective clinical study using a solid phase ELISA.

Since the recognition of mixed connective tissue disease (MCTD) as a separate clinical entity (8, 9), a number of different procedures have been described for serological differentiation between MCTD and other chronic inflammatory connective tissue diseases. Previously, we have described an enzyme-linked immuno-sorbent assay (ELISA) for the semiquantitative detection of anti-ENA (extractable nuclear antigen) antibody in serum and other biological fluids (10). In order to determine the diagnostic value of this technique we decided, prospectively, to

use it for anti-ENA antibody detection in patients, hospitalized at a department of internal medicine, subspecialized in rheumatology, allergology, and immunological medicine.

MATERIAL AND METHODS

Patients

Sera from 383 patients were tested over a period of 6 months (Table 1). 326 (85 %) had diseases that could be classified within rheumatology, allergology and/or immunological

medicine. The remaining patients suffered from a variety of unselected internal medical disorders.

The patients with rheumatological diseases were diagnosed and classified according to internationally accepted criteria. Definite or classical rheumatoid arthritis was diagnosed using the ARA criteria (7). Primary Sjögren's syndrome was diagnosed if the patients had at least two of three objective criteria for keratoconjunctivitis sicca and xerostomia (Copenhagen criteria) (4), in the absence of other chronic inflammatory connective tissue diseases. For systemic lupus erythematosus the 1982 ARA diagnostic criteria were applied (11). The diagnosis of MCTD was made in patients

with combined features of systemic lupus erythematosus, Sjögren's syndrome, polymyositis and/or progressive systemic sclerosis together with a high anti-RNP (ribonucleoprotein) antibody titre in the serum (9). Other diseases were likewise carefully diagnosed, using generally accepted clinical and paraclinical criteria. Laboratory data including ESR, concentrations of B-haemoglobin, -eosinophilocytes, -neutrophilocytes, -lymphocytes, -thrombocytes, P-albumin, -IgG, -IgA, -IgM, -creatinine, and titres of antinuclear antibodies (ANA) (semiquantitative immunofluorescence technique); circulating immune complexes and anti-IgG (titre according to Waaler technique) were recorded at the time of anti-ENA antibody determination.

Table 1

Material of patients and controls examined with enzyme-linked immunosorbent assay (ELISA) technique for antibody against extractable nuclear antigen (ENA)

Diagnosis	No patients	♀	♂	Age (years)		No. ENA pos.	No. RNP pos.	No. Sm pos.	Immunoglobulin classes		
				mean	range				IgA	IgG	IgM
Blood donors	101	43	58	40	19-64	9 (8.9 %)	0	9 (8.9 %)	3	9	0
Rheumatoid arthritis	59	38	21	53	30-83	5 (8.5 %)	0	5 (8.5 %)	1	3	2
Collagenosis NUD	49	36	13	48	19-81	10 (21.3 %)	2 (4 %)**	8 (16 %)	2	4	7
Primary Sjögren syndr.	29	27	2	39	31-81	4 (13.8 %)	1 (3 %)*	3 (10.3 %)	2	2	3
SLE	27	26	1	42	19-72	6 (22.2 %)	1 (3.7 %)*	5 (18.5 %)	0	3	6
Arthralgia	20	13	7	33	19-85	-	-	-	-	-	-
Arthrosis	12	11	1	58	48-60	-	-	-	-	-	-
Periarteritis nodosa	6	4	2	55	37-69	-	-	-	-	-	-
Psoriatic arthritis	6	4	2	38	21-58	-	-	-	-	-	-
Polymyalgia rheumatica	4	3	1	64	56-73	-	-	-	-	-	-
Uric arthritis (gout)	4	1	3	49	33-65	-	-	-	-	-	-
Progressive systemic sclerosis	3	3	0	44	34-54	-	-	-	-	-	-
Mb. Bechterew	2	0	2	41	31-51	-	-	-	-	-	-
Temporal arteritis	2	0	2	72	66-78	-	-	-	-	-	-
Dermatomyositis	1	1	0	51	51	-	-	-	-	-	-
MCTD	5	5	0	46	29-55	5 (100 %)	5 (100 %)	-	2	5	1
Total	229					30	9	21	5	12	18
Allergic	97	52	45	35	11-76	10 (10.3 %)**	-	10 (10.3 %)	6	7	6
Neoplastic	9	7	2	44	31-71	-	-	-	-	-	-
Various internal med.	34	24	10	46	13-82	-	-	-	-	-	-
No definitive diagn.	14	7	7	40	25-72	1 (7 %)	-	1 (7 %)	-	1	-
Total	154	90	64	165	11-82	11 (7.1 %)	-	11 (7.1 %)	6	8	6

* One case highly positive.

** Two cases highly positive.

Controls

Serum samples from 101 blood donors equally distributed within the age range 19–64 years were examined for anti-ENA antibody (Table 1). The results were used for calculation of a normal reference range.

Anti-ENA-antibody determination with ELISA

The ELISA for detection of serum antibodies and the modification applied for serum anti-ENA antibody determination have been described elsewhere (2, 10).

In this ELISA buffer extractable bovine nuclear antigen is linked to a solid phase (polystyrene microtitre plates) and 10 µl serum samples from patients and controls are then added. Formation of antigen-antibody complexes indicating presence of antibody in a sample is visualised by spectrophotometry after adding peroxidase-conjugated rabbit antibody against α , γ and μ chains and a peroxidase substrate with colour indicator. In the modification employed by us, all positive samples were retested and also reexamined with and without RNase treatment of the antigen. All sera were analysed blindly in quadruplicates together with normal controls and a daily standard consisting of serum from the same donor. Slight day-to-day variations were corrected by multiplying donor values with the factor:

$$\frac{\text{fixed standard}}{\text{titre of daily standard}}$$

A range for healthy persons was calculated as mean values ± 2 SD of observations in the control group, and values exceeding that range were considered abnormal and positive. Values exceeding (mean value + 2 SD) $\times 3$ were by definition considered highly positive.

Antibodies against the RNase-sensitive part of ENA are termed anti-RNP (-ribonucleoprotein) antibodies. Antibodies against the RNase resistant part of ENA are termed anti-Sm (Smith) antibodies.

RESULTS

Of the 101 blood donors nine (8.9 %, 3 females, 6 males) had a positive anti-ENA antibody test (Table 1). All the positive blood donor values were below the highly positive limit. There was no difference between the age groups. All nine were Sm-positive and had positive IgG-ENA antibody and three also had positive IgA-ENA antibody. None had anti-RNP antibody.

In the group of 229 patients with various chronic inflammatory connective tissue diseases 30 (13.1 %) had positive anti-ENA antibody titre. In 21 of these the antibody was of Sm type, and the remaining nine had anti-RNP antibody. In patients with rheumatoid arthritis and with primary Sjögren's syndrome the prevalence of positive anti-ENA antibody reaction did not differ from that of normal healthy persons. Patients with MCTD had highly positive values of anti-RNP antibody. However, such elevated values were also found in primary Sjögren's syndrome (1 case) systemic lupus erythematosus (1 case) and in the group of unclassified chronic inflammatory connective tissue diseases (2 cases).

In the group of 154 patients with non-rheumatoid internal medical diseases, including patients with allergic disorders, 11 (7 %) had a positive anti-ENA antibody reaction (Table 1), solely the Sm type in all cases. Two cases (allergic disorders) showed highly positive values. In three of these 11 cases the antibody was of IgM class only. The remainder all had IgG anti-Sm antibody, and six had raised IgA anti-Sm antibody as well. The frequency of positive serological reactions was not different from that found in the control group.

Calculation of the diagnostic value for MCTD of a positive anti-ENA antibody test in a group of patients with rheumatic diseases showed $5 : (5 + 25) \times 100 \% = 17 \%$. In the same group the diagnostic value of highly positive RNP reactions is $5 : (5 + 4) \times 100 \% = 56 \%$.

DISCUSSION

The clinical diagnosis of MCTD can be extremely difficult if unassisted by serological

investigation, since it is a syndrome composed of symptoms and signs shared with and resembling systemic lupus erythematosus, primary Sjögren's syndrome, progressive systemic sclerosis, and polymyositis. However, a correct diagnosis is not only of academic interest, because it has been emphasized (8, 9) that patients with MCTD and high RNP antibodies may have a better prognosis, as distinct especially from systemic lupus erythematosus. There is no agreement on this view (1, 6, 13). As MCTD seems to be distinguishable from other chronic inflammatory connective tissue diseases by a particular high serum titre of anti-RNP antibody, determination of autoantibodies in patients under evaluation should include quantitative anti-ENA antibody detection with differentiation between antibodies to RNP- and Sm-antigens.

It is of importance to note that the diagnostic value of a given test is determined by a complex interaction between the diagnostic sensitivity and specificity and the prevalence of the disease. In very seldom diseases, such as MCTD, it is of the utmost importance that a diagnostic test has a high predictive value. A diagnostic value for MCTD of 56 % for a highly positive anti-RNP antibody reaction may therefore seem a little disappointing. A high anti-RNP antibody value does not exclude one of the other, more common, chronic inflammatory connective tissue diseases, e.g. primary Sjögren's syndrome or systemic lupus erythematosus. From a practical clinical point of view therefore, a positive and even a highly positive anti-RNP antibody reaction should not induce a definite stop for further diagnostic procedures.

The prevalence of positive anti-ENA antibody reactions was nearly the same among blood donors and among patients with various medical and allergic disorders. In most cases the antibodies were directed against the RNase resistant antigen, i.e. anti-Sm antibodies.

The positive anti-ENA reactions may explain some of the positive ANA reactions seen among this category of patients and controls, as previously described by Wiik (12), who found that the prevalence of positive ANA reactions in-

creased with age and that approximately 8 % in comparable groups had positive ANA reactions in low titre. Based on the present investigation we conclude that anti-ENA antibodies occur in approximately 10 % of blood donors and patients with various rheumatic, allergic and medical disorders. High levels of anti-RNP antibodies are seen particularly in patients with MCTD, but may occur in other chronic inflammatory connective tissue diseases as well.

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