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Antinuclear factor (ANF) test—its diagnostic value

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THE antinuclear factor (ANF) test has gained considerable popularity in recent years as an aid to diagnosis of various diseases that may have an underlying basis of autoimmunity. However, when faced with the diagnosis of such diseases, most clinical laboratories still depend almost exclusively on the well-known lupus erythematosus (L.E.) cell test.

It is generally agreed that the L.E. cell test and the ANF test both detect the same factors[1] or antibodies, and the difference lies only in the experimental design to detect such antibodies. The ANF test is based on an immunofluorescent technic, and as such is much more sensitive than the L.E. cell test, so that it may be positive in a much wider variety of disease processes thought to be of possible autoimmune origin. Because of this latter property of the ANF test, it has been considered by some investigators[2] as one of the rather valuable screening tests in the diagnosis of autoimmune diseases.

The ANF test was introduced in the Cleveland Clinic laboratories about three years ago, and the main purpose of this study was to analyze and to correlate clinically the first 500 consecutive ANF tests performed during the year 1967. Since in a majority of the cases the L.E. cell tests were performed also, a further comparison between the results of these two tests and the patient's clinical disease was possible.

Materials and methods

Various technics employing nuclear material of different tissues have been reported for performing the ANF test. These procedures include tumor imprints, nuclei of hepatic or renal tissue sections, peripheral leukocyte smears, mouse spleen imprints, granulocytes, and lymphocytes. The technic employed at the Cleveland Clinic laboratory during the period these tests were performed was that of Svec[3] and was essentially as follows.

Human splenic tissue obtained at the time of operation was immediately cut into pieces about 2 by 2 cm, and then stored frozen at −20 C. At the time of the test, the splenic tissue block was thawed, and slide imprints were prepared by gently touching one surface of a microscopic slide to the splenic tissue. The slides were dried in air, and a drop of the patient's serum inactivated at 56 C for 30 min was then put on the splenic imprint. The slides were incubated in a moist

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closed container for 20 min, and then rinsed with three changes of buffer (FTA hemagglutination buffer pH 7.3, from Baltimore Biological Laboratories), and finally placed in fresh buffer for 15 min. The excess buffer was removed, and the slides were then overlaid with appropriate dilution of fluorescent antihuman γ-globulin (from Hyland Laboratories), and incubated in a moist closed container for 20 min. The slides were finally rinsed, in three changes of buffer, and placed in fresh buffer for 15 min. The excess buffer was removed, and then the slides were mounted with buffered glycerin (9 parts glycerin—1 part phosphate buffer 0.1 molar, pH 7.2) and were read for fluorescence in a Zeiss fluorescent microscope. Positive fluorescence was apparent as an intense apple-green coloration of the nuclei, nuclear membranes, and/or nucleoli. When a positive result was obtained with the undiluted serum, appropriate dilutions of the serum in saline were made up to 1:160, and the tests were repeated in the usual fashion. Lack of nuclear fluorescence was interpreted as a negative result.

The L.E. cell test was performed as follows. Peripheral blood was drawn and allowed to clot. Within 24 hr the clot was macerated and the blood strained through gauze, after which it was centrifuged for 5 min at 2500 rpm. The serum was removed, and smears were made from the buffy coat, which were then stained with Wright's stain and read.

The pattern of fluorescence in the ANF test can occur in four different forms—diffuse homogeneous, shaggy or membranous, speckled, and nucleolar. The particular pattern of fluorescence has been reported by some investigators to have a certain correlation with the type of autoimmune disease. However, not all reports are in agreement with this finding. In our study, the diffuse nuclear fluorescence was observed most commonly (*Fig. 1*), and usually we have not

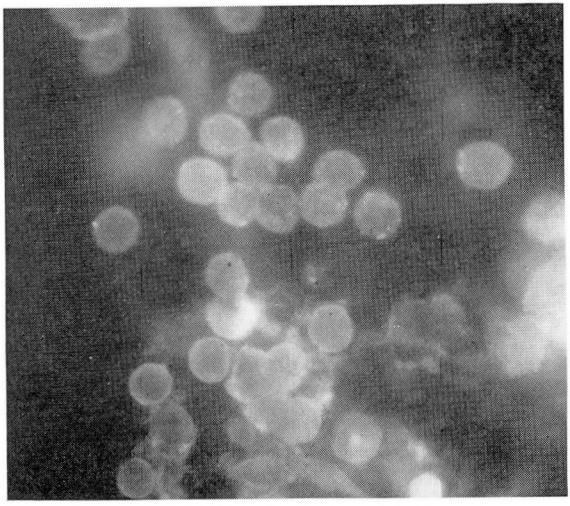

Fig. 1. Photomicrograph showing typical positive fluorescence of splenic lymphocyte nuclei; magnification × 1200.

Table 1.—*Correlation between results of the ANF test and the L.E. cell test*

Groups (results of tests)	Results,* number of patients			
	ANF test		L.E. cell test	
	Positive	Negative	Positive	Negative
I (+ANF + L.E. cell)	45	0	45	0
II (+ANF − L.E. cell)	59	0	0	51
III (−ANF + L.E. cell)	0	3	3	0
IV (−ANF − L.E. cell)	0	353	0	313
Total	104	356	48	364

* In the separation of various groups, the cases in which the L.E. cell test was not done (48) were assumed to be negative for the L.E. cell test; 40 of these cases were in group IV, and 8 in group II.

made a special effort to correlate the pattern of fluorescence with the clinical disease.

The 500 ANF tests were performed on 460 patients whose ages ranged from 2 to 89 years. There were 159 males and 301 females. All of the tests were ordered on the basis of clinical suspicion of an immunologic disease.

Of the 500 ANF tests, 124 (about 25 percent) were positive; whereas, of the 412 L.E. cell tests performed on the same patients, only 48 (11.6 percent) were positive. The results of the L.E. cell tests and of the ANF tests were correlated in the manner as shown in *Table 1.*

Group I—*Positive ANF tests and positive L.E. cell tests.* There were 45 patients in this group, and the distribution of patients according to the primary diseases is summarized in *Table 2.* For 29 of these patients the diagnosis of systemic lupus erythematosus was established by clinical and by laboratory means. For the other 16 patients, either the diagnosis of systemic lupus erythematosus was not clearly established or the patient had some other disease process generally thought to be in the autoimmune category.

Table 3 summarizes the incidence of positive ANF tests and positive L.E. cell tests in regard to patients with some of the autoimmune diseases.

Group II (*Table 4*)—*Positive ANF tests and negative L.E. cell tests.* There were 59 patients in this group, most of whom had clinical manifestations of diseases that are generally classified as possibly autoimmune.

Group III—*Negative ANF tests and positive L.E. cell tests.* In this group there were three patients: two patients with rheumatoid arthritis, and one patient with scleroderma.

Group IV—*Negative ANF tests and negative L.E. cell tests.* This group included 353 patients with various primary diseases, of which some of the more important were: rheumatoid arthritis (34); glomerulonephritis (33); scleroderma (13); discoid lupus erythematosus (12); cirrhosis (8); polymorphous light eruption (8);

Table 2.—*Diagnoses of 45 patients in group I (positive ANF test and positive L.E. cell test)*

Diagnosis	Patients, number
Systemic lupus erythematosus	29
Rheumatoid arthritis	7
Suspected systemic lupus erythematosus	2
Lupus erythematosus diathesis	1
Probable atypical systemic lupus erythematosus	1
Chronic active hepatitis	1
Postnecrotic cirrhosis	1
Hyperlipemia	1
Polymyositis	1
Tertiary syphilis (also had hypertension; on methyldopa treatment)	1
Total	45

Table 3.—*Incidences of positive ANF test and L.E. cell test in regard to certain autoimmune diseases*

	Patients, number					
		ANF test			L.E. cell test	
Diagnosis	Total	Positive (%)		Negative	Positive (%)	Negative
Systemic lupus erythematosus (S.L.E.)	33	31	(94)	2	28 (84.8)	25
Suspected or questionable S.L.E.	10	8	(80)	2	4 (40)	6
Rheumatoid arthritis	50	14	(28)	36	9 (18)	41
Scleroderma	18	5	(27.7)	13	1 (5.5)	17
Discoid lupus erythematosus	13	1	(7.6)	12	0 (0)	13

dermatomyositis (7); multiple sclerosis (6); suspected or family history of systemic lupus erythematosus (6); lymphocytic lupus erythematosus, restricted to skin (5); demyelinating disease (5); polymyositis (4); Raynaud's phenomenon (4); erythema nodosum (4); cadaveric kidney transplant (3); Sjögren's syndrome (3), and idiopathic thrombocytopenic purpura (1).

Discussion

Among those patients for whom the diagnosis of systemic lupus erythematosus was established beyond doubt, by clinical and by laboratory means, the incidence of positive ANF tests (94 percent), and the incidence of the positive L.E. cell tests (84.8 percent) were both high. Similar results have been reported by other investigators.[4] It is also apparent from the results in *Table 3* that perhaps

Table 4.—*Diagnoses of 59 patients in group II (positive ANF test, negative L.E. cell test)*

Diagnosis	Patients, number
Rheumatoid arthritis	8
Scleroderma	8
Probable or questionable systemic lupus erythematosus	5
Systemic lupus erythematosus	4
Glomerulonephritis	4
Sjögren's syndrome	2
Chronic hepatitis	2
Fever of undetermined origin	2
Dermatomyositis	1
Polymyositis	1
Amyloidosis	1
Miscellaneous*	21
Total	59

* This group included one case each of the following: retrobulbar neuritis, erythema multiforme, gouty arthritis, hypertension (on guanethidine sulfate therapy), traumatic arthritis, chronic renal failure, myasthenic syndrome, temporal giant-cell arteritis, chronic polyangiitis, syncope, lymphocytic lupus erythematosus, sickle-cell trait, chronic dermatitis, vasculitis, erythema nodosum, arterial and arteriolar nephrosclerosis, discoid lupus erythematosus, fibrositis, olecranon bursitis, myoclonic seizures (on diphenyl-hydantoin therapy), and osteoporosis of right elbow.

the greatest value of the ANF test lies in the study of patients who have some disease process, clinically not definitely lupus erythematosus, but generally thought to be of an autoimmune nature. Thus, among those patients with suspected or questionable systemic lupus erythematosus the incidence of positive ANF tests was 80 percent as compared with a 40 percent incidence for the positive L.E. cell test. Among patients with rheumatoid arthritis and scleroderma, the incidence of positive ANF tests was significantly higher than that for the L.E. cell tests (*Table 3*). Among patients with discoid lupus erythematosus, however, the difference in the incidence of positive tests between the ANF test and the L.E. cell test was not significant. In this last respect, our results do not agree with those of others,[5] who report an incidence of positive ANF tests as high as 35 percent among patients with discoid lupus erythematosus.

The higher incidence of positive ANF tests, among patients with autoimmune diseases, is consistent with the view that the ANF test is more sensitive in detecting the antinuclear antibodies than is the L.E. cell test, although both of these tests detect the same antinuclear antibodies in the patient's serum. It is not clear to us why for three patients (*Table 1*) the ANF test was negative while the L.E. cell test was definitely positive on more than one occasion. It may be possible that certain inhibitory factors in the patients' sera, the nature

of which remains unknown at the present time, may have been responsible for the negative ANF tests.

Of the 59 patients with positive ANF tests and negative L.E. cell tests, it was interesting that a majority of those patients had disease processes generally thought to be of the autoimmune type. Thus, our results suggest that the ANF test is helpful in diagnosing systemic lupus erythematosus, when the test is performed in conjunction with the L.E. cell test, and perhaps the ANF test is of greater importance because it is an excellent screening test for the evaluation of autoimmune disease processes in general.

Summary

The results of 500 consecutive antinuclear factor (ANF) tests performed at the Cleveland Clinic during 1967 were compared with the results of the L.E. cell tests performed, and their relationship to some of the probable and possible autoimmune diseases was studied. The incidence of positive ANF tests was slightly higher among patients with systemic lupus erythematosus, and significantly higher among patients with suspected or questionable systemic lupus erythematosus, rheumatoid arthritis, and scleroderma than that of the positive L.E. cell tests. It is suggested that the ANF test is of great value as an aid in the diagnosis of systemic lupus erythematosus and as a screening test for some of the other autoimmune diseases.

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