ANTINUCLEAR FACTOR IN RHEUMATOID ARTHRITIS ITS INCIDENCE AND CLINICAL SIGNIFICANCE

BY

D. J. WARD, G. D. JOHNSON, AND E. J. HOLBOROW

M.R.C. Rheumatism Research Unit, Canadian Red Cross Memorial Hospital, Taplow, Bucks

The antinuclear factor (ANF) test has been used in the diagnosis of systemic lupus erythematosus (SLE) for some years since it was first described in 1957 (Friou, Finch, and Detre, 1957; Holborow, Weir, and Johnson, 1957) and in this Unit it has been positive in every case of clinically active lupus erythematosus tested. ANF has also been found in the sera in some cases of rheumatoid arthritis but the incidence of such a finding has varied from centre to centre, being as high as 36 per cent. and even 65 per cent. in some centres (Hall, Bardawil, Bayles, Mednis, and Galins, 1960; Alexander, Bremner, and Duthie, 1960). The test has been performed at the M.R.C. Rheumatism Research Unit, Taplow, for over 5 years on a large series of well-documented cases of rheumatoid arthritis seen at Hammersmith Hospital or Taplow. The purpose of the present study was to determine (a) the frequency of a positive result by the method used in our laboratory and (b) its clinical significance, if any.

Material and Method

The cases studied were selected as meeting the criteria for classical and definite rheumatoid arthritis according to the American Rheumatism Association classification (Ropes, Bennett, Cobb, Jacox, and Jessar, 1959). Sera taken from 273 cases during the 6-year period, 1958-1963, have been studied in retrospect and efforts have been made to repeat the test on one or more occasions in as many cases as possible. Over 500 tests have been performed on these cases. L.E.-cells were looked for in most cases but were not found. In no case was there a rash to suggest SLE and neither was the clinical course in keeping with this diagnosis.

The method for determining the presence or absence of antinuclear factors is an immunofluorescent technique using frozen sections of calf thyroid as nuclear antigen. Full details of the method and a critical appraisal of the test are given by Holborow and Johnson (1964). In all cases a 1:10 dilution of serum was used and all tests were performed by the same person (G.D.J.). No attempt

was made to find the end-point of nuclear fluorescence by a titration procedure. Using this method, the incidence of a definite positive test in sera from normal individuals taken from a population survey is less than 1 per cent. and even weak positives occur in less than 3 per cent.

Results

Considering only the first test carried out on each patient, we found that 220 (81 per cent.) of the 273 cases studied gave a negative result, 44 (16 per cent.) were definite positives, and nine (3 per cent.) were weak positives. Weak positives are subsequently considered as positives. When subsequent tests were also taken into account 211 (77 per cent.) remained negative throughout, 34 (13 per cent.) remained positive throughout, and 28 (10 per cent.) varied, having both positive and negative results on different occasions.

Clinical Features

The patients have been considered in these three groups and a comparison of the basic clinical features is given in Table I (opposite).

The mean follow-up period in these three groups since the first test has been 2.4, 2.2, and 3 years respectively.

At the time of the initial test the duration of arthritis was similar in all three groups, being more than 5 years in just over 70 per cent. The average age of the patients in the three groups was also similar (52, 51, and 52 years). The male:female sex ratio was 1:2.5, 1:3.8, and 1:6.

Functional capacity was graded 1 to 5 where Grade 1 represents normal function, Grade 2 slight impairment, Grade 3 moderate impairment, Grade 4 severe impairment (chair-bed existence), and Grade 5 complete incapacity (confined to bed); the initial mean functional capacity was 2.7, 3.0, and 2.5 in the three groups respectively.

ANTINUCLEAR FACTOR

307

TABLE I
INCIDENCE OF ANF IN CASES OF CLASSICAL AND DEFINITE RHEUMATOID ARTHRITIS

ANF Result	Negative throughout		Positive throughout		Varying Negative and Positive	
	No.	per cent.	No.	per cent.	No.	per cent.
No. of Cases (Total 273)	211	77	34	13	28	10
Mean Follow-up	2.4		2.2		3	
Average Age (yrs) .. .	52		51		52	
Sex Ratio (male: female) ..	1: 2.5		1: 3.8		1: 6	
Mean Grade of Functional Capacity ..	2.7		3.0		2.5	
No. with Rheumatoid Arthritis for 5 yrs ..	147	70	24	70	21	75
Erosions	204	97	34	100	28	100
Nodules*	97	46	28	82	22	79
Positive DAT**	137	65	28	82	24	86

Significant difference between incidence in negative and positive groups * ($P = < .001$)
** ($P = < .05$)

Erosions were present in nearly all cases.

Nodules and a positive sheep cell agglutination test were more common in patients having at least one positive ANF test and the differences are statistically significant by the χ^2 test.

Table II shows that certain other associated clinical features are more frequent in patients having a positive ANF test. Vascular and eye lesions, Felty's syndrome, and infections all had a higher incidence in the positive group than in the negative group and the differences are statistically significant. The difference is also significant when the positive and mixed groups are combined and compared with the negative group.

Vascular lesions were diagnosed if one or more of the following features were present: (1) nail-fold thrombosis and ischaemic skin lesions, (2) ischaemic neuropathy, (3) positive arteriogram, (4) positive biopsy (Bywaters and Scott, 1963). A history of Raynaud's phenomenon alone was not accepted as

sufficient evidence. Fourteen (7 per cent.) of the negative group had vascular lesions, compared with ten (29 per cent.) of the positive and thirteen (46 per cent.) of the mixed group.

Ocular lesions totalled twelve (6 per cent.) in the negative group, thirteen (38 per cent.) in the positive group, and four (14 per cent.) in the mixed group. Keratoconjunctivitis sicca was the predominant lesion and in each case the diagnosis was confirmed by our consultant ophthalmologist, Mr. W. K. Smiley.

Felty's syndrome (chronic polyarthritis with splenomegaly and white counts persistently below 5,000/cu. mm.) was present in two (1 per cent.) of the negative, seven (21 per cent.) of the positive, and two (7 per cent.) of the mixed group.

Infections, chiefly exacerbations of bronchitis and septic lesions, were present at the time of one of the tests in 25 (12 per cent.) of the negative group, thirteen (38 per cent.) of the positive group, and seven (25 per cent.) of the mixed group.

TABLE II
ASSOCIATED CLINICAL FEATURES IN CASES OF CLASSICAL AND DEFINITE RHEUMATOID ARTHRITIS
RELATED TO ANTINUCLEAR FACTOR RESULT

ANF Result		Negative Throughout		Positive Throughout		Varying Negative and Positive	
		No.	Per cent.	No.	Per cent.	No.	Per cent.
Vascular Lesions		14	7	10	29	13	46
Ocular Lesions	Kerato-conjunctivitis sicca ..	8	12	11	38	2	14
	Episcleritis	2		2		1	
	Scleromalacia perforans ..	1		0		0	
	Iritis	1		0		0	
	Iridocyclitis	0		0		1	
Felty's Syndrome		2	1	7	21	2	7
Infections		25	12	13	38	7	25

In each category: $P = < .001$ comparing negative with positive, and $P = < .001$ comparing negative with combined positive and mixed groups.

Relation of ANF to Clinical Course

Apart from the effect of associated features on prognosis, the finding of a positive ANF test did not appear to indicate that the arthritis would pursue a more rapidly downhill course. Functional capacity deteriorated in approximately 30 per cent. of all groups by one, or at the most two grades. Nineteen of the negative group and five of the positive group died (Table III). It will be noted that sepsis in various forms was present in a high proportion of patients dying in both groups.

Because of the possibility of therapy having an effect on the result of ANF testing, the groups were also reviewed in the light of current treatment. Steroids were being taken by 34 per cent. of the negative, 34 per cent. of the positive, and 47 per cent. of the mixed cases. There was little difference between groups in the percentage treated with gold within the 12 months preceding each test. Although no correlation could be found between therapy and the ANF result, drug reactions were statistically more frequent in the positive (15 per cent.) and mixed (29 per cent.) groups than in the negative group (7 per cent.).

The courses of two patients, one from the positive group and one from the mixed group are illustrated in Figs 1 and 2 (opposite).

Case 1, a woman aged 28 at the onset of rheumatoid arthritis in 1945, had generalized nodular, erosive, sero-positive arthritis when seen at Taplow in 1959. The ANF test has remained positive since then. She has vascular lesions in the form of nail-fold thromboses and a maculo-papular erythematous rash on the medial aspect of the knees. A nodule biopsy confirmed vascular changes. The patient developed kerato-conjunctivitis sicca but, despite the ocular and vascular lesions, her functional capacity has not deteriorated. She started to take prednisone after the first ANF test and has continued on this medication. Three subsequent ANF tests have been performed and they have remained positive. It is interesting to note, however, that the DAT has become negative. L.E.-cells have not been found on any of four occasions.

Case 2, a man aged 48 at the onset of nodular, erosive, sero-positive rheumatoid arthritis in 1952, has had vascular lesions since 1954 and recently recurrent infections of the toes. The ANF test has varied between positive and negative but has not been influenced by steroid therapy. Changes in DAT did not correlate

TABLE III
CAUSES OF DEATH IN 24 CASES

ANF	Cause of Death	No. of Cases	
Negative	Secondary amyloid disease { widespread with renal failure with bronchopneumonia	1 1 1	19
	Mesenteric arteritis { with visceral infarcts with septicaemia	1 1	
	Myocardial infarct	2	
	Myocardial ischaemia with bronchopneumonia	1	
	Renal failure (pyelo-nephritis)	2	
	Bronchopneumonia and macroglobulinaemia	1	
	Purulent bronchitis	1	
	Peptic ulcer { haematemesis perforation	2	
	Marrow depression (? gold)	1	
	Rheumatic heart disease	1	
	Subarachnoid haemorrhage	1	
	Meningitis	1	
	?(Died at home, no <i>post mortem</i>)	1	
Positive	Empyema with myocardial infarct with septic arthritis	1 1	5
	Septicaemia and septic arthritis	1	
	Chronic bronchitis and congestive cardiac failure	1	
	Chronic myeloid leukaemia	1

Fig. 1.—Course of disease in Case 1.

Fig. 2.—Course of disease in Case 2.

with those in ANF, but the patient became sero-negative after a course of gold in 1961. The gold was discontinued after twelve injections because of a rash which lasted until 1963. His functional capacity shows little deterioration.

Summary and Conclusions

The results of repeated ANF tests in 273 patients with classical or definite rheumatoid arthritis have been studied in retrospect. Considering initial tests only, 81 per cent. were negative and 19 per cent. positive. Taking all tests into account, 77 per cent. of the initial 273 cases remained negative, 13 per cent. remained positive, and 10 per cent. varied, having both positive and negative results. This should be compared with an incidence of less than 1 per cent. of positive tests found in a normal population using the method described, and an incidence of 100 per cent. in 62 cases of clinically active SLE.

The finding of a positive test does not seem to imply that the arthritis will pursue a less favourable course. Nodules and a positive DAT are encountered more commonly in the positive group and there is a significantly higher incidence of vascular lesions, infections, ocular lesions (especially keratoconjunctivitis sicca), and Felty's syndrome. The

two latter conditions are particularly frequent in patients with a persistently positive ANF test.

In some cases the results of the test may vary from year to year. It has not been found possible to correlate these changes with any simultaneous change in clinical or laboratory findings nor with any alteration in therapy. It is, however, apparent that these patients have a lower incidence of ocular lesions and of Felty's syndrome than those with a consistently positive test.

This study is being extended prospectively so that in future all cases of classical and definite rheumatoid arthritis seen at this hospital will have an annual ANF test.

We are grateful to Mr. W. K. Smiley for his ophthalmological opinion.

REFERENCES

- Alexander, W. R. M., Bremner, J. M., and Duthie, J. J. R. (1960). *Ann. rheum. Dis.*, **19**, 338.
- Bywaters, E. G. L., and Scott, J. T. (1963). *J. chron. Dis.*, **16**, 905.
- Friou, G. J., Finch, S. C., and Detre, K. D. (1957). *Fed. Proc.*, **16**, 413.
- Hall, A. P., Bardawil, W. A., Bayles, T. B., Mednis, A. D. and Galins, N. (1960). *New Engl. J. Med.*, **263**, 769.

- Holborow, E. J., and Johnson, G. D. (1964). *Arthr. and Rheum.*, (in press).
- , Weir, D. M., and Johnson, G. D. (1957). *Brit. med. J.*, 2, 732.
- Ropes, M. W., Bennett, G. A., Cobb, S., Jacox, R., and Jessar, R. A. (1959). *Ann. rheum. Dis.*, 18, 49.

Ces études seront continuées et désormais tous les malades atteints d'arthrite rhumatismale classique et définie vus dans notre hôpital, seront soumis tous les ans au test ANF.

El factor antinuclear en la artritis reumatoide

SUMARIO

Se estudiaron retrospectivamente los resultados de los tests para el factor antinuclear (test ANF) en 273 enfermos con artritis reumatoide clásica o definida. Al considerar sólo los tests iniciales, se hallaron un 81 por ciento negativos y un 19 por ciento positivos. Al tomar en consideración todos los tests, un 77 por ciento de los 273 casos quedaron negativos, un 13 por ciento quedaron positivos y un 10 por ciento variaron en ambos sentidos. Se compara esto con la frecuencia de menos de un por ciento en la población normal y del 100 por ciento en 62 casos de lupus eritematoso disseminado y activo clínicamente.

Un test positivo no quiere decir que el curso de la enfermedad sera menos favorable. Nódulos de Heberden y una reacción de Waaler-Rose positiva se ven con más frecuencia en el grupo positivo que comporta también un número significativamente mayor de lesiones vasculares, infecciones, lesiones oculares (en particular la querato conjunctivitis sicca) y el síndrome de Felty. Las dos últimas afecciones se ven con una frecuencia particular en enfermos con el test ANF siempre positivo.

En ciertos casos los resultados del test pueden variar cada año. No se pudieron correlacionar estos cambios con alteraciones simultáneas en los dominios clínico, terapéutico o de laboratorio. No cabe duda, sin embargo, que la frecuencia en estos enfermos de lesiones oculares y del síndrome de Felty es menor que en enfermos que acusan un test constantemente positivo.

Se intenta continuar este estudio y se anticipa un test ANF anual de todos los enfermos con artritis reumatoide clásica o definida, vistos en nuestro hospital.

Facteur antinucléaire dans l'arthrite rhumatismale

RÉSUMÉ

On a étudié retrospectivement les résultats des tests pour déceler le facteur antinucléaire (test ANF) chez 273 malades atteints d'une arthrite rhumatismale classique ou définie. Si l'on considère les tests initiaux seulement, 81 pour cent. étaient négatifs et 19 pour cent. positifs. Lorsqu'on prend en considération tous les tests, 77 pour cent. des 273 cas demeuraient négatifs, 13 pour cent. demeuraient positifs et 10 pour cent. variaient, donnant des résultats aussi bien positifs que négatifs. On compare cela à la fréquence de moins d'un pour cent. pour la population normale et de 100 pour cent. de 62 cas de lupus érythémateux disséminé, cliniquement actif.

Le fait qu'un test est positif ne veut pas dire que le pronostic est moins favorable. Les nodosités d'Heberden et la réaction de Waaler-Rose positive sont plus fréquentes dans le groupe positif et on y trouve une incidence significativement plus grande des lésions vasculaires, des infections, des lésions oculaires (particulièrement la *kérato-conjunctivitis sicca*) et du syndrome de Felty. Les deux dernières affections sont particulièrement fréquentes chez des malades ayant le test ANF constamment positif.

Dans certains cas les résultats du test peuvent varier d'un an à l'autre. On n'a pas pu trouver de rapport entre ces variations et les variations cliniques, thérapeutiques ou de laboratoire. Il est toutefois apparent que la fréquence chez ces malades des lésions oculaires ou du syndrome de Felty est moindre que chez des malades accusant un test toujours positif.