

SIGNIFICANCE OF ANTINUCLEAR FACTORS IN OLDER PERSONS

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Antibodies to a variety of tissues and tissue extracts have been demonstrated in the serum of older persons (Goudie, Anderson, and Gray, 1959; Irvine, 1965). The occurrence of antinuclear factors (ANF) in the ageing has been pointed out by Beck (1963) and Litwin and Singer (1965). These studies, however, have dealt with populations composed of both well and sick individuals and have not related the determination of ANF to disease. Litwin and Singer (1965), and Cammarata, Rodnan, Fennell, Cestello, and Creighton (1964) have related the occurrence of antigamma globulin factors (AGGF) to chronic disease other than connective tissue diseases and have also suggested that they (the AGGF) may be related to changes which they found in immunoglobulins. Recent experimental data suggest the ANF may be more widespread than previously estimated, and that they may occur more frequently in older experimental animals (Barnett and Vaughan, 1966).

The availability of a population of older persons stimulated us to test the incidence of ANF and to evaluate its presence as related to disease, to ageing *per se*, and to changes in the serum immunoglobulins.

Material and Methods

Subjects.—The persons studied were residents of a retirement home who were selected on the basis of reasonably good health and adequate income. The establishment is an urban apartment house and attracts active, retired individuals of moderate means. Married

couples or individuals live in their own apartments with a common dining room; a dietitian and staff physician are available, and there is also an infirmary.

Of the 256 residents, 161 (131 women and 30 men) agreed to undergo an examination including blood tests. 10 months later 131 of them (109 women and 22 men) were re-tested.

The subjects' ages ranged from 59 to 98 years (mean 75). A complete medical history was taken and a physical examination was made by one of us (RFW), with a special examination of the musculoskeletal system.

Laboratory studies included haematocrit, white blood cell count, examination of a blood smear, erythrocyte sedimentation rate (Westergren), and VDRL test for syphilis in addition to the following specific tests:

Antinuclear Factor (ANF) was determined using cryostat-cut rat liver sections layered with the patients' serum diluted 1:5 with phosphate-buffered saline (pH7.0) and counter stained with fluorescein-labelled goat antihuman globulin.* Slides were interpreted by three observers independently and graded from 0 to 4+ on the intensity of fluorescence.

Rheumatoid Factor, which is here considered as anti-gamma globulin factor (AGGF), was sought by three methods: slide latex agglutination,† tube latex agglutination (Singer and Plotz, 1956), and sensitized sheep cell agglutination (SSC).

Immunoglobulin Levels (IgG, IgA, IgM) were determined by diffusion on agar plates containing antisera to specific immunoglobulins (Fahey and McKelvey, 1965)†. Pooled normal sera were used to establish a reference curve, and all measurements were performed in duplicate.

Controls.—For comparison, the tests for antinuclear factor, rheumatoid factor, and immunoglobulin levels were performed on the sera of 150 college students coming to the Health Service of the University of Washington with various minor complaints. This group consisted of 64 females and 86 males ranging in age from 18 to 25 years.

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Results

The results are shown in Tables I and II. ANF was present in 24 (21 women and 3 men) of the 161 in the older group, and the test was positive in greater than trace amount in nine of the 24. Six of these nine (all women) had a definable disease: rheumatoid arthritis (2); possible systemic lupus erythematosus (2); neoplasia (2). Brief case reports of these six female patients (Nos 29, 10, 15, 9, 78, and 44) are given below*; besides these six,

ANF was present in fifteen women and three men (11 per cent.).

ANF was present in only four of the control group of 150 college students (2.5 per cent.), all in trace amount. This differs significantly from the group of older subjects ($P < 0.001$).

The 131 older subjects who were re-tested 10 months later included nineteen in whom ANF was originally present, and fifteen of these were still positive, five being the five surviving subjects with recognized disease. In addition, eight sera originally negative now showed ANF (Table II).

TABLE I

ANF AND AGGF IN OLDER AND YOUNGER SUBJECTS ON INITIAL TESTING†, EXCLUDING SIX OLDER WOMEN WITH KNOWN DISEASE

Series	Sex	No. of Cases	ANF	AGGF	
				Slide	Tube
Retirement Home	Male	30	3	4	3
	Female	125*	15	38	7
	Total	155	18	42	10
College Students	Male	86	2	2	2
	Female	64	2	1	0
	Total	150	4	3	2

† Six subjects with recognized disease excluded.

Case Reports*

Patient No. 29, a 79-year-old farmer's widow, had presented one year before the study with migratory polyarthritis of the shoulders, knees, elbows, and finger joints. The erythrocyte sedimentation was 50 mm./hr (Westergren) but rheumatoid factor was not present. She was treated with gold sodium thiomalate without benefit and subsequently developed myalgia, finger-tip ulcerations, malar erythema, palatal ulcers, and proteinuria. At this point, serum obtained for this study was strongly positive (+ 4) for ANF. An L.E. preparation was negative, renal biopsy revealed arteriolar nephrosclerosis, and serum levels of muscle enzymes were ele-

TABLE II

ANF AND AGGF INDIVIDUAL RESULTS IN ALL OLDER SUBJECTS WHO GAVE POSITIVE RESULTS AT FIRST AND/OR SECOND EXAMINATION

Patient No.	Sex	ANF		AGGF		Diagnosis	
		1965	1966	Slide	S.P.		
29	F	+4	+4	+	--	? SLE	
10	F	+2	+2	--	--	? SLE	
78	F	2	2	+	+	Rheumatoid Arthritis	
9	F	3	3	--	--	Lymphosarcoma	
15	F	+2	Died	--	--	Carcinoma	
44	F	1	1	--	--	Rheumatoid Arthritis	
8	M	Tr*	2	--	--	No recognized disease	
144	F	2	4	--	--		
13	F	2	--	--	--		
81	F	1	--	--	--		
34	F	Tr	ND	--	--		
49	M	Tr	1	--	--		
151	F	Tr	Tr	+	--		
5	M	Tr	ND	+	+		
30	F	Tr	1	--	--		
64	F	Tr	Tr	--	--		
79	F	Tr	--	+	--		
109	F	Tr	Tr	+	--		
11	F	Tr	2	--	--		
127	F	Tr	--	--	--		
126	F	Tr	Tr	--	--		
168	F	Tr	ND	+	--		
176	F	Tr	ND	+	--		
33	F	Tr	--	--	--		
72	F	--	Tr	+	--		No recognized disease
76	M	--	2	--	--		
110	F	--	+1	+	--		
133	M	--	Tr	--	--		
142	F	--	2	--	--		
143	M	--	Tr	--	--		
153	F	--	3	+	--		
160	F	--	Tr	+	--		

*Trace

ND Not Done

†VDRL also positive

-- Negative

vated. She was treated with prednisone 15 mg./day with rapid improvement in her symptoms.

Patient No. 10, a 75-year-old physician's widow, had been seen 2 years before the study for pleurisy, recurrent pulmonary infiltrates, and a deep ulcer on the dorsum of the left foot which developed without injury or venous insufficiency. Physical examination revealed no abnormalities. The ANF was 2+, an L.E. preparation was negative, and the erythrocyte sedimentation rate was 10 mm./hr.

Patient No. 15, an 85-year-old YMCA secretary, had ANF 2+ at the initial study. Within 3 months she was found to have carcinoma of the uterus which spread rapidly; she died soon thereafter, and was not included in the second series of tests.

Patient No. 9, a 79-year-old missionary's wife, had localized lymphosarcoma found at laparotomy one year before the study. Subsequently, the serum was positive on both occasions, demonstrating 3+ nucleolar staining for ANF.

Patients 78 and 44, both retired schoolmistresses, one aged 64 and the other 69 years, had long-standing rheumatoid arthritis with multiple joint involvement.

The 155 patients without disease are grouped by age and sex in Table III. The tests were positive more often in women than in men. There was no trend to increasing prevalence of ANF with age.

Antigamma globulin factors were found with greatest frequency with the slide latex method and less often with the Singer-Plotz test (Table I). Both tests were significantly more often positive in the older group than in the college students.

Only one serum, from No. 78, a woman with rheumatoid arthritis, was also positive in the SSC test.

The correlation of AGGF with ANF was slight (Table II).

The only serum in which the VDRL test was positive (No. 51) was also positive for ANF and AGGF; this patient had no recognized disease.

Mean immunoglobulin levels of the 161 in the older group and of fifty of the younger group are recorded in Table IV. The mean IgA level of the older group is greater than that of the younger, but the difference is significant only in the older males. The sera with ANF and AGGF do not have greater levels of immunoglobulins. There was no difference with increasing age.

TABLE IV
IMMUNOGLOBULINS (mg./ml.)

Group		No. of Cases	IgG	IgM	IgA
Young	50	1300	110	230
Older	Females	131	1230	110	270
	Males	30	1310	70	350
	Total	161	1240	100	290
Older with AGGF	..	42	1300	130	290
Older with ANF	..	17	1310	140	310

Discussion

Of the older persons studied, 11 per cent. were found to have ANF compared with 2.5 per cent. of the younger control group. The prevalence was greater in older females than in males, as was also found by Beck (1963) and Seligmann, Cannat, and Hamard (1965). At a serum dilution of 1:4, Beck found that 32 per cent. of random hospitalized females gave positive tests compared with 19 per cent. of males. In Seligmann's study of 55 presumably normal blood donors between the ages of 60 and 80, 20 per cent. of the females and 13 per cent. of the males were positive.

TABLE III
ANF AND AGGF IN OLDER INDIVIDUALS WITHOUT RECOGNIZED DISEASE
GROUPED BY AGE AND SEX

Age (yrs)	Sex	No. of Cases	ANF		AGGF			
			No.	Per cent.	Slide		Tube	
					No.	Per cent.	No.	Per cent.
60-69	Male	4	1	25	0	0	0	0
	Female	28	2	7.4	3	14	1	4
70-79	Male	15	0	0	2	10	2	13
	Female	67	10	15	28	41	3	4
80+	Male	11	2	20	2	18	2	18
	Female	30	3	10	7	23	2	7
Total		155	18		42		10	

It is noteworthy that six of the nine older subjects with greater than trace-positive ANF tests had some form of disease. Of nineteen subjects originally positive and available for re-testing, fifteen were still positive, and these included the five surviving patients with recognized disease. Excluding these five, there were thus ten individuals in whom the ANF remained positive; in six the degree of positivity had increased and in four it was the same. An additional eight subjects, negative at the first testing, were positive at the second (Table II). The reasons for these changes were not evident in the subject's clinical status. There is no clear evidence that ANF reactivity increases with age *per se*. These variations in a 10-month period suggest that unrecognized environmental factors such as intercurrent viral infections may influence the result.

Antigamma globulin factors were found in 26 per cent. of the older patients compared with 2 per cent. of the younger group, using the slide test (Table IV). Heimer, Levin, and Rudd (1963), using the same test, found that 42 per cent. of the inhabitants of an old age home were positive. The good health of the patients in this series makes the lower incidence of AGGF comparable to that of the patients reported by Litwin and Singer (1965) of whom 20 per cent. were positive. In our series the 70 to 80-year age group had a higher incidence of AGGF than the 60 to 70-year group, but this trend did not continue into the ninth decade.

No obvious relationship was noted between these factors and clinical disease. Except for one positive SSC test in a patient with rheumatoid arthritis, the AGGF of the older persons was of the "non-rheumatoid" variety, as has been found with liver disease (Dresner and Trombly, 1959), tuberculosis (Singer, Plotz, Peralta, and Lyons, 1962), and bacterial infections (Williams and Kunkel, 1962). Since the SSC test is the most specific test for rheumatoid factor, AGGF is a better term for the protein

responsible for latex agglutination in these older subjects.

Immunoglobulin levels in the older group showed little difference from those in the younger group. There was no significant change with increasing age or correlation with the presence of ANF or AGGF. The older males in this study showed a consistently higher level of IgA than the older females or the younger controls. Unlike Litwin and Singer (1965), we failed to show any increase in IgG with increasing age, nor was the presence of abnormal macroproteins, M-components, suggested by disproportionately elevated values for individual immunoglobulins.

Summary

ANF and AGGF of the non-rheumatoid type were found more frequently in a group of 161 older healthy retired subjects than in a group of 150 college students.

Six of nine older subjects whose serum contained ANF in greater than trace amount had evidence of disease (malignancy in two, rheumatoid arthritis in two, and possible systemic lupus erythematosus in two). ANF was also present in eighteen older individuals who had no evidence of disease.

Re-testing 131 available individuals of the older group 10 months later showed changes from positive to negative in four of them and from negative to positive in eight. This variation suggests that ANF in older individuals, particularly in trace amount, may be due to unrecognized environmental changes. A persistently positive test, particularly in greater than trace amount, suggests the presence of connective tissue disease or malignancy.

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L'importance des facteurs antinucléaires chez des personnes âgées

RÉSUMÉ

Le facteur antinucléaire (*ANF*) et le facteur antigamma-globuline (*AGGF*) furent trouvés plus souvent dans un groupe de 161 vieillards sains que dans un groupe de 150 étudiants.

Six sur neuf sujets âgés dont le sérum contenait plus qu'une trace d'*ANF* présentèrent des signes de maladie (néoplastique chez deux d'entre eux, rhumatismale arthritique chez deux autres et de lupus érythémateux disséminé possible chez les deux restants). L'*ANF* fut aussi présent chez 18 personnes âgées ne manifestant aucun signe de maladie.

Un examen répété dix mois plus tard du sérum des 131 personnes âgées disponibles révéla un changement de la réaction positive à la négative chez quatre d'entre eux et de la négative à la positive chez huit d'entre eux. Cette variation suggère que l'*ANF* chez des personnes âgées, surtout lorsqu'il s'agit d'une trace, peut être dû à des changements méconnus du milieu ambiant. Une réaction toujours positive, surtout lorsqu'on en trouve plus qu'une trace, suggère la présence d'une maladie du tissu conjonctif ou d'une néoplasie.

La importancia de los factores antinucleares en personas de edad avanzada

SUMARIO

El factor antinuclear (*ANF*) y el factor antigamma-globulínico (*AGGF*) fueron encontrados con más frecuencia en un grupo de 161 ancianos sanos que en un grupo de 150 estudiantes.

Seis de los nueve ancianos cuyo suero contuvo más que huellas de *ANF* presentaron evidencia de enfermedad: neoplástica en dos, artritis reumatoide en otros dos y un posible lupus eritematoso diseminado en los dos últimos. Se reveló también el *ANF* en 18 ancianos sin manifestación alguna de enfermedad.

A repetir el test en 131 ancianos disponibles se notó un cambio de la reacción positiva a la negativa en cuatro de ellos y de la negativa a la positiva en ocho. Esta variación sugiere que en personas de edad avanzada la presencia del *ANF*, particularmente si se trata sólo de huellas, se puede deber a cambios poco conocidos del medio ambiente. En cambio, una reacción persistentemente positiva, cuando se encuentra más que huellas, sugiere la presencia de una enfermedad del tejido conectivo o de una neoplasia.