AGE-RELATED RHEUMATOID FACTORS
INCIDENCE AND CHARACTERISTICS

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The prevalence of the RA factor in sera of healthy young or middle-aged subjects has been found to be approximately 4 per cent. During the past few years, however, it has become increasingly clear that positive reactions are much more frequent when subjects 65 years of age and older are tested (Table I). Review of these studies reveals that the prevalence of positive reactions is consistently greater in aged than in young persons. There is considerable variation among populations from different geographical

areas and also according to the different techniques used in testing for the RA factor.

The present work compares the prevalence of RA factor in the serum of a well-defined hospitalized population with that in a healthy ambulatory population and in a group of patients seen at a rheumatology out-patients department; it also compares the results obtained with a Hyland RA slide test with and without inactivation of the serum, with a modification of this slide test (Watson, 1965) with a

TABLE I
INCIDENCE OF RA FACTOR IN ELDERLY PERSONS. SUMMARY OF THE LITERATURE

Authors	Dates	Geographical Area	Number of Subjects Tested	Age (yrs)	Per cent. Positive reactions		
					Hyland RA-test	Latex-Fixation Test	Waalser-Rose Test
Ball and Lawrence	1961	Leigh (U.K.) Wensleydale (U.K.)	201 178	65 65			11.9
Heimer and others	1963	New York (U.S.A.)	50 121	Control 65	46	4 42	2 2
Waller and others	1964	Richmond (U.S.A.)	2,828 2,536 97	0-29 30-59 60-89	3.08 4.3 27.8		0.07 0.24 2.06
Milazzo, Cattaneo, and Invernizzi	1964	Milan (Italy)	50 110	25-64 64	6 47.2		12 15.5
Jacqueline, Podliachouk, and Eyquem	1965	Savoy (France)	270 248	20-69 70		1.1 10.9	1.1 12.5
Litwin and Singer	1965	New York (U.S.A.)	235	Controls 30-91		2.6 25.4	2.9 14.9
Adler, Abramson, Goldberg, Elkan, and Hador	1967	Jerusalem (Israel)	60 F 56 M	65 65		11.6 6.2	1 0
Cammarata, Rodnan, and Fennell	1967	Pittsburgh (U.S.A.)	165 135 25	65-79 80-89 90		17 13 24	
De Blécourt, Westendorp Boerma, and Vorenkamp	1967	Gröningen (Netherlands)	195	65		8.2	8.8
Mikkelsen, Dodge, Duff, and Kato	1967	Michigan (U.S.A.)	272	70		11.4	
Present work	1968	Leuven (Belgium)	68 144	30-59 60-95	19 33.3	5.9 9	1.5 7

qualitative and quantitative reading, and with a modified Waaler-Rose test (Podliachouk, Eyquem, and Jacqueline, 1958).

Material and Methods

The hospitalized population comprised 212 psychiatric females aged 30 to 94 years, the healthy ambulatory population 96 blood donors aged 15 to 65 years (48 males and 48 females), and the rheumatic control population 83 patients aged 17 to 82 years (37 males and 46 females).

The following serological tests for RA factor were performed on the serum specimens:

(1) *Hyland RA slide test*: Serum specimens diluted 1:20 in glycine buffer were mixed with the Hyland RA reagent and read after one minute.

(2) *Hyland RA slide test after inactivation of sera*: Before mixing with the reagent, the 1:20 diluted serum specimens were heated to 56°C. for 30 minutes.

(3) *Modified RA slide test* (Watson, 1965):

(a) *Preliminary screening test*: The serum specimens were previously heated to 56°C. for 30 minutes and the test performed on serum diluted 1:16;

(b) *Quantitative test*: Sera with a positive result in the screening test were serially diluted in glycine buffer in order to obtain a quantitative reading.

(4) *Modified Waaler-Rose test* (Podliachouk and others, 1958).

Results

Hospitalized Female Population

A summary of the results of the various serological tests by age groups performed on serum samples appears in Table II.

With the Hyland RA slide test there was a high frequency of positive results in all age groups, with a trend towards a higher frequency in the aged, 33 per cent. in those aged 60 years and over and 19 per cent. in those aged less than 60 years. Inactivation of the serum decreased the percentage of positive results in all age groups, but the trend towards higher frequency with age remained, and the difference (20.1 per cent. above 60, and 5.9 per cent. below 60) became statistically significant ($P < 0.001$).

With the Watson screening test 47 per cent. of the older group were positive and 17.5 per cent. of the

TABLE II
PERCENTAGE DISTRIBUTION OF POSITIVE RA-FACTOR TESTS IN 212 HOSPITALIZED FEMALES, BY AGE

Age group (yrs)	..	..	..	30-39	40-49	50-59	60-69	70-79	80-89	30-59	60-94	30-94
No. of Subjects	..	..	..	16	26	26	73	49	23	68	144	212
Hyland RA				6.2	27	19	31.5	35	35	19	33.3	28.5
Hyland RA after inactivation of sera				0	3.8	11.5	18	21	22	5.9	20.1	15.5
Watson screening				12.5	11.5	27	46.5	46	52	17.5	47	37.5
Watson quantitative				12.5	11.5	23	31.5	14.5	39	16.2	27	23.5
	≥ 1/32			6.2	0	11.5	9.6	8	8.7	5.9	9	8
	≥ 1/64			0	0	3.8	2.7	0	0	1.5	1.4	1.4
	≥ 1/128			0	0	0	1.4	0	0	0	0.7	0.4
	≥ 1/256			0	0	0						
Modified Waaler-Rose				0	3.8	3.8	16.5	16.5	13	3	16	11.7
	≥ 1/32			0	3.8	0	6.8	6.2	8.7	1.5	7	5.2
	≥ 1/64			0	0	0	2.7	0	4.3	0	2.1	1.4

TABLE III
PERCENTAGE DISTRIBUTION OF POSITIVE RA FACTOR TESTS IN 87 INSTITUTIONALIZED AND 93

Age Group (yrs)	..	..	..	..	..	..	20-29	30-39
Population Studied	..	..	..	..	..	..	Institutionalized	Institutionalized
No. Tested	..	..	..	..	..	..	20	23
Hyland RA							0	4.3
Hyland RA after inactivation of sera							0	4.3
Watson screening							0	8.7
Watson quantitative							0	4.3
	≥ 1/32						0	0
	≥ 1/64						0	0
	≥ 1/128						0	0
	≥ 1/256						0	0
Modified Waaler-Rose							5	0
	≥ 1/32						0	0
	≥ 1/64						0	0

younger group ($P < 0.001$). The age difference disappeared in the quantitative Watson test. At a titre of 1: 128, 1.4 and 1.5 per cent. positive results were observed, a titre of 1: 256 was seen only once in 144 sera of subjects aged 60 years and more, and none in 68 sera of those below 60 years.

Of the 212 subjects, only 5.2 per cent. were found to have a modified Waaler-Rose test positive at a titre of 1: 32 or more. There was again a difference in the percentage of positive reactors between the older (7 per cent.) and younger (1.5 per cent.) subjects.

Healthy Ambulatory Population

With all test techniques a trend towards higher frequency of positive results in the aged subjects was noted. Below age 50 no flocculation was found in a serum dilution of 1: 64 or higher with the modified latex test nor of 1: 32 or higher with the modified

Waalser-Rose test. A comparison of the distribution of positive results in 87 of the institutionalized population and 93 of the healthy ambulatory population revealed a higher incidence of positive results with the latex tests and a lower incidence with the Waaler-Rose test in the institutionalized group (Table III).

Rheumatic Patients

According to the criteria of the ARA (Ropes, Bennett, Cobb, Jacox, and Jessar, 1959), these were classified in three groups (Table IV). 83 per cent. of those with definite or classical rheumatoid arthritis and 31 per cent. of those with other rheumatic diseases had a positive Hyland RA screening test. Inactivation of the sera brought no difference in the rheumatoid arthritis groups but the percentage of positive results was halved in the group not suffering from rheumatoid arthritis.

In 68 and 55 per cent. of the definite and classical

TABLE IV
PERCENTAGE DISTRIBUTION OF POSITIVE RA FACTOR TESTS IN 83 PATIENTS SUFFERING FROM RHEUMATIC DISEASES, CLASSIFIED BY THE ARA CRITERIA

Diagnosis		Rheumatoid Arthritis		Other Rheumatic Diseases
		Definite or Classical	Probable	
Mean Age (yrs)		48.9	48.7	48.6
No. Tested		47	7	29
Hyland RA		83	57	31
Hyland RA after inactivation of sera		85	57	14
Watson screening		89	57	17
Watson quantitative	≥ 1/32	79	57	14
	≥ 1/64	68	43	6.9
	≥ 1/128	55	14	0
	≥ 1/256	51	0	0
Modified Waaler-Rose	≥ 1/16	72	28	17
	≥ 1/32	64	14	7
	≥ 1/64	55	0	0

II

HEALTHY AMBULATORY (NON-INSTITUTIONALIZED) POPULATIONS, AGED 20 TO 64 YEARS

40-49		50-59		60-64	
Institutionalized	Non Institutionalized	Institutionalized	Non Institutionalized	Institutionalized	Non Institutionalized
26	26	26	18	19	6
27	3.6*	19	5.5	31.5	16.6
3.8	0	11.5	11.1	26.3	16.6
11.5	11.5	27	16.6	57.8	16.6*
11.5	0	23	11.1	31.5	16.6
0	0	11.5	11.1	10.5	16.6
0	0	3.8	5.5	5.2	0
0	0	0	0	5.2	0
3.8	7.7	3.8	22.2*	26.3	33.3
3.8	0	0	16.6	5.2	16.6
0	0	0	11.1	5.2	0

* $P < 0.01$

rheumatoid arthritis groups the Watson latex test titre was 1: 64 and 1: 128 or more respectively, compared with 6.9 and 0 per cent. in the group with other rheumatic diseases.

With the modified Waaler-Rose test a titre of 1: 32 or more was found in 64 per cent. of the rheumatoid arthritis group and 7 per cent. group with other rheumatic diseases.

Discussion

Our finding of a high frequency of RA factor in the sera of elderly people is in agreement with the observations of other authors who have reported increased amounts of serum antiglobulin factors in large series of subjects (Table I). In all our test techniques a trend towards increased frequency with advancing age was noted. The highest percentage of positive reactions was found using latex particles coated with human γ -globulin. When serum was examined by means of sensitized human red cell agglutination test, in which the γ -globulin coating is of non-human origin, the number of positive reactions was much lower, although still greater in older than in younger persons. Heimer, Levin, and Rudd (1963) and Waller, Toone, and Vaughan (1964) reported the same results.

Inactivation of the sera by incubation at 56° C for 30 minutes markedly decreased the percentage of positive latex-fixation reactions in the non-rheumatic groups, but no difference was noted in the sera of patients suffering from rheumatoid arthritis. Inactivation of the serum increases the specificity of the latex-fixation test by eliminating a heat-labile agglutinator which is responsible for a number of positive results in the elderly.

When serum was examined quantitatively the difference between young and old subjects decreased with increasing titres. Aged sero-reactors had a predominantly low range of titres in contrast with patients suffering from rheumatoid arthritis where the majority had high titres. Titration of the sera seems to be necessary to differentiate the positive reactions of the "normal" aged from the positive reactions occurring in rheumatoid arthritis patients.

In the hospitalized population, the incidence of positive latex reactions was higher and that of positive Waaler-Rose reactions lower than in an age-matched healthy ambulatory population (Table III). De Blécourt, Boerma, and Vorenkamp (1967) and Litwin and Singer (1965) observed a difference in occurrence of RA factor between institutionalized and non-institutionalized groups. Ball and Lawrence (1961) found differences in the numbers of posi-

tive reactions in urban and rural populations. De Blécourt and others (1967) confirmed this finding in the Netherlands.

The biological significance of the presence of a high incidence of antiglobulin factor in the serum of aged people is not clear. According to Lichtman, Vaughan, and Hames (1967), chronic infection, which is more frequent in older individuals, could be responsible for a stimulation of antiglobulin production. On the other hand, it is known that ageing is also associated with an increased frequency of biological false-positive reactions for antinuclear factor, thyroglobulin antibodies, and VDRL-reagents. In the light of these findings, the increased incidence of RA factor in old age may perhaps relate to a piling up of autoantibodies.

A third hypothesis is that the heat-stable inhibitors in the serum, which inhibit the agglutinating effect of RA factor, decrease with advancing age, as was proposed by Klein (1964). Another possible explanation for the high incidence of positive RA factor in the aged might be that this reflects an increasing incidence of rheumatoid arthritis with age. Suggestive evidence for this explanation can be found in population studies, where a higher incidence of rheumatoid arthritis was noted on follow-up in the sero-positive group than in the sero-negative group (Ball and Lawrence, 1961; Laine, 1966). Against this hypothesis is the fact that the sex distribution of the positive reactions in the aged is approximately equal, in contrast to the predominance of rheumatoid arthritis in females.

Regardless of the true cause of the increased incidence of positive reactions for RA factor in serum samples from the aged, the hazards of using serological tests in the diagnosis of rheumatoid arthritis in elderly persons should be recognized. With inactivation of the sera and quantitative measurements of the positive screening tests, the hazards of false positive reactions in the elderly can be reduced to a minimum. In our hands, the RA latex test by the Watson procedure is specific for rheumatoid arthritis from a titre of 1: 64 and the modified Waaler-Rose test from a titre of 1: 32. With these titres less than 10 per cent. "false positive" reactions in the very aged were found, and 68 per cent. of the definite or classical rheumatoid arthritis patients were sero-positive.

Summary and Conclusion

The distribution of RA factor detected by different techniques was studied in a group of 308 persons, ranging in age from 15 to 94 years, and not suffering from rheumatoid arthritis, 83 patients attending a

rheumatology clinic serving as a control group. It was found that an important number of normal persons had a positive RA factor reaction and that the frequency increased with advancing age.

Inactivation of the sera reduced the frequency of positive reactions in normal people, especially the aged, but did not affect the presence of RA factor in patients suffering from rheumatoid arthritis. With quantitative measurements it was possible to differentiate the "false positive" reactions from the positive reactions found in rheumatoid arthritis.

A predominantly low range of titres was observed in aged sero-reactors and a high range of titres in the rheumatoid arthritis patients.

The biological significance of the presence of a high incidence of antiglobulin factor in the sera of aged persons has been discussed.

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Facteurs rhumatoïdes en relation avec l'âge**RÉSUMÉ**

La distribution du facteur RA découverte par différentes techniques a été étudiée chez un groupe de 308 personnes âgées de 15 à 94 ans qui n'étaient pas atteintes de polyarthrite rhumatoïde, 83 malades soignés à un dispensaire rhumatologique servant comme témoins. On a trouvé qu'un important nombre de personnes normales donnait une réaction positive au facteur RA et que cette fréquence augmentait avec l'âge.

L'inactivation du sérum réduisait la fréquence des réactions positives chez les sujets normaux, spécialement chez les vieillards, mais ne l'affectait pas chez les malades atteints de polyarthrite rhumatoïde. Par des titrages il a été possible de différencier entre les réactions "pseudo-positives" et les réactions positives trouvées dans la polyarthrite rhumatoïde. Une gamme de titres bas avait été observée chez les séro-réactifs âgés et une gamme de titres élevés chez les patients atteints de polyarthrite rhumatoïde.

La signification biologique d'une incidence marquée du facteur antiglobuline dans le sérum des personnes âgées a été discutée.

**Factores reumatoïdes relacionados con la edad
Incidencia y características****SUMARIO**

La distribución del factor RA detectado por distintas técnicas fue estudiado en un grupo de 308 personas, cuyas edades oscilaban entre los 15 y los 94 años, y que no sufrían de poliartritis reumatoide, sirviendo de testigos 83 pacientes que asistían a una clínica reumatológica. Se descubrió que un número importante de personas normales tenía una reacción positiva al factor RA, y que la frecuencia aumentaba con el paso de los años. La inactivación de los sueros redujo la frecuencia de las reacciones positivas en personas normales, especialmente las de más edad, pero no afectó la presencia del factor RA en pacientes que padecían de poliartritis reumatoide. Empleando medidas cuantitativas fue posible diferenciar las reacciones "falsopositivas" de las reacciones positivas halladas en la poliartritis reumatoide. Se observó una variedad de títulos predominantemente bajos en los ancianos seroreactores, y una alta variedad de títulos en los pacientes con poliartritis reumatoide.

Se ha discutido el significado biológico de la presencia de una alta incidencia de un factor antiglobulina en los sueros de personas ancianas.