ROMANIAN J. GERONT. GERIATRICS, 1980, 1, 1, p. 133—141

AUTOIMMUNE PHENOMENA IN THE ELDERLY

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Summary. Studies were carried out on 300 apparently healthy subjects (180 women and 120 men) from Bucharest, ranging in age from 40 to 90 years, on the presence of smooth muscle (SMA), antinuclear (ANA) and anticytoplasmic antibodies (ACA) by indirect immunofluorescence, antialbumin antibodies (AAA) tested by agarose gel immunodiffusion with glutaraldehyde polymerized human serum albumin, and immunoglobulin concentrations by single radial immunodiffusion.

The results showed an increase with age in the incidence of autoantibodies, especially in women, while in men a decrease in the prevalance of autoantibodies was registered in the 7th and 8th decades, in agreement with the influence of autoimmune phenomena on the death rate in males.

AAA increased in both men and women as compared to the other categories of autoantibodies, but showed a different association tendency AAA-SMA in women and AAA-ANA in men, suggesting a different etiopathogeny for some subclinical liver diseases.

Slight increase in IgG and IgA concentrations and decrease in IgM values with age were also noticed.

INTRODUCTION

Studies performed in apparently healthy individuals have revealed an increase with age in the frequency of antinuclear antibodies (ANA) [1—3], smooth muscle antibodies (SMA) and rheumatoid factors [2, 4].

The concomitant raised incidence of infections and cancer described in the elderly, as well as in naturally or therapeutically induced immunodeficient states, lend support to the theory of T lymphocyte deficiency associated with aging [1, 5, 6]. A defect in the suppressor T cell population, however, would ascribe pathogenic significance to the different categories of autoimmune phenomena and to the association of autoantibodies with cardiovascular disease and mortality with age observed by Mackay [3] and by Roberts-Thompson et al. [4]. On the other hand, the increased incidence of autoantibodies in the aged might reflect pathologic alterations of endothelial and parenchymal cells, especially in the arteries, liver and kidney following different metabolic, infectious or toxic aggressions accumulating in time.

It is well known that besides age and sex dependence, the incidence of autoantibodies registers geographical differences correlated with genetic and environmental factors. Hence, we considered it of interest to investigate the frequency

of autoantibodies and immunoglobulins concentration in 300 normal subjects from Bucharest, ranging in age from 40 to 90 years.

MATERIALS AND METHODS

Subjects. Sera were obtained from 180 women and 120 men ranging in age from 40 to 90 years, in whom no overt hepatic or other clinical impairment was observed. Case distribution according to sex and age is presented in table 1. Sera were kept at −20°C till analyses were performed.

TESTS FOR AUTOANTIBODIES

The indirect immunofluorescence technique was used [7] for testing autoantibodies to nuclei (ANA), to smooth muscle (SMA) and to the cytoplasm of gastric parietal and kidney tubular cells, i.e. anticytoplasmic antibodies (ACA) characterized by a fine granular and diffuse cytoplasmic fluorescence.

Rat heart, kidney and stomach were cut in 4 μ thick sections in a cryostat at −20°C. All tissue fragments on the slides were first layered with test serum diluted 1:10 in phosphate buffered saline (PBS) at pH 7.3, and then with rabbit anti-human immunoglobulin serum conjugated with fluorescein isothiocyanate. The sections were mounted in 10% PBS in glycerol and examined in fluorescence microscopy (HBO 200 lamp). Sera which gave off fluorescence at a 1:10 dilution were considered positive.

Detection of antialbumin antibodies (AAA) was performed by immunodiffusion (ID) with glutaraldehyde polymerized human serum albumin (ID—HSAP), using the method described by Lenkei and Gheție [8].

An amount of 20 mg human serum albumin (HSA) (Kabi, Stockholm) was dissolved in 0.9 ml PBS 0.1 M pH = 6.8 and 0.1 ml of a 2.5% glutaraldehyde solution was added. The mixture was incubated for 2 hrs at room temperature and further dialysed against PBS for 3 hrs with frequent changes of buffer.

The glutaraldehyde treated HSA solution (HSAP) (20 mg/ml) was mixed with an equal volume of untreated HSA (40 mg/ml) dissolved in 0.2 M carbonate buffer (pH = 9) and further incubated for 1 h at 37°C and overnight at +4°C. This solution of copoli-HSA was used for ID in serial dilutions ranging from 1.250 μg/ml to 19.9 μg/ml. The lowest concentration of copoli-HSA giving a precipitation with the undiluted patient's serum was considered to reflect AAA concentration in the respective serum. Positivity was considered to begin with 625 μg/ml.

Quantitative determinations of serum immunoglobulins (IgG, IgA, IgA) were performed by the Mancini technique [9].

HBs Ag positivity was determined by the counter current electrophoresis method [10].

Statistical analysis. The results were expressed as the arithmetic mean of N values. The standard errors were calculated and the standard errors of the mean (SEM) were used to indicate the statistical significance of the results.

RESULTS

HBs Ag positivity. As can be seen in Table 1, HBs Ag distribution had an irregular aspect probably due to the small number of cases in each group. The

greatest HBs Ag incidence was observed in the 71—80-year-old groups (6.9 % in females and 5.7% in males), no HBs Ag positivity being recorded in the 61 — 70 and 81—90-year-old groups.

Table 1

Age and sex distribution of 300 clinically healthy subjects from Bucharest; HBsAg presence according to sex and age groups

Sex	Number of cases by age (years)				
	41—50	51—60	61—70	71—80	81—90
Females	25(1)*	34(0)	36(0)	58(4)	27(0)
Males	13(0)	26(1)	28(0)	34(2)	18(0)

* In brackets number of HBsAg positive cases.

Autoantibodies. General data referring to the incidence of autoantibodies are presented in Table 2.

Table 2

Autoantibodies and HBsAg incidence in 300 normal subjects grouped according to sex

Subjects	No. of cases	Mean age (years)	Autoantibodies (%)				
			ANA	SMA	ACA	AAA	HBsAg (%)
Females	180	62.9	3.5	15.4	14.3	32.9	2.9
Males	120	66.6	8.9	15	9.7	31.1	2.5

Similar AAA and SMA frequencies were recorded in both sexes, significant differences appearing only in the other categories of autoantibodies. Thus, the incidence of ANA was higher in males (8.9 as against 3.5% in females, $p \leqslant 0.02$), and that of ACA higher in females (14.3 as against 9.7% in males; $p \leqslant 0.02$).

The incidence of different autoantibodies according to sex and age groups is given in Fig. 1a, b, c, d.

As can be seen in Fig. 1a, ANA showed in both sexes a continuous increase in incidence between 40 and 60 years. While in males the increase continues in the seventh decade followed by a decrease in prevalence in the eighth decade, in females decrease in the incidence supervenes a decade earlier.

The same parallelism between the curves corresponding to the prevalence of autoantibodies in females and males between the ages of 40 and 60 was noted for ACA (Fig. 1b) with a slightly greater incidence in females. After 60 years a contrary evolution of ACA incidence was recorded: increase in the seventh decade followed by a sharp decrease in the eighth decade, and decrease in the seventh decade followed by a steep increase in the eighth decade in males.

SMA had a fairly constant frequency between 40 and 60 years in both sexes, somewhat greater in males; but an inverse evolution of SMA incidence was noticed (Fig. 1c) in the seventh and eighth decade, i.e. an abrupt rise in females (40% SMA positivity in the 80—90 years age-group) and a corresponding decrease in males (7% in the same age-group).

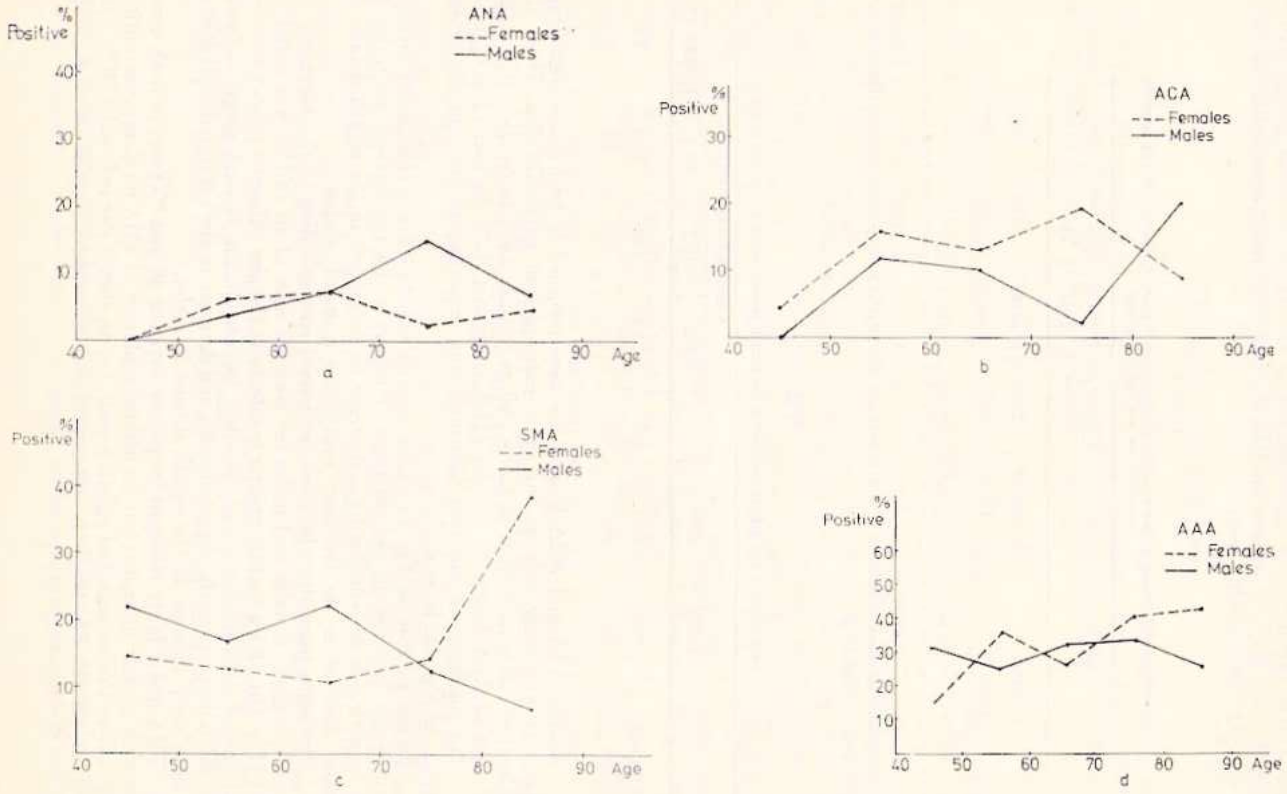

ANA
Females
Males
% Positive
40
30
20
10
40
50
60
70
80
90 Age
a
ACA
Females
Males
% Positive
30
20
10
40
50
60
70
80
90 Age
b
SMA
Females
Males
% Positive
40
30
20
10
40
50
60
70
80
90 Age
c
AAA
Females
Males
% Positive
60
50
40
30
20
10
40
50
60
70
80
90 Age
d

Fig. 1

AAA (Fig. 1 d) had an almost equal incidence in males in all age-groups, while a rise was observed in females from 12% in the 40—50 age-group to 40% after 81 years.

The general incidence of autoantibodies and their association are represented in Fig. 2.

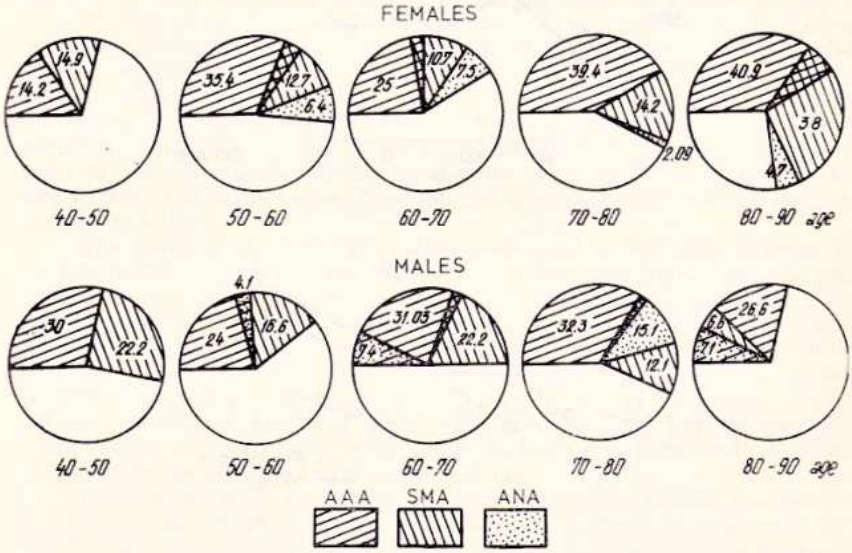


Fig. 2

An increase with age in the prevalence of autoimmune phenomena is evident in females (29.1% in the 41—50 age-group and up to 78.6% in the 81—90 age-group). This phenomenon is not registered in males, where a clear fall in the incidence of autoantibodies was observed between 51—60 and 80—90 years.

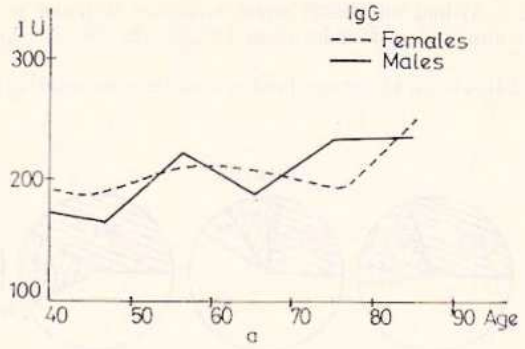
The prevalent association noticed in females was between AAA and SMA while in males AAA were associated with ANA.

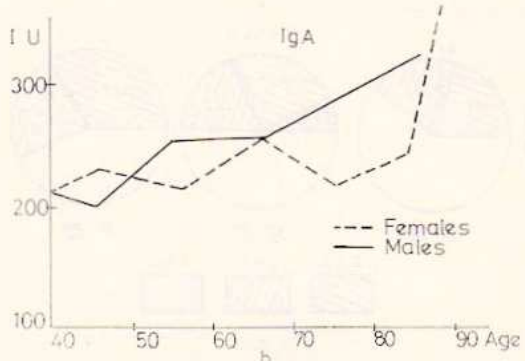
Immunoglobulins. In general, serum immunoglobulin concentrations register similar levels in both sexes and each age-group (Fig. 3).

An increase in serum IgG and IgA with age was observed, more marked in the case of IgA (Fig. 3 b). IgM levels presented a constant and significant decrease with age in females.

DISCUSSION

The general incidence of ANA (5%) obtained in our group of 300 subjects is comparable to that recorded by Hooper et al. in 1969—1972 [11] in the 3492 individuals constituting the Caucasian rural community of Busselton (Western Australia), respectively 5.5%; it differs, however, from this study and from the well-


IgG
I U
300
200
100
Females
Males
40
50
60
70
80
90 Age
a


IgA
I U
300
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Females
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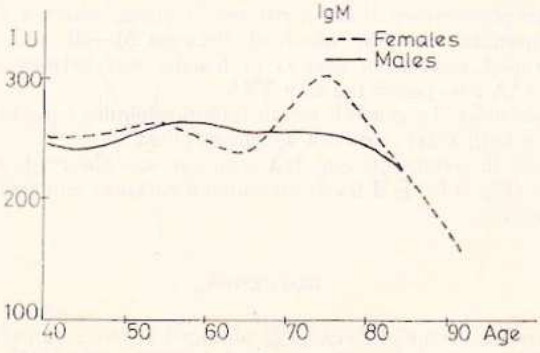
b


IgM
I U
300
200
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Females
Males
40
50
60
70
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90 Age
c

Fig. 3

known tendency of females toward developing autoantibodies and autoimmune diseases [12], in that a significant higher frequency of ANA was recorded in males than in females (8.8 and 3.5% respectively). A possible explanation for this observation will be given further on.

The parallel increase in the incidence of ANA in both females and males in the fourth-to-sixth decade is also in agreement with numerous other studies which show an increase with age in autoimmune phenomena in both sexes [13, 14].

The net sharp decrease registered in the incidence of ANA in octogenarian males may be interpreted as due to a higher death rate of males positive for ANA. Thus, in a follow-up study of the Busselton population continued during 1972—1975 in order to estimate the death rate in subjects with different autoantibodies Mackay et al. [15] observed among men with autoantibodies an excess mortality rate from vascular causes and from cancer among men with rheumatoid factor, these autoimmune phenomena having no death predicting value in women. It is also of interest that the autoantibody reaction associated with nuclei involved the greatest risk [4].

The relatively constant SMA incidence observed in both sexes between the ages of 40 and 70 years is in agreement with the Busselton study. However, SMA were registered with higher frequencies than in the Busselton population. This lack of correlation of SMA incidence with age was interpreted as being possibly due to a masking effect provoked by the induction of SMA in the course of an endemic infection [15]. A terminal, steep rise was noticed in our group of women, concomitantly with the aspect shown in Fig. 2, i. e. the greater number of autoimmune reactions in women in the 81—90 years age-group corresponding to a lower immune response in men, which could also be explained by a pathogenic role of autoantibodies in diseases causing death in men.

Nevertheless, in the present study a sharp terminal rise in SMA was observed in women in the 81—90 age-group. This phenomenon should be interpreted with care in view of the relatively small number of cases forming the latter age-group.

An age and sex dependence was observed in the prevalence of ACA. Thus, the incidence of ACA constantly registered higher frequencies in women than in men, except in the last decade when a divergent evolution was noticed (Fig. 1 b). The incidence of ACA also increased with age in women from the fourth-to-seventh decade, while in men a decrease in ACA prevalence was observed in the preceding decade, concomitantly with a decrease of SMA.

The group with the presence of ACA probably included sera with low antigastric parietal cell and antithyroid cell antibody titers; this appears to be in keeping with the well-known predisposition of females toward developing these categories of autoantibodies. ACA may be assumed to have a certain protective role in women, helping to eliminate the necrotic products resulting from cellular destruction more accentuated in the aged. Thus, the favourable role of ACA was observed in patients with diseases of the liver, in which the presence of ACA was associated with a better hepatocellular function [16].

High AAA titers were registered in females, their prevalence being age-dependent, and an almost flat curve recorded in men. Many studies have revealed the value of AAA in the diagnosis of liver cell dysfunction, these autoantibodies appearing in children and adults strictly as a consequence of liver alterations [17, 18, 19].

As can be noticed in fig. 2, a good correlation was registered between AAA and SMA in women, the phenomenon being also observed in studies performed in

1065 patients and apparently healthy subjects [20]. This association points to the appearance of AAA in women as a result of liver cell dysfunction, accentuated with age. Studies carried out by Thompson and Williams [21] and Skaunic et al. [22] also furnished arguments lending support to liver functional alterations in the elderly; the liver status may be very different in the diseased population, since it depends to a great extent on nutritional habits and environmental conditions. The association of AAA with SMA in the presence of a fairly low HBs Ag incidence in these aged individuals also noticed in other studies [23] argue for the role of autoimmune phenomena in the maintenance of discrete liver cell alterations in females.

The predominant association observed in men was between AAA and ANA, the maximum incidences of both these autoantibodies being in the seventh decade, concomitantly with the highest HBs Ag incidence (Table 1 and Fig. 2). This association of AAA and ANA suggests the possibility of viral and microbial infections as the principal factors involved in the production of these antibodies in men, probably also dependent on liver cell alterations.

The data shown in figure 2 illustrate the general increased incidence of autoantibodies with age in females, and a net decrease in males after the eigth decade, supporting the hypothesis of autoantibodies contribution to the death rate in men.

As regards immunoglobulins, our results are in agreement with the data obtained by Schwick and Mecker [24] who in their study on a group of blood donors found an increase in IgG with age and a significant fall in IgM levels, and with those of Cassidy et al. [25], who observed an increase with age in IgG and IgA concentrations.

Résumé. On a testé 300 sujets de Bucarest, âgés entre 40 et 90 ans, sans phénomènes pathologiques cliniques évidents. Par la technique d'immunofluorescence indirecte, on a mis en évidence, dans le sérum des sujets testés, les autoanticorps antinucléaires (ANA), anticytoplasmiques (ACA) et anti-muscle lisse (SMA), et par immunodiffusion avec albumine polymérisée à glutaraldéhyde les autoanticorps antialbumine (AAA). On a aussi testé le niveau des immunoglobulines sériques (IgG, IgA, IgM).

On a souligné l'augmentation de l'incidence d'autoanticorps avec l'avancement en âge, spécialement chez les femmes, pendant que chez les hommes une baisse de la prévalence des autoanticorps a été enregistrée pendant la 7e et 8e décennie, conformément à l'influence déjà connue des phénomènes autoimmuns sur les décès des hommes.

Les AAA ont présenté une prévalence augmentée tant en ce qui concerne les hommes que les femmes comparativement aux autres catégories d'autoanticorps. On a observé une tendance différente en ce qui concerne les associations des autoanticorps, respectivement AAA-SMA chez les femmes et AAA-ANA chez les hommes; cela suggère une étiopathogénie différente concernant les maladies sous-cliniques du foie les plus fréquemment rencontrées.

On a observé avec l'avancement en âge une faible augmentation de la concentration des IgG et IgA et une diminution de la valeur de l'IgM.

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