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The prevalence of autoantibodies in an elderly sub-Saharan African population

R. NJEMINI*†, I. MEYERS*, C. DEMANET‡, J. SMITZ§, M. SOSSO† & T. METS* **Geriatric Unit, Academic Hospital, Free University Brussels (VUB), Belgium, †Department of Surgery, Academic Hospital, University of Yaounde 1 (UY1), Cameroon, ‡Immunology Laboratory, Academic Hospital, Free University Brussels (VUB), Belgium, §Radioimmunology Laboratory, Academic Hospital, Free University Brussels (VUB), Belgium*

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SUMMARY

In the present prospective, census-based study we have investigated the prevalence of organ-specific and non-organ-specific autoantibodies (AAb) in 152 unselected Cameroonians aged 60 years and older living in the community. AAb were detected in 49% of the participants. Non-organ-specific AAb (47%) predominated over organ-specific AAb (7%). Anti-TPO, anti-Tm, anti-Tg and anti-PC AAb were completely absent. RF was the most frequent AAb, being found in 57 (38%) cases. The prevalences of anti-SMA and RF were significantly higher in women than in men (respectively, $P = 0{\cdot}023$ and $P = 0{\cdot}016$). Higher serum concentrations of gammaglobulins were accompanied by a higher prevalence of RF ($P < 0{\cdot}0001$) and a lower prevalence of ANA ($P = 0{\cdot}036$). The overall prevalence of AAb was higher in the filaria-infected (60%) compared to the non-infected (42%) participants ($P = 0{\cdot}046$). There was no significant influence of the vitamin D status, number of pregnancies, physical activity or medication use on the prevalence of AAb. In this study a heterogeneous pattern for the presence of the various AAb was found. Some AAb, which are commonly encountered in other studies on elderly subjects, were completely absent in this population. This diversified pattern of AAb prevalence therefore argues in favour of exogenous influences in the occurrence of AAb in elderly populations.

Keywords autoantibodies elderly infection lifestyle sub-Saharan Africa

INTRODUCTION

Ageing is associated with important changes in multiple areas of immune function, but to date no single mechanism has emerged as being responsible for all the observations. Paralleling the functional deterioration of the immune responsiveness to exogenous antigens is an increase in autoreactivity [1–4]. Some authors, however, have not confirmed the increased prevalence of autoantibodies (AAb) in elderly individuals [5,6]. It is estimated that 10–15% of seemingly healthy elderly individuals have increased serum levels of AAb. These AAb have been hypothesized to play a role in the general deterioration of older subjects through their participation in subclinical chronic tissue damage [7–10]. Environmental factors including drugs, diet [11], physical activity or underlying diseases [12], capable of eliciting or potentiating an autoimmune response, may play a proportionately larger role in the aged. A closely related factor that confounds the study of normal ageing is the use of medication. Goodwin *et al*. [13] have shown that non-steroidal anti-inflammatory agents, specifically indomethacin and piroxicam, inhibit by 50% *in vitro* production of IgM rheumatoid factor (RF). These same authors [14] have also shown that serum levels of RF in patients with rheumatoid arthritis are suppressed significantly by the administration of a non-steroidal anti-inflammatory agent. Also, elderly individuals often suffer from nutritional deficiency, especially protein calorie malnutrition. Studies of the immune status in patients have shown a high correlation between malnutrition and decreased immune responses as measured by delayed skin reactivity [15–17]. In contrast to the enormous amount of literature on the immune responsiveness of elderly from industrialized countries, only scant data are available on the elderly in developing countries. In the present study we have investigated the prevalence of organ-specific and non-organ-specific AAb in a geographically confined but unselected population of elderly living in the community in Cameroon. Some factors that could influence the prevalence of AAb, such as diet, lifestyle and environment, were evaluated.

Correspondence: Prof Dr Tony Mets, Geriatric Unit, Academic Hospital, Free University Brussels (VUB), Laarbeeklaan 101, B-1090 Brussels, Belgium.

E-mail: germst@az.vub.ac.be

PARTICIPANTS AND METHODS

Setting

The study was conducted in Cameroon for the purpose of evaluating the health status of the elderly population in sub-Saharan

Africa. Participants were recruited from the Ntam health area, situated in the predominantly rural South-west province, adjoining six rural villages near the small town of Kumba. This area was selected for its well-structured health-care system.

Participants

A census was organized in order to be able to contact all the elderly living in the area, having a population of 4267. All those indicated to be 60 years of age or older were invited to participate. Initially, 331 (7·8% of the population) people (183 (55%) men and 148 (45%) women) were located. Each person was visited at home and information about the project was provided. Sixty-seven (20·2%) people could not participate: 12 (3·6%) had died; 21 (6·3%) were younger than 60 years; 10 (3%) refused to participate; 24 (7·3%) were continuously absent. The remaining 264 participants (79·8%) signed the informed consent sheet, as approved by the ethical committee of the University of Yaounde 1, Cameroon. Upon further control of administrative documents and further questioning, 91 other people were deemed to be younger than 60 years and were excluded in the course of the study. Some participants were lost due to subsequent refusal ($n = 17$) and decease ($n = 4$), bringing the number of people who could be evaluated for the present study to 152. They represent 69·4% of the 219 people aged 60 years or more located originally. There were 92 men (61%) and 60 women (39%), aged between 60 and 86 years (median age 66 years). There was a difference with respect to gender between the 331 participants initially addressed and the 152 subjects taken into account for this study (a drop in the percentage of women from 45% to 39%). Sociodemographic background information for all participants (education, number of pregnancies, lifestyle and living conditions), medical history, current medical and functional status and diet pattern were obtained by questioning the participants and by physical examination. Most participants were involved in subsistence farming. As such physical activity was quantified based on the time needed to walk to their fields as well as the time spent on the fields. Body mass index (BMI) was calculated as weight (kg)/length^2 (m^2).

Methods

Venous blood was obtained after overnight fasting. Erythrocyte sedimentation rate (ESR) was performed according to Westergren (normal values: <10 mm/h for men and <20 mm/h for women). Red blood cell (RBC) count was performed using counting chambers. Fresh blood smears were microscopically screened for the presence of filarial parasites. After centrifugation, sera were stored at –20°C. One part of the samples was transported in a cooler box to the laboratories of the University of Yaounde, where total serum protein concentration (normal value: 6·3–8·2 g/dL) was determined by the biuret colourimetric method and electrophoretic analysis was performed on a cellulose acetate gel. After separation and colouration with Ponceau S, the gels were scanned and the proportion and concentration of albumin (normal value: 3·7–5 g/dL) and gammaglobulin (normal value: 0·5–1·5 g/dL) were calculated. The other part of the serum was shipped on dry ice to Brussels, where the remainig analyses were performed in the clinical laboratories of the Free University of Brussels (VUB). Sera were stored no longer than 6 months before determination except for serum C-reactive proteins (CRP), which was performed after storage for a period of up to 2 years. Tests for antinuclear antibodies (ANA) were performed by indirect immunofluorescence (IIF) on HEp-2 (human epithelial cell line 2) cells as substrate and titres of 1/40 or higher were considered positive [1]. ANA positive sera were also examined for the presence of several ANA specificities. Anti-ds-DNA antibodies were determined by IIF [1] employing crithidia luciliae as a substrate (Biomedical Diagnostics bmd, Brugge, Belgium). A titre of 1/20 or higher was considered positive. Antibodies against Smith (Sm), nuclear ribonucleoprotein (nRNP), Sjögren's syndrome A (SSA) and B (SSB), and topoisomerase 1 antigens were tested by immunoblotting (ANA Blot System II, Gull Laboratories, the Netherlands). The results were read visually by aligning the reference bands of the test strips to the ones of the control strips and comparing any positive bands to the bands appearing on the control [18]. For the detection of antiparietal cell antibodies (PCA), antismooth muscle antibodies (SMA) and antimitochondrial antibodies (MA), an IIF test system that utilizes as substrate mouse kidney and stomach sections (MARD X Diagnostics, CA, USA) was used [19]. Sera were scored as positive when titres were ≥1/40. RF was detected by the N latex RF kit (Dade Behring, Marburg GmbH, Germany), as used by Teo *et al*. [20]. RF concentrations ≥22 IU/ml were detectable and were considered positive. The Thymune tanned turkey red blood cell agglutination test (Murex Biotech Ltd) was used for the semiquantitative determination of antithyroglobulin (Tg) and antithyroid microsome (Tm) AAb [21]. The test was considered positive when at least one of the wells containing serum showed more than 50% agglutination. Sera were tested for the presence of antithyroid peroxidase (TPO) AAb using kits from Brahms Diagnostica GmbH, Berlin [22]. Anti-TPO positivity was established at >100 U/ml. The presence of anti-intrinsic factor (IF) antibodies (Diagnostic Products Corporation, Los Angeles, USA) was determined according to the manufacturer's insert. The serum levels of 25-OH vitamin D (Diasorin Inc., USA) and vitamin B_{12} (Becton Dickinson Immunodiagnostics, USA) were determined by radioimmunoassay methods as in Hollis [23] and Cooper *et al*. [24], respectively. A value of more than 0·21 μg/l and 15 μg/l was considered normal for vitamin B_{12} and 25-OH vitamin D, respectively. Normal values and cut-off points were applied as indicated in the manufacturers' prescriptions. For samples whose values were below the detection limit, the value at the detection limit was considered for calculations.

Antimalarial antibody concentration was determined in the clinical laboratory of the Institute of Tropical Medicine (Antwerp, Belgium). Antimalarial antibodies were tested by an IIF [25] using antigens from the Institute of Tropical Medicine and an antihuman IgGAM conjugate. Titres ≥1/40 were considered positive.

CRP was quantified by immunonephelometry using the N high sensitivity CRP kit [26] obtained from Dade Behring, Marburg GmbH, Germany. Values <4 mg/l were considered normal.

Fresh skin snips, taken from the lower extremities were screened microscopically for the presence of filarial parasites.

The estimation of the consumption of proteins of animal origin was based upon a questionnaire about the intake of meat, poultry, eggs, fish, crayfish and dairy products. Correct amounts of intake could not be reported by the participants, but we obtained an estimate of the number of intakes per year (*N*/y). From scientific tables [27], the protein content of these principal nutrients was taken in to account. A formula was derived where, for each product, the protein content was multiplied by the fre-

quency of consumption. This procedure gives the following formula for the estimation of total protein intake of animal origin:

'Protein coefficient' = milk (*N*/y)*30 + meat (*N*/y)*15
+ poultry (*N*/y)*20 + eggs (*N*/y)*13
+ fish (*N*/y)*18 + crayfish (*N*/y)*17·4

Statistical analysis

Fisher's exact test and chi-squared test were used for comparison of numbers in groups. To test the approximation of population distribution to normality, column statistics (with statistical package Prism 3·0) as well as probability plots were used. Averages were compared using Student's *t*- test or ANOVA. Continuous variables were compared by Pearson or Spearman correlation. For data that were not normally distributed, the non-parametric Wilcoxon signed rank test and the Mann–Whitney test were applied. All *P*-values were two-sided unless otherwise stated; values < 0·05 were considered statistically significant.

RESULTS

Prevalence of AAb

The prevalence of the various AAb in the sera of our participants is summarized in Table 1. The prevalence was diversified, with some AAb being completely absent while others were common. No AAb against thyroid tissue (anti-TPO, anti-Tm, anti-Tg) nor parietal cells were encountered. RF was the most common AAb, being found in 57 (38%) cases; in 12 (8%) participants it was present at a low (22–29 IU/ml), in 20 (13%) at an intermediate (30–59 IU/ml) and in 25 (16%) at a high concentration (≥60 IU/ml). ANA were found in 14 (9%) participants, generally at low (1/40–1/80) titres; a high titre (>1/320) was found in only one case. Several patterns of nuclear staining were observed: fine speckled in four (3%) cases, nucleolar in four (3%) cases and coarse speckled in one (1%) case. Staining of peripolar mitotic spindles was seen in six (4%) cases. When ANA were found to be present, subtyping was performed. AAb against ds-DNA, Sm, nRNP, SSA, SSB and topoisomerase 1 were absent in each case. With this technique, however, other, unidentified bands of antibodies were demonstrated in all the tested samples (whether ANA positive or negative). These uncharacterized bands were of variable localization and intensity. Comparison of the bands from the sera of our participants failed to demonstrate any similarity. Of the 11 (7%) participants for whom anti-IF AAb were present, values were low in all cases.

Anti-SMA and RF were significantly more prevalent in the women (respectively, $P = 0{\cdot}023$ and $P = 0{\cdot}016$) than in men. For the other AAb the gender difference was not significant.

Table 1. Prevalence of various autoantibodies in the serum of the participants according to gender: N(%)

Autoantibody	Total ($n = 152$)	Men ($n = 92$)	Women ($n = 60$)	Difference men *versus* women
Anti-TPO	0 (0)	0 (0)	0 (0)	n.s.
Anti-Tm	0 (0)	0 (0)	0 (0)	n.s.
Anti-Tg	0 (0)	0 (0)	0 (0)	n.s.
RF	57 (38)	27 (29)	30 (50)	$P = 0{\cdot}016$
ANA	14 (9)	10 (11)	4 (7)	ns
Anticentromere	0 (0)	0 (0)	0 (0)	n.s.
Anti ds-DNA	0 (0)	0 (0)	0(0)	ns
Anti-ENA	0 (0)	0 (0)	0 (0)	ns
Anti-SMA	14 (9)	4 (4)	10 (17)	$P = 0{\cdot}023$
Anti-MA	1 (1)	0 (0)	1 (2)	n.s.
Anti-IF	11 (7)	9 (10)	2 (3)	n.s.
Anti-PCA	0 (0)	0 (0)	0 (0)	n.s.

TPO, thyroid peroxidase; Tm, thyroid microsome; Tg, thyroglobulin; RF, rheumatoid factor; ANA, antinuclear antibody; ENA, extractable nuclear antigen; SMA, smooth muscle antibody; MA, mitochondrial antibody; IF, intrinsic factor; PCA, parietal cell antibody; n.s., not significant.

AAb associations

Approximately half of the participants (49%) had serum AAb. As shown in Table 2, 13% of the participants had two AAb types; none had more than three of the tested AAb. The most frequent AAb association was between SMA and RF (seven cases, 5%). There was no relationship between RF and the other AAb. Non organ-specific AAb were predominant and were significantly more prevalent in women ($P = 0{\cdot}043$) compared to men.

Variables potentially influencing the prevalence of AAb

Data on filaria prevalence were available for 146 participants; 60 (41%) of them had a positive skin snip and/or blood smear. There was a trend for women to be more frequently infected by filariasis than men ($P = 0{\cdot}051$). No significant differences were found in the prevalence of the various AAb according to the filariasis status, but the overall prevalence of AAb was higher in the filaria-infected (60%) than in the non-infected (42%) participants ($P = 0{\cdot}046$).

All the participants had antimalarial antibodies in a titre of at least 1/640. There was a positive and significant relationship between the antimalarial antibody concentration and the serum level of RF ($r = 0{\cdot}210$, $P = 0{\cdot}01$). There was no relationship between the antimalarial antibody titre and the occurrence of the other AAb.

The average weight and BMI of the women and men were 50·7 kg (s.d. 11·9), 21·3 (s.d. 4·2) and 54·8 kg (s.d. 9·3), 20·4 (s.d. 2·9), respectively. Both weight and BMI correlated negatively with age in women (respectively, $r = -0{\cdot}275$; $P = 0{\cdot}033$ and $r = -0{\cdot}267$; $P = 0{\cdot}039$) and in men (respectively, $r = -0{\cdot}312$; $P = 0{\cdot}002$ and $r = -0{\cdot}276$; $P = 0{\cdot}008$). Thirteen (9%) participants had a BMI of more than 25 and thus could be considered as being overweight; 33 (22%) had a BMI <18 and thus were underweight. No relationship existed between the BMI and the occurrence of AAb.

The diet of the participants was predominantly vegetarian, comprising mainly beans, manioc, sweet potatoes and plantains. Many of them used proteins of animal origin very rarely. Using the 'protein coefficient' as a parameter, a widespread distribution of the total yearly animal protein intake was obtained. The serum albumin concentration was positively associated to the 'protein coefficient' ($r = 0{\cdot}182$; $P = 0{\cdot}030$, one-sided). There was a positive relationship between the antimalarial antibody titre and the 'protein coefficient' ($r = 0{\cdot}301$; $P = 0{\cdot}001$). The 'protein coefficient' was not related to the age of the participants or to the prevalence of AAb. Comparison of the separate diet components and the occurrence of AAb revealed no clear relationship.

Serum gammaglobulins were often present in high concentrations in the participants. Their average value was 2·1 g/dL (s.d.

1·0). When gammaglobulin concentrations were grouped according to quartiles, a significant positive relationship occurred for RF (prevalence of 16% in the lowest and 68% in the highest quartile; $P < 0·0001$) and a negative relationship for ANA (prevalence of 18% in the lowest and 3% in the highest quartile; $P = 0·036$) (Table 3). The gammaglobulin concentration was not related to the age, the gender of the participants, the 'protein coefficient' or to the presence of filaria.

Most women had been pregnant several times. The median number of pregnancies was five; 11 (18%) women had never been pregnant. There was no influence of the number of pregnancies on the prevalence of AAb.

The average 25-OH vitamin D serum level was 21·1 μg/l (s.d. 7·7). Thirty-seven (24%) participants had below normal serum levels (on average 12·5 μg/l (s.d. 3·2)). There were no significant differences in the prevalence of AAb between the participants having normal and decreased 25-OH vitamin D serum levels.

Low vitamin B_{12} serum levels were found in seven (5%) participants (on average 0·20 μg/l (s.d. 0·02)). None had anti-IF AAb. Of the 145 participants with normal vitamin B_{12} serum levels, 11 (8%) had anti-IF AAb.

Various pathological conditions were encountered in the participants. Although a large number of them complained of chronic joint pain, only four had a clear history of chronic inflammatory joint disease. For most participants (92%) the physical examination of the thyroid gland was normal; five participants (3%) had a diffuse goitre, five (3%) had a solitary nodule and two (1%) had a polynodular thyroid gland.

Data on the medication (modern or traditional) taken by the participants were recorded. Seventy-three of 149 (49%) participants were on medication. Forty-four participants (30%) were receiving more than one medication. Seventeen participants (11%) were also receiving traditional treatment. The most commonly used medications were paracetamol (30 participants, 20%) and chloroquine (26 participants, 17%). There was no difference in the prevalence of AAb between the participants on medication and those that were not taking medication.

One hundred and one participants (75%) were involved in subsistence farming. Their average time spent daily for physical activity was 7·6 h (s.d. 3·5). There was no relationship between physical activity and the prevalence of AAb.

Inflammatory parameters

The level of inflammation was evaluated by the ESR and the serum level of CRP. The average ESR was 77 mm/h (s.d. 37). One hundred and forty-four of 148 participants (97%) had an elevated sedimentation rate. The ESR was correlated with the serum gammaglobulin concentration ($r = 0·454$; $P < 0·0001$) and with the RBC count ($r = -0·228$; $P = 0·005$). The ESR was significantly higher ($P < 0·0001$) in participants with RF (average 98, s.d. 33) compared to those without RF (average 65, s.d. 34). The same observation was seen in participants with anti-IF AAb (average 104, s.d. 27) compared to those without anti-IF AAb (average 75,

Table 2. Type and association of autoantibody N(%) according to gender

	Total ($n = 152$)	Men ($n = 92$)	Women ($n = 60$)	Difference men *versus* women
Presence of AAb	75 (49)	40 (43)	35 (58)	n.s.
1 AAb	54 (36)	30 (33)	24 (40)	n.s.
2 AAb	20 (13)	10 (11)	10 (17)	n.s.
3 AAb	1 (1)	0 (0)	1 (2)	n.s.
Organ-specific AAb	11 (7)	9 (10)	2 (3)	n.s.
Non organ-specific AAb	72 (47)	37 (40)	35 (58)	P = 0·043
Organ-specific and non organ-specific AAb	8 (5)	6 (7)	2 (3)	n.s.

AAb, autoantibodies; n.s., not significant.

Table 3. Prevalence of autoantibody in the different quartiles of serum gammaglobulin concentration (g/dL)

AAb	First quartile <1·5 $N = 38$	Second quartile 1·5–1·9 $N = 38$	Third quartile 2–2·6 $N = 38$	Fourth quartile >2·6 $N = 38$	Difference
RF	6 (16)	12 (32)	13 (34)	26 (68)	$P < 0·0001$
ANA	7 (18)	5 (13)	1 (3)	1 (3)	$P = 0·036$
SMA	5 (13)	3 (8)	2 (5)	4 (11)	n.s.
IF	1 (3)	3 (8)	3 (8)	4 (11)	n.s.

AAb, autoantibodies; RF, rheumatoid factor; ANA, antinuclear antibody; SMA, smooth muscle antibody; IF, intrinsic factor; n.s., not significant.

s.d. 37; $P = 0{\cdot}019$). There was also a positive and significant correlation between the ESR and the antimalarial antibody titre ($r = 0{\cdot}222$, $P = 0{\cdot}003$). There was no relationship between the ESR and the prevalence of the other AAb.

The average CRP serum concentration was 12·1 mg/l (s.d. 25). Seventy-one of 150 participants (47%) had an elevated CRP serum level. There was no relationship between the serum CRP level and the prevalence of AAb, antimalarial antibody titre or filarial infection.

DISCUSSION

A large body of literature indicates that various kinds of AAb are common in the sera of elderly people [28]. The reasons for this increased prevalence of AAb are not well understood. It has been suggested that there is an age-related decline in both function and control of the immune system, and that some of the features of senescence may even be the result of these processes [29]. Table 4 gives an overview of the prevalence of AAb in the elderly reported in the literature [1–5,19–21,30–42]. Most of these studies have been performed on selected elderly populations, unlike the one we describe here. Our study population is peculiar in that it concerns elderly people from a developing country, with a completely different lifestyle and living conditions than those from industrialized countries. Care was taken to contact, by census, all elderly people living in a geographically defined area; we estimate that about two-thirds participated in the study. Because the participants were unselected and represent an important proportion of the elderly in this area, our results are not affected by selection bias and appear applicable to the general population of adults older than 60 years in this region. Women, however, were less represented (39%), although above the age of 60 years they are expected to represent the majority of the population. This same observation was reported in another study on the sub-Saharan African population [43]. During the study, women had a higher dropout rate than the men. Sociocultural factors have probably influenced their participation. In the rural areas of Cameroon, women are more involved in the local markets and in fieldwork and therefore it is unlikely to meet them at home. Besides, most women are unwilling to participate without immediate benefit. Also, in certain polygamous families, either all the wives had to participate or none at all.

In agreement with other reports, the serological profile of our elderly population was found to contain several AAb, including RF (38% of the participants), ANA (9%), anti-SMA (9%), anti-IF (7%) and anti-MA (1%). Forty-nine per cent of the subjects were positive for at least one test. Some authors (Table 4) have described a similar high prevalence.

The most frequent AAb in our subjects was RF. Other reports [1,2,4,20] have described prevalences from 9 to 41% in sera of large groups of older subjects (Table 4) using various methods, including the N latex RF kit, as we did. The present observation is in agreement with the prevalence of 41·3% and 30% reported by Ruffatti *et al*. [1] and Hijmans *et al*. [33], respectively. Both studies concerned elderly people in European countries. The 38% prevalence of RF in the present study is at variance with the figure of 9·4% and 14% reported, respectively, by Oyenyinka *et al*. [2] in elderly Nigerians and Goodwin *et al*. [42] in old subjects in New Mexico. This might be due to the fact that both study populations were subjected to exclusion criteria such as diabetes [42], infection and inflammation [2]. The clear increase in the prevalence of RF seen with increasing levels of gammaglobulin ($P < 0{\cdot}0001$) indicates that the high prevalence of RF is due at least partly to atypical reactions. The high frequency of RF could not be explained by increased prevalence of rheumatoid arthritis since only four (3%) of our participants had chronic inflammatory joint disease. High concentrations of gammaglobulin in African populations have been described frequently. They are supposed to be elicited by chronic infections, such as malaria, filaria, intestinal or systemic worm infestations or fungal infections of the skin and the scalp. It should be noted that in the present population both malaria and systemic filariasis were endemic. A direct relation between gammaglobulin concentration and the presence of filaria, however, was not found. All participants had high titres of antimalarial antibodies.

Antinuclear antibodies were present in 9% of the participants, which is comparable to most other reports, but low compared to others showing up to 33% [20]. In agreement with other reports in older people [1,3,6], ANA were characterized by low titres and several staining patterns. In regard to the ANA specificities, we could not detect the presence of antibodies directed to ds-DNA, Sm, nRNP, SSA, SSB or topoisomerase 1 antigens. Others have reported higher frequencies of anti-SSA [44,45] and antids-DNA [46] in aged humans. With the technique we used, however, other unidentified bands were encountered. This same observation was reported by Manoussakis *et al*. [4] in ANA positive individuals. The presence of undefined bands in both ANA positive and ANA negative participants in our study might indicate that they are non-specific. Furthermore, the dissimilarity between the bands of our participants, unlike in Manoussakis' case, supports this idea. Also, it should be noted that our participants have a completely different lifestyle and less favourable living conditions. As such, exposure to the enormous variety of antigens of infectious and parasitic origin might stimulate their system to produce non-specific antibodies. Further studies are currently under way to clarify this point.

Detection of anti-SMA in 9% of our aged subjects was consistent with other reports, indicating a prevalence of 0–18% (Table 4).

Anti-MA, with a prevalence of 1%, was almost non-existent in our subjects. This agrees with most other reports, describing 0–4·9%, even though Chakravarty *et al*. [32] reported a prevalence of 13% in a healthy elderly British population.

The 7% of participants having anti-IF AAb in this population is high. We found, however, no relationship with hypovitaminosis B_{12}. No anti-PC AAb were found, indicating that autoimmune gastritis must have a low prevalence in this population. An age-dependent prevalence of anti-PC AAb has been established by a number of authors [32,47].

Surprisingly, antithyroid AAb were not found in this elderly population. This cannot be ascribed to technical problems, since control samples from the kits, run together with the target samples, were clearly positive. Furthermore, in the laboratory where the tests were performed using the same technique, clinical samples were regularly positive: in a 3-month period, 39% (216 of 547), 21% (20 of 96), 6% (six of 96) and 41% (126 of 306), were positive for anti-TPO, anti-Tm, anti-Tg and anti-PC AAb, respectively. Hackett *et al*. [48] have reported that the prevalence of thyroid AAb in individuals without thyroiditis increases with age. In our population only 12 (8%) of the participants had an enlarged thyroid gland at clinical examination.

Table 4. Prevalence of autoantibodies (%) reported in the literature

Principal author	Anti-TPO	Anti-Tm	Anti-Tg	RF	ANA	Anti-SMA	Anti-MA	Anti-IF	Anti-PCA	Age	Number	Population
Present study	0	0	0	38	9	9	1	7	0	≥60	152	Cameroon†
Oyeyinka *et al*. [2]				9·4	8·3					>65	212	Nigeria‡
Ricardo *et al*. [30]					11·4					>65	419	Japan
Azizah *et al*. [19]					6·9	0	4·9		0·9	31·7(mean)	101	Malasia‡
Teo *et al*. [20]				16	33					>65	96	Singapore§
Delepesse *et al*. [31]		6	8·4		5·1	3·9				57(average)	134	Belgium¶
Moulias *et al*. [3]			8		10					≥90	150	France¶
Chakravarty *et al*. [32]				10	5	18	13		10	>60	100	UK‡
Hijmans *et al*. [33]		17	12	30	18	6	0	0	7	>95	65	the Netherlands‡
Guiseppina *et al*. [34]			26		11				18	>71	54	Italy‡
Velluzzi *et al*. [35]	9·6									38(mean)	91	Italy¶
Ruffatti *et al*. [1]				41·3	15·3					>65	300	Italy††
Manoussakis *et al*. [36]				20·2						>67	119	Greece‡
Manoussakis *et al*. [4]				14·1	31·1					>67	64	Greece‡
Perez l *et al*. [37]				11·8	18·6	16·6	1			>65	102	Spain‡
Andersen *et al*. [38]	6·8		5·1							>100	59	Denmark†
Andersen P. [39]					25	10				>60	40	Denmark‡
Ockhuizen *et al*. [40]		6			8	0	2		6	>60	51	USA¶
Roderick *et al*. [41]				13	19					>65	300	USA‡
Goodwin *et al*. [42]				14	18					>65	279	USA‡
Hallgren *et al*. [21]			17	21						>60	124	USA‡
Pandey *et al*. [5]			8		10				12	>50	727	USA¶

TPO, thyroid peroxidase; Tm, thyroid microsome; Tg, thyroglobulin; RF, rheumatoid factor; ANA, antinuclear antibody; SMA, smooth muscle antibody; MA, mitochondrial antibody; IF, intrinsic factor; PCA, parietal cell antibody; blanks, not performed. †General population, ‡selected individuals based on medical illnesses and drugs, §patients, ¶healthy, ††selected according to Senieur protocol.

There was a predominance of non-organ-specific AAb (47%) compared to organ-specific AAb (7%). With the exception of a few studies, Table 4 also shows a similar predominance. A combined presence of organ-specific and non-organ-specific AAb was found in only six of 40 (15%) AAb positive men and in two of the 35 (6%) AAb positive women.

RF ($P = 0{\cdot}016$) and anti-SMA ($P = 0{\cdot}023$) were more frequent in women than in men. This might have contributed to the overall increased prevalence of non organ-specific AAb in women ($P = 0{\cdot}043$) compared to men.

The area where the study was performed is endemic for malarial and filarial parasites. There was a positive and significant correlation between antimalarial antibody titre and the serum level of RF as well as 'protein coefficient'. Some authors [49] have reported that RF is produced during active or chronic malarial infection and that it might contribute non-specifically to the enhanced clearance of plasmodia. It is also well known that chronic exposure to malarial parasites can lead to polyclonal stimulation of immunoglobulin cells and thus to an increased frequency of AAb [50]. The polyclonal B-cell activation that takes place during the course of infection may appear as a result of successive waves of antigen-specific B-cell activation [51]. All participants had high levels of antimalarial antibodies. Differences in the antimalarial antibody serum levels were not accompanied by differences in the prevalence of the other AAb.

Women are more exposed to filarial infection than men, due probably to their responsibility for laundering at lake sites. This could explain the higher prevalence of filarial infection in women, which was almost significantly different ($P = 0{\cdot}051$) from that in men. The overall prevalence of AAb was higher in the filaria-infected compared to the non-infected participants ($P = 0{\cdot}046$). This again could be due to polyclonal activation of immunoglobulin products as a result of the infection [52]. A shift in the balance between Th1 and Th2 cells in falaria-primed individuals is thought to be the cause of immune activation [53]. It was decided not to investigate amoebiasis and helminthic infections since they are ubiquitous in this population.

Ninety-seven per cent of the participants had elevated ESR which mainly reflects their inflammatory status. A significantly higher ESR was seen in participants having RF ($P < 0{\cdot}0001$) and anti-IF AAb ($P = 0{\cdot}019$). Serum CRP had no relationship with the prevalence of AAb, antimalarial antibody titre or filarial infection.

Thirty-seven subjects had hypovitaminosis D. There were no significant differences in AAb prevalence according to their 25-OH vitamin D serum level.

The protein intake of animal origin was estimated by calculating a 'protein coefficient' from the questionnaire. The significantly higher serum levels of albumin ($P = 0{\cdot}030$) with increasing levels of 'protein coefficient' supports the validity of the applied technique. There was a positive and significant relationship between the 'protein coefficient' and the antimalarial antibody titre. A normal protein and energy diet has been reported to favour parasitaemia, resulting in a higher antigen load and higher serum antibody concentrations [54].

Seventy-three of 149 (49%) participants were on medication. There was no difference in the prevalence of AAb between the participants on medication and those that were not taking medication.

No relationship was found between BMI, physical activity or the number of pregnancies and the prevalence of AAb.

In conclusion, the study reveals that certain AAb (RF, ANA, anti-SMA, anti-IF and anti-MA) were present in this elderly population at low titres, while other AAb, all organ-specific (anti-TPO, anti-Tg, anti-Tm and anti-PC AAb), were absent. There thus appears to be a marked heterogeneous pattern for the presence of the various AAb in this elderly population from sub-Saharan Africa. The AAb pattern is different from that commonly encountered in other elderly populations. Taken together these data indicate that external factors, such as chronic infection, might be more important contributors in the autoimmune phenomenon than age-related disturbances of the immune system.

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