Evaluation of the association of autoantibodies with mortality in the very elderly: a cohort study

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Objective. To evaluate whether autoantibodies in the absence of rheumatic diseases increase the risk of mortality among very elderly subjects who are otherwise in good functional condition.

Methods. Autoantibodies were measured in 1987 in 156 elderly nursing home residents (median age 84 yr) who were followed subsequently over 14.6 yr.

Results. Eleven subjects had anticardiolipin antibodies, 30 had rheumatoid factor and 19 had antibodies to single-stranded DNA (ssDNA). Other autoantibodies were more rare. During follow-up, 144 subjects died. Adjusting for age as a time-dependent covariate, the hazard ratio for death was 0.71 [95% confidence interval (CI) 0.38–1.32] for anticardiolipin antibodies, 0.93 (95% CI 0.60–1.41) for rheumatoid factor, 1.08 (95% CI 0.65–1.79) for antibodies to ssDNA, and 0.99 (95% CI, 0.70–1.41) for any autoantibody. Hazard ratios were similar when adjusted also for sex and clinical conditions.

Conclusion. Our results exclude the possibility that the autoantibodies evaluated increase substantially the risk of death among very elderly subjects in good functional condition.

Key words: Autoantibodies, Mortality, Elderly, Anticardiolipin antibodies, Rheumatoid factor.

Both organ-specific and non-specific autoantibodies are seen frequently in the absence of an autoimmune disease among elderly subjects\cite{1-7}. Apparently, the prevalence of autoantibodies increases steadily between the fifth and early ninth decades but may decrease to lower levels thereafter\cite{7}. The clinical meaning of these autoantibodies is uncertain. For a long time the question has been posed whether the presence of autoantibodies may confer an increased risk of death that is independent of other risk factors\cite{8}. Such an increase in mortality has recently been suggested in some studies, mostly in association with anticardiolipin antibodies\cite{9,10}, especially in the setting of vascular disease, and mortality has been linked mostly with excess cardiovascular deaths. Some evidence also exists for an increased risk of death in the presence of ‘false positive’ elevated rheumatoid factor titres\cite{11}. Prior studies have targeted mostly middle-aged individuals or the elderly in the sixth and seventh decades and have addressed mostly selected populations. There are no longitudinal data on unselected populations of very elderly subjects. In this study, we examined whether autoantibodies influenced the risk of death among a cohort of very elderly individuals for whom follow-up was available over a period of almost 15 yr.

Methods

Study subjects

The subjects were aged 67 yr and over, were residents of a middle-class nursing home in Athens, were in good functional condition and had no major medical problem suggestive of imminent risk of death at the time of autoantibody evaluation. Thus subjects with cancer, severe renal impairment, severe heart failure, cirrhosis, severe respiratory disease and stroke were not eligible. The subjects were recruited for an autoantibody study...
prevalence study in 1987 [1]. Details of this study have been presented elsewhere along with results on the subjects who had no mention of any medical disease at all and were receiving no prescription drugs or daily non-prescription drugs [1]. For the present study, we included all subjects who had autoantibody measurements. Vital status was last updated in March 2002, translating to a total follow-up of 14.6 yr after the time of autoantibody measurements.

Autoantibody measurements
Details on autoantibody determinations have been presented elsewhere [1, 2]. In brief, rheumatoid factor was measured by latex fixation and considered positive if the titre was 1:40 (40 IU/ml) or higher. Antibodies to extracted cellular antigens Ro (SSA), La (SSB), Sm and U1RNP were measured by counterimmunoelectrophoresis against calf thymus extract. The levels of immunoglobulin (Ig) G and IgM antibodies to single-stranded DNA (anti-ssDNA), double-stranded DNA (anti-dsDNA) and cardiolipin (anticardiolipin) were determined by quantitative isotype-specific solid-phase enzyme-linked immunosorbent assays (ELISA). The results were expressed as a binding index calculated by dividing the binding units of every sample by the mean binding units of the control group plus three standard deviations (for the anti-ssDNA and anti-dsDNA assays) or four standard deviations (for the anticardiolipin assays) and multiplying by 100. According to this formula, a binding index of 100 was defined as the cut-off point. This cut-off value is approximately equivalent to 15 U/ml for cardiolipin antibodies and 7 U/ml for anti-dsDNA antibodies.

Statistical analysis
The age of subjects with and without each autoantibody was compared with t-tests. Time-to-event analyses for death were performed with Kaplan–Meier plots with the log-rank test and with Cox models. Kaplan–Meier plots were examined to ensure that proportional hazards would be appropriate. All the Cox models used an adjustment for age as a time-dependent covariate [12], as age is by far the strongest predictor of death in very elderly subjects. We assessed separately the effects on overall mortality of each autoantibody that had been found in more than 10 subjects as well as the effect of having any autoantibody detected.

For autoantibodies that were seen in 10 subjects, there was 80% power to detect a hazard ratio of approximately 2.5 for mortality at \( z = 0.05 \), assuming that 90% of the subjects would die during follow-up. For autoantibodies seen in 20, 30 and 50 subjects, under the same assumptions there was 80% power to detect hazard ratios of approximately 2.0, 1.8 and 1.65 respectively. Thus the study was adequately powered to rule out associations that would be of clinically relevant magnitude.

Besides age, we performed analyses adjusting also for sex, the presence of any medical condition, and specific medical conditions, such as diabetes mellitus, mild heart failure, coronary artery disease, and mild renal impairment. Age was always used as a time-dependent covariate.

Analyses were conducted in SPSS 10.0 (SPSS, Chicago, IL, USA). All \( P \) values are two-tailed.

Results
Of 157 subjects with autoantibody measurements, no vital status information could be retrieved for one; thus 156 subjects were included in the analysis. Of these, only four were known to be still alive in March 2002. Another eight had moved from the nursing home at some point and their current vital status was unknown afterwards; thus their follow-up was censored at the time they left the nursing home. The other 144 subjects were known to have died during follow-up.

The mean age at the time of autoantibody measurement was 82.2 yr (s.d. 6.2) and the median age was 84 yr (interquartile range 78–86). There were 26 male subjects (16.7%). Thirty-eight subjects (24.4%) had at least one recorded medical condition, including histories of diabetes mellitus (\( n = 21 \)), peripheral vascular disease (\( n = 1 \)), coronary artery disease (\( n = 4 \)), mild renal impairment (\( n = 10 \)), psychosis (\( n = 1 \)), tuberculosis (\( n = 2 \)), uncontrolled hypertension (\( n = 1 \)), mild heart failure (\( n = 3 \)), modestly severe chronic obstructive pulmonary disease (\( n = 1 \)), malaria (\( n = 1 \)), dengue fever (\( n = 2 \)), paraproteinaemia (\( n = 1 \)) and dementia (\( n = 1 \)).

In the whole population, the median (interquartile range) titres were 40 (28–53) for IgG anticardiolipin antibodies, 39 (22–49) for IgM anticardiolipin antibodies, 25 (12–45) for IgG anti-ssDNA, 31 (17–58) for IgM anti-ssDNA, 9 (4–19) for IgG anti-dsDNA antibodies and 6 (0–16) for IgM anti-dsDNA antibodies. Anticardiolipin antibodies were seen in 11 subjects (IgG in six, IgM in five), rheumatoid factor was seen in 30 (I:40 in 18 subjects, I:80 in six, I:160 in three, and I:320, I:640 or I:1280 in one patient each), anti-ssDNA antibodies in 19 (IgG in five, IgM in 12, both in two), anti-dsDNA antibodies in five (IgG in two, IgM in three), anti-U1RNP in one and anti-Ro in two; anti-La or anti-Sm antibodies were not seen in any subject. A total of 54 subjects (34.6%) had at least one of these antibodies.

The mean age of subjects with anticardiolipin antibodies tended to be slightly lower than that of those without such antibodies, but this was not formally significant (79.4 yr vs 82.4 yr, \( P = 0.11 \)). No similar strong trend was seen in relation to rheumatoid factor (81.5 yr vs 82.4 yr, \( P = 0.47 \)), anti-ssDNA (82.0 yr vs 82.2 yr, \( P = 0.88 \)) or any autoantibody (81.8 yr vs 82.4 yr, \( P = 0.59 \)). Anticardiolipin antibodies tended to be less frequent in subjects older than 85 yr (1/42) than in those aged 85 or younger (10/115), but the difference was not formally significant (\( P = 0.29 \)). A similar non-significant trend was seen for rheumatoid factor (5/42 vs 25/114, \( P = 0.18 \)).

The median survival in the study cohort was 3.0 yr (interquartile range, 1.0–6.9). As shown in Fig. 1, there was no strong evidence that any of the evaluated autoantibodies was associated with an increased risk of death in Kaplan–Meier analyses without adjustment for other factors. If anything, there was a trend towards reduced mortality in the presence of anticardiolipin antibodies (log-rank \( P = 0.13 \)), but this could be due to the modest difference in age at baseline.

Analyses adjusting for age (Table 1) also showed that no antibody seemed to increase the risk of death and the 95% confidence intervals easily excluded hazard ratios that would correspond to a modest increase in risk, such as a doubling of mortality. Conversely, the confidence
FIG. 1. Kaplan–Meier plots for mortality according to the presence or absence of (a) anti-cardiolipin antibodies, (b) rheumatoid factor, (c) anti-ssDNA antibodies, and (d) any autoantibody among those assayed (see Methods). In each graph, the survival of subjects with autoantibodies is shown with a thick line and the survival of subjects without autoantibodies is shown with a thin line. $P$ values according to the log-rank test are 0.13, 0.64, 0.93 and 0.73 respectively.

TABLE 1. Association of autoantibodies with the risk of death with various adjustments

<table>
<thead>
<tr>
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<th>Age only</th>
<th>Age + any medical condition</th>
<th>Age + specific conditions</th>
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<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>$P$</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>ACL</td>
<td>0.71 (0.38–1.32)</td>
<td>0.27</td>
<td>0.71 (0.38–1.34)</td>
</tr>
<tr>
<td>RF</td>
<td>0.93 (0.60–1.41)</td>
<td>0.72</td>
<td>0.93 (0.61–1.43)</td>
</tr>
<tr>
<td>Anti-ssDNA</td>
<td>1.07 (0.65–1.79)</td>
<td>0.78</td>
<td>1.08 (0.65–1.79)</td>
</tr>
<tr>
<td>Any antibody$^b$</td>
<td>0.99 (0.70–1.42)</td>
<td>0.96</td>
<td>0.99 (0.70–1.41)</td>
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$^a$Age was used as a time-dependent covariate in all models and it was always highly statistically significant, with a hazard ratio of 1.06 (95% CI 1.03–1.09) per yr; specific conditions include diabetes mellitus, renal impairment, congestive heart failure, and coronary artery disease.

$^b$Of those assayed (see Methods).

HR, hazard ratio; CI, confidence interval; ACL, anticardiolipin antibodies; RF, rheumatoid factor.
intervals also excluded an effect towards decreasing mortality by half, with the exception of anticardiolipin antibodies, where such a protective effect could not be excluded. When all autoantibodies were considered, the hazard ratio was almost exactly 1, suggesting no effect at all, either detrimental or protective. Sex was not associated with the risk of death in this cohort (hazard ratio 0.91) and analyses adjusting also for sex yielded similar results (not shown). Analyses adjusting for the overall presence of medical conditions and for specific medical conditions, such as diabetes mellitus, mild renal impairment, coronary artery disease and mild congestive heart failure, yielded very similar results for the effects of autoantibodies (Table 1). Similar results were obtained when analyses were limited to the 118 subjects who had no medical condition recorded and were receiving no prescription drugs or daily non-prescription drugs (not shown).

Discussion

This cohort study with longitudinal follow-up over almost 15 yr demonstrates that the presence of autoantibodies such as anticardiolipin antibodies, rheumatoid factor and anti-ssDNA antibodies does not increase the mortality risk among very elderly subjects without major medical conditions suggestive of imminent death. Thus measuring autoantibodies in very elderly subjects without clinical evidence for autoimmune disease is certainly not indicated.

Previous studies have suggested that anticardiolipin antibodies are independent predictors of mortality among patients with peripheral vascular disease and venous thromboembolism [9, 10]. The excess mortality has been attributed to an increased incidence of fatal cardiovascular events or pulmonary embolism. These studies targeted selected populations that already had a history of vascular disease and were overall substantially younger than the cohort that we studied. Anticardiolipin antibodies have been linked to the atherosclerosis process in several studies and they may be contributors to coronary events, both primary and recurrent [13, 14], in middle-aged populations. It is unclear whether these effects are strong enough to make a significant impact on mortality at a population level. Even if they are, they are likely to be limited to the older middle-aged and the younger elderly. The prevalence of anticardiolipin antibodies may decrease in the very elderly and they may no longer be associated with a bad prognosis. In fact, in our study we even observed a non-significant trend for longer survival in the presence of anticardiolipin antibodies. A decrease in the prevalence of anticardiolipin antibodies in very elderly subjects may be compatible with a ‘survivor’ effect, if subjects with anticardiolipin antibodies are at high risk of dying in late middle age and early old age and therefore may not survive to the late 80s and beyond. Alternatively, the phenomenon could be consistent with immune changes due to senescence, and a similar effect has been reported for thyroid autoantibodies [7].

The presence of rheumatoid factor in the absence of autoimmune disease has also been described in the literature as a predictor of overall mortality. A population survey in Finland [11] observed a hazard ratio of 1.74 after adjusting for age and smoking, which may be confounders for the presence of rheumatoid factor. The excess mortality was due to death from cardiovascular causes. The survey included subjects over 30 yr old, and thus it targeted an age range very different from that in our study. Our cohort shows that rheumatoid factor is unlikely to confer any meaningful increase in the risk of death among the very elderly. Rheumatoid factor may often be assayed routinely in such elderly populations, but this is not useful at all in most cases.

We found no association of anti-ssDNA with overall mortality. Anti-ssDNA antibodies are frequently seen in elderly people but there is no reason why they should be measured on a routine basis. When all autoantibodies were considered, the overall hazard ratio was very close to 1.0, suggesting absolutely no effect on overall mortality. It is not known why all these autoantibodies seem clinically innocuous in the elderly. Some experimental evidence is available showing that the induction of experimental autoimmune disease, such as systemic lupus erythematosus in BALB/c mice, becomes more difficult with increasing age. Autoantibodies are induced in older mice at the same titres or at titres that are slightly lower than in young mice, but clinical manifestations (proteinuria and neutropenia) in older mice are much milder [15]. Autoantibodies alone may not suffice to induce pathogenetic processes in very elderly subjects.

Our study targeted an elderly population without major health problems. Autoantibodies may be more frequent in elderly populations in poorer health and may be affected by exogenous influences, such as exposure to infectious agents [16]. Several studies have shown a lack of correlation with clinical autoimmune disease [17, 18]. Despite the fact that in a few patients the onset of an autoimmune disease, such as rheumatoid arthritis, systemic lupus erythematosus or antiphospholipid syndrome, may occur in their old age [19–21], these cases are rare, and for the vast majority of elderly subjects autoantibodies do not herald autoimmune disease. Anticardiolipin antibodies in particular are more commonly seen in elderly patients with deilities, in particular stroke and multi-infarct dementia [5, 22]. However, even in such settings their clinical implication is uncertain.

We should also acknowledge that in this study we only examined overall mortality and did not seek to investigate whether specific autoantibodies may increase specific types of mortality, e.g. cardiovascular or cerebrovascular mortality. However, most deaths in these very elderly subjects could not be attributed with certainty to specific causes and thus no reliable cause-specific data were available. This is very common in populations of this age range. More importantly, in the
absence of overall effects of mortality, the perusal of specific subgroups of deaths may lead to spurious findings due to multiple comparisons and data dredging. The conclusions of this study should not necessarily be extrapolated to other organ-specific autoantibodies. However, unless evidence accumulates showing that other organ-specific autoantibodies may have a prognostic role in very elderly subjects, we would caution against their routine evaluation in the absence of very specific clinical indications.

References