Brief report

Blood Biomarkers and Functional Disability Among Extremely Longevoous Individuals: A Population-Based Study

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Abstract

Background. Several blood biomarkers have been linked to functional disability, a health problem in general populations. However, there are limited data for evaluating the potential association of biomarkers with functional disability in an extremely longevous (95+) population.

Methods. We used data from 420 extremely longevous individuals from the Rugao longevity cohort, a population-based association study conducted in Rugao, a longevity town in China. Functional disability was assessed by the Katz Index of Independence in activities of daily living. Blood biomarkers, including serum lipid, lipoprotein cholesterol, serum albumin, and lymphocyte count, were correlated with activities of daily living.

Results. Among extremely longevous women, following the degree of functional disability, serum albumin and lymphocyte count decreased significantly (all p for trend < .001). In a univariate model, serum albumin (β = −0.279, p < .001), lymphocyte count (β = −0.187, p < .001), and neutrophil count (β = 0.140, p = .012) were found to be significantly associated with activities of daily living in women. After adjustment for other covariates, the significance remained. Notably, multivariate regression analysis revealed independent effects of all the three biomarkers on activities of daily living (β = −0.242, −0.185, and 0.143, all p < .05). We did not observe any association in men.

Conclusions. We found significant associations between serum albumin, lymphocyte count, and neutrophil count and physical disability even after adjustment for potential confounders in extremely longevous women, which call for further study. The findings provide preliminary but crucial clues for future studies specifically aimed at exploring the longitudinal relationships of interest before proceeding with interventions.

Key Words: Biomarker—Functional disability—Extremely longevous—Serum albumin.
As the population ages rapidly in recent decades, many health problems have gained public attention. Functional disability appears to be particularly crucial. As aging-related diseases change, functional disability has been linked to mental disorders (1), mortality (2), survival (3), and higher health care costs, resulting in great familial and societal burdens. Identification of its modifiable related factors (ie, biomarkers) is important and imperative for providing potential clues for public interventions and long-term clinical care.

Recently, blood biomarkers in different systems associated with chronic diseases (ie, albumin, lymphocyte count, interleukin-6, dehydroepiandrosterone sulfate) have been linked to functional disability in several studies (4–11). However, common to most of these studies was the relatively young age of the study participants. In fact, with the rapid increase in life expectancy, the oldest old have been the fastest growing segment of the population. More and more of the oldest old individuals have suffered from functional disability with advanced aging. Considering the vulnerability and the potential unique pattern of related factors compared with the general populations, this special age group deserves more attention. Nevertheless, to the best of the authors’ knowledge, only two studies in Japan had explored the relationship between biomarkers (serum albumin) and functional disability in the oldest old populations (12,13). Moreover, because only one (5,7,8,11–13) or a few biomarkers (4,6) were observed in aforementioned studies, appraisements and screening of the effects of various biomarkers representing status of diverse pathophysiologic systems on physical functioning could be substantially helpful for our comprehensive understanding.

Based on data from the Rugao longevity cohort, this study evaluated the potential associations between 16 blood biomarkers and functional disability among extremely longevous (aged 95+ years, EL) individuals. In terms of the unique characteristic of this age group, this study intends to shed light on the potential modified risk factors for poor physical performance in the EL individuals through regular laboratory testing, which may offer possible direction for long-term clinical care and public interventions.

**Methods**

**Study Population and Procedures**

We used data from EL individuals enrolled in the Rugao longevity cohort, a population-based association study conducted between December 24, 2007 and February 29, 2008 in Rugao, a famous longevity town of Jiangsu Province, China. Descriptions in detail had been provided elsewhere (14). Briefly, after a strict four-step age verification, we recruited 463 (103 men and 360 women) participants aged 95+ years with a response rate of 71.6% (sampling frame = 755). No significant difference in age or gender ratio was found between nonresponders and respondents (all p > .05). The exclusion criteria were as follows: missing data for functional disability, acute infections, known malignant tumor, severely physically or mentally ill, or taking anti-inflammatory drug (ie, nonsteroidal antiinflammatory drugs) in previous 12 months. Eventually, 420 eligible participants (97 men and 323 women) with relatively good health remained in this study.

As described previously (14), a structured questionnaire was administered by trained field staff that delved into areas including demographic characteristics, histories of chronic disease (ie, chronic obstructive pulmonary disease, coronary heart disease, malignant tumor), functional disability (assessed by the Katz Index of activities of daily living [ADL]), and medications (ie, anti-inflammatory drug). Written informed consent was obtained from each participant or a member of his or her immediate family. The Human Ethics Committee of Fudan University School of Life Sciences approved the research.

**Blood biomarkers**

According to previous relevant studies (4–8), some blood biomarkers, including total cholesterol, total triglyceride, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, blood hemoglobin, serum albumin, and lymphocyte count, that were extensively investigated in relation with aging and physical outcomes were considered in this study, for little knowledge exists regarding their relationship to ADL among EL individuals. We also included other rarely investigated biomarkers, such as serum uric acid, total bilirubin, direct bilirubin, alanine transaminase, glutamic oxaloacetic transaminase, red blood cell count, white blood cell count, platelet count, and neutrophil count (9,12,13), which were routinely examined in clinical diagnosis to establish potential novel associations with ADL. All biomarkers were determined by standard laboratory techniques (performed by a technician in the biochemistry laboratory of Jiangsu Rugao Hospital of Traditional Chinese Medicine).

**Functional disability**

Functional disability was assessed by the Katz Index of ADL, which was modified from the original scale and has been extensively used to evaluate the functional status of older adults. The Katz Index is based on six daily tasks: eating, dressing, bathing, indoor transferring, going to the toilet, and cleaning oneself afterward (15). Each task has the following three response alternatives: strongly independent, somewhat dependent, and strongly dependent, with a score of 1, 2, and 3 points, respectively. Lower scores indicated better physical functioning. Based on the total summed scores, a nominal variable with three categories of functional disability, “none” (total score = 6), “moderate” (total score = 7–9), “severe” (total score ≥ 10), was constructed.

**Covariates**

Covariates were collected from the structured questionnaire, including demographics (age, education levels, marital status), lifestyles (smoking, drinking status), body mass index, and numbers of chronic diseases (4).

**Statistical Analyses**

We calculated the means ± standard deviation (SD) or median (interquartile range) of biomarkers according to ADL categories. The presence of a linear trend of biomarkers among three ADL categories was evaluated by defining a linear contrast in the one-way analysis of variance (nonnormal biomarkers were transformed to Z score with a mean of 0 and SD of 1). Univariate and multivariate regression analysis were performed to test the associations of biomarkers with dependent variables (ADL). Variables (biomarkers and ADL) were transformed to Z score with a mean of 0 and SD of 1, and regression coefficients (standardized) were documented. All the analyses were carried out separately for men and women in consideration of the possible gender difference suggested by other studies (6). SAS software (version 9.3; SAS Institute, Cary, NC) was used for all analyses. A p value of <.05 (two-tailed) was considered statistically significant.

**Results**

The means (±SD) of age were 97.0 ± 1.9 years (range, 95–103 years) for men and 97.4 ± 2.1 years (range, 95–107 years) for women. About 12.4% and 35.7% of men were currently married and literate (≥1 years education), respectively, and the proportions were 2.8% and 6.8% in women. Approximately 45.4% and 53.6% of men reported a history of smoking and alcohol consumption, and the corresponding percentages were 11.8% and 34.1% for women. The
mean value of body mass index was 22.2 kg/m² for men and 21.4 kg/m² for women. The numbers of chronic diseases were 1.9 in both genders. Among men, 55.7% had “none;” 22.7% had “moderate;” and 22.6% had “severe” ADL disability. The corresponding proportions in women were 46.4%, 23.2%, and 30.3%, respectively. The means ± SD or median (interquartile range) of blood biomarkers according to ADL categories were presented in Table 1. Among women, following the degree of functional disability, serum albumin and lymphocyte count decreased significantly (all \( p \) for trend < .001). No other significant changing trend in women or men was found in the descriptive statistics for 16 blood biomarkers.

Next, in the univariate model, we observed that 3 of 16 biomarkers, including serum albumin, lymphocyte count, and neutrophil count, were significantly associated with ADL in women, with a regression coefficient (\( \beta \), standardized) of -0.279 (\( p < .001 \)), -0.187 (\( p < .001 \)), and 0.140 (\( p = .012 \)), respectively (Supplementary Table 1). We then examined the relationships between the three identified biomarkers and ADL by using multivariate regression analysis. After further adjustment for other covariates (ie, age, education levels), the significances of the observed associations remained (Table 2, Model 2). To test the presence of independent effects of the three biomarkers on ADL, we adjusted for them in Model 3. For example, for serum albumin, the other two biomarkers, lymphocyte and neutrophil count, were added in the full model (with all other variables held constant). We found that there were independent effects of the three biomarkers on ADL (\( \beta = -0.242, -0.185, \) and 0.143, all \( p < .05 \)) in women. We did not observe any association in men (Supplementary Table 1).

**Discussion**

The significant and important findings of this brief report is that of the 16 biomarkers, serum albumin, lymphocyte count, and neutrophil count were found statistically significantly associated with functional disability even after adjustment for potential confounders among EL women in China. From the observations of this study, we suggest that the three biomarkers could be potentially useful markers of physical disability of EL women, which calls for further study.

Our findings of the association between serum albumin and functional disability are consistent with previous studies (4,6,12,13). Serum albumin serves certain vital physiologic roles in health and well-being for many organs. For example, it maintains the intravascular oncotic pressure and acts as a transport protein for fatty acids. Albumin has been considered as a conventional marker of nutritional status in the elderly population, but is still controversial (16). A recent study found that among community-living Chinese aged 90 and older, lower albumin was associated with poor nutritional status (17). This finding indicates that the observed association between albumin and functional disability in our study might be partially explained by malnutrition, in consideration of the reasonably homogeneous racial characteristics of study participants. To verify this speculation, a combination of biomarkers (ie, serum albumin) and other indicators provided by various methods (ie, 24-hour recall method) are recommended to evaluate one’s nutritional adequacy in future investigations.

Generally, a serum albumin level of less than 35 g/L (3.7% in women of this study) was defined as malnutrition in the elderly population. However, researchers found that signs of malnutrition (ie, declines in muscle strength) could occur even within the normal range of albumin levels. Therefore, from the perspective of interrelated biological mechanisms, skeletal muscle loss or depletion, even inflammatory conditions, may mediate the observed albumin–functional disability association (4,18), which warrants further exploration.

In this study, lymphocyte count showed an inverse association with functional disability. To our best knowledge, this association has not yet been reported in the oldest old. Obviously, little is known about the underlying mechanisms. With respect to negative relations between low lymphocyte count and mortality in older adults (19), according to Izaks and colleagues (19), “a low lymphocyte count may not only indicate an exhaustion of the lymphoid cell line but may also be the marker of a decline in other physiological functions.” It is then intriguing to elaborate the findings of our study. We suggest that, to some extent, the association of lymphocyte count with ADL can be considered as an effective compensation of the previous study on mortality. That is, participants with low lymphocyte count within physiological range reported severe functional disability that may eventually cause their deaths. Obviously, the assumption needs confirmation provided by longitudinal data.

Neutrophil count was the third biomarker that significantly associated with ADL in this study. Unfortunately, the inverse relation was relatively weak. We failed to observe the significant change trend of neutrophil count according to ADL categories. This may be attributed to the decreased power triggered by artificially transforming continuous variables (ADL) into ordinal categorical variable when performing an analysis of variance. To date, it has been postulated that the cytokines, oxidation metabolites, and free radicals produced by neutrophils may cause oxidative damages to various tissues (ie, muscles) and organ systems (20), which may have an impact on physical functioning, that is, ADL, frailty. That is why several studies reported an inverse association between neutrophil count and physical activity (one of five specific criteria assessing frailty) (9). Nevertheless, the exact molecular mechanism is still unclear, and thus, the postulation remains future decipherments.

Notably, no association of serum lipid, lipoprotein cholesterol with functional disability was observed in this study. In addition to differences in measurements, the disparities may be attributed to participants’ characteristics (6), such as age. It is well documented that, the EL individuals, a highly epidemiological selected age group, have escaped, delayed, or survived many age-related diseases (ie, stroke, myocardial infarction) (21) caused by common risk factors including dyslipidemia, resulting in a favorable lipid profile, which does not differ evidently enough for us to detect the potential association. To some degree, this is the reason that the EL individuals were widely used as a model for healthy aging studies.

The strengths of this study include the population-based approach, the reasonably large sample size of EL individuals, and the observations of independent effects of different biomarkers on functional disability. An inherent limitation of this study is the cross-sectional design, and thus, no causal inferences could be made. In addition, the lack of a nutritional assessment (ie, Mini-Nutritional Assessment) does not allow for fully appreciating the nature of the relationship between biomarkers and ADL disability in this study. Finally, the negative results of men might be attributed to the smaller sample size relative to women, although it is not that few, especially considering the very advanced age. The findings in women imply the inability to directly apply these results to men, which might represent a limitation of the study. We hope that future studies could confirm our initial findings and extend them to various longevous populations, especially in populations with a large sample size of males.

In conclusion, for the first time, we found significant associations between serum albumin, lymphocyte count, and neutrophil count and physical disability even after adjustment for potential
Table 1. Blood Biomarkers of the EL Men (97.0 ± 1.9 y old) and Women (97.4 ± 2.1 years old) From the Rugao Longevity Cohort (Jiangsu, China) According to ADL Categories

<table>
<thead>
<tr>
<th>Biomarkers*</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. None (ADL = 6)</td>
<td>Moderate (7 ≤ ADL &lt; 10)</td>
<td>Severe (ADL ≥ 10)</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>4.33 ± 0.84</td>
<td>4.56 ± 1.01</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>0.75 (0.58–0.96)</td>
<td>0.87 (0.67–1.28)</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.35 (1.18–1.56)</td>
<td>1.13 (1.06–1.33)</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>2.23 (1.86–2.70)</td>
<td>2.49 (1.87–2.98)</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>129.0 (120.0–139.0)</td>
<td>138.0 (113.0–148.0)</td>
</tr>
<tr>
<td>Serum albumin (g/L)</td>
<td>43.3 ± 3.7</td>
<td>41.0 ± 6.8</td>
</tr>
<tr>
<td>SUA (μmol/L)</td>
<td>329.0 (256.0–378.0)</td>
<td>326.5 (263.0–400.0)</td>
</tr>
<tr>
<td>TBIL (μmol/L)</td>
<td>15.2 (11.3–18.7)</td>
<td>11.8 (8.8–14.7)</td>
</tr>
<tr>
<td>DBIL (μmol/L)</td>
<td>4.0 (3.0–5.2)</td>
<td>3.3 (2.4–4.1)</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>12.0 (10.0–16.0)</td>
<td>13.0 (9.0–18.0)</td>
</tr>
<tr>
<td>GOT (U/L)</td>
<td>26.0 (23.0–31.0)</td>
<td>25.5 (23.0–30.0)</td>
</tr>
<tr>
<td>RBC count (×10¹²/L)</td>
<td>4.0 (3.7–4.4)</td>
<td>4.0 (3.7–4.4)</td>
</tr>
<tr>
<td>Neutrophil count (×10⁹/L)</td>
<td>3.1 (2.4–3.6)</td>
<td>3.1 (2.5–3.8)</td>
</tr>
</tbody>
</table>

Notes: ADL = activities of daily living; ALT = alanine transaminase; DBIL = direct bilirubin; EL = extremely longevous; GOT = glutamic oxaloacetic transaminase; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; RBC = red blood cell; SUA = serum uric acid; TBIL = total bilirubin; TC = total cholesterol; TG = total triglyceride; WBC = white blood cell.

* Normally distributed biomarkers were expressed as means ± standard deviation (SD); nonnormal biomarkers were expressed as median (interquartile range) and were transformed to Z score with a mean of 0 and SD of 1 for the parametric test.

† The numbers in "none," "moderate," and "severe" (for TC) subgroups were 54, 22, and 21, respectively. There was a little difference in the proportions when applying to other biomarkers.

‡ The presence of a linear trend was evaluated by defining a linear contrast in the one-way analysis of variance.

§ The numbers in "none," "moderate," and "severe" (for TC) subgroups were 150, 75, and 97, respectively. There was a little difference in the proportions when applying to other biomarkers.
Table 2. The Associations of Serum Albumin, Lymphocyte Count, and Neutrophil Count With ADL Among the EL Women (974±2.1 years old) From the Rugao Longevity Cohort, Jiangsu, China

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>β* (SE)</td>
<td>p Value</td>
</tr>
<tr>
<td>Serum albumin*</td>
<td>321</td>
<td>-0.279</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>(kg/m²)</td>
<td>(0.054)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphocyte count*</td>
<td>317</td>
<td>-0.187</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>(cells/μL)</td>
<td>(0.055)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutrophil count*</td>
<td>319</td>
<td>0.140</td>
<td>0.012</td>
</tr>
<tr>
<td>(cells/μL)</td>
<td>(0.055)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Notes: ADL = activities of daily living; EL = extremely longevous; SE = standard error. Model 1: unadjusted (a univariate model). Model 2: adjusted for age, education levels, marital status, smoking and drinking status, body mass index, and sum of chronic conditions (including visual and hearing impairment, arthritis, diabetes, respiratory diseases [ie, chronic obstructive pulmonary disease], cardiovascular diseases [ie, hypertension, coronary artery disease], fracture, and stroke). Model 3: add the other identified biomarkers (ie, for serum albumin, add neutrophil and lymphocyte count) to Model 2. Body mass index was calculated as weight divided by height in squared (kg/m²).

*Variables (biomarkers and ADL) were transformed to Z score with a mean of 0 and standard deviation (SD) of 1, and regression coefficients (standardized) were documented.

confounders among EL women. The initial cross-sectional findings provide primary but crucial clues for future studies specifically aimed at exploring the longitudinal relationships of interest before proceeding with interventions.

Supplementary Material
Supplementary material can be found at: http://biomedergerontology.oxfordjournals.org/

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References