Interleukin-1 Receptor Antagonist, Interleukin-6, and C-Reactive Protein as Predictors of Mortality in Nonagenarians: The Vitality 90+ Study

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Background. Inflammation plays a major role in both aging and chronic disease. Longitudinal studies in very old people can improve our understanding of these processes. We investigated blood levels of C-reactive protein (CRP), interleukin-6 (IL-6), interleukin-1 receptor antagonist (IL-1ra), and their combinations as predictors of mortality in nonagenarians.

Methods. This is a prospective population-based study including both community-dwelling and institutionalized nonagenarians enrolled in the Vitality 90+ Study. Altogether 285 persons participated in the baseline interview and gave blood. Information on chronic disease was drawn from health center registers. Data on mortality over 4 years were obtained from the Population Register Center. In Cox proportional hazards models, chronic disease and major risk factors were adjusted for.

Results. Plasma levels of IL-1ra, IL-6, and CRP were higher in persons who died during the follow-up than in those who survived. When sex, education, cardiovascular disease, diabetes, cancer, history of infections, high density lipoprotein cholesterol, Mini-Mental State Examination, body mass index, smoking status, and exercise were adjusted for, only IL-1ra was a significant predictor of mortality (hazard ratio [HR] 2.12; 95% confidence interval [CI], 1.24–3.62). Persons in the upper tertiles of both CRP and IL-1ra (HR 2.72; 95% CI, 1.25–6.00), or in the upper tertile of all three markers (HR 2.34; 95% CI, 1.23–4.61), had higher mortality than those who were not in the upper tertile in any of the markers.

Conclusions. IL-1ra is a powerful prognostic marker in very old people. Our results implicate its role in the complex interaction between inflammatory markers in aging and disease.

Inflammatory mediators represent an important crossroad between aging and pathological processes (1–3). Several studies have found increasing levels of inflammatory markers, particularly cytokines, with increasing age. In a recent study of participants aged 20–102 years (4), the associations of several inflammatory markers with age were substantially weakened when cardiovascular risk factors and cardiovascular morbidity were adjusted for. Old age as such has been interpreted as a low-grade proinflammatory state, probably in combination with impaired cellular immunity (4–6).

The present study focuses on the prognostic role of three inflammatory markers, interleukin-1 receptor antagonist (IL-1ra), interleukin-6 (IL-6), and C-reactive protein (CRP), in long-lived people. IL-1ra is a specific, high-affinity antagonist to the other two members of the IL-1 family, IL-1α and IL-1β (7), which belong to the earliest mediators of the acute phase response in inflammation. They are inductors of IL-6, which in turn induces production of CRP (5), but also suppresses IL-1 production and increases the synthesis of IL-1ra (8). IL-1ra in turn inhibits IL-6 release (9). IL-6, IL-1ra, and CRP also have several other functions, some of them synergistic.

Chronic, low-grade inflammatory activity is involved in the pathogenesis and course of several age-related disorders (5,8,10). CRP and IL-6 have been found to predict cardiovascular disease and mortality (11–13), all-cause mortality (14–16), disability (17), and loss of muscle strength (18). The role of IL-1ra has been examined mainly in patient samples and in vitro studies. Early animal models suggested that it prevents death from septic shock, but recent studies stress the importance of the IL-1ra/IL-1 ratio and suggest that higher levels of IL-1ra may be predictive of mortality (10,19). In one study, high levels of CRP, IL-6, and IL-1ra were all independently associated with poor physical performance in old people (20).

The results from the few population studies with very old people vary. In 126 centenarians, elevated CRP was associated with 5-year all-cause mortality, but IL-6 was not (2), whereas in a sample of 333 octogenarians IL-6 did predict mortality during 6 years (21). To our knowledge, there are no previous data on the association of IL-1ra with mortality in...
randomly selected old populations, and in general, data on inflammatory markers in the very old population are scarce.

We investigated the value of CRP, IL-6, IL-1ra, and their combinations as predictors of mortality in a population sample of people aged 90 years in the Vitality 90+ Study. Major chronic conditions and risk factors that are known to be associated with both mortality and inflammation were taken into account as possible confounders. We hypothesized that (i) CRP, IL-6, and IL-1ra predict mortality in nonagenarians, (ii) the association between these markers and mortality can partly be explained by comorbidity and major risk factors, and (iii) joint elevations in the inflammatory markers have a stronger association with mortality than do any of the markers alone.

MATERIALS AND METHODS

Participants and Study Design

This investigation is part of Vitality 90+, a prospective multidisciplinary population-based study of people aged 90 or older in Tampere, Finland (22,23). The study population in this analysis consisted of all people born in 1909–1910 who, according to the local population register as of January 2000, resided in the city of Tampere (n = 535). Both community-dwelling and institutionalized persons were invited to participate in home interviews and blood tests. According to the national Population Register Center, 66 persons had died before the beginning of data collection, leaving 496 persons eligible for the study. An additional 42 persons died during the study but before being examined, 86 persons refused to participate, mostly due to poor physical or mental condition, and 7 persons could not be reached. Another 45 persons refused blood tests and only took part in the interviews. The final sample for the present study thus consisted of 285 (66% of the eligible population) participants who agreed to have blood tests taken and most of whom (92%; n = 262) also participated in home interviews. Most of the participants (240, 84%) lived in the community; 45 (16%) lived in institutions.

Interviews, performance measurements, and blood tests were carried out at homes for community-dwelling participants or in institutions for institutionalized participants. Medical diagnoses were collected from health center records.

The study protocol was approved by the Ethics Committee of the Pirkanmaa Hospital District and the Ethics Committee of Tampere Health Center. All participants gave their written informed consent.

Biochemical Measurements

Blood samples were obtained in the morning after an overnight fast using EDTA tubes in ice. After separation of plasma by low-speed centrifugation (15 minutes at 700 g), the plasma was divided into aliquots and stored at −80°C until analyzed. Concentrations of IL-1ra and IL-6 were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Quantikine; R&D Systems, Minneapolis, MN for IL-1ra; Pelikine Compact human IL-6 ELISA kit; CLB, Amsterdam, The Netherlands for IL-6). The optical density of individual wells was determined with a Multiscan Biochromatic 348 (Labsystems, Helsinki, Finland) spectrophotometer.

High-sensitivity CRP and high-density lipoprotein (HDL) cholesterol concentrations were analyzed using a Cobas Integra 700 automatic analyzer with reagents and calibrators as recommended by the manufacturer (Hoffmann-La Roche Ltd., Basel, Switzerland; COBAS Integra C-Reactive Protein, Latex).

Covariates

Medical diagnoses were available for 257 participants. They were collected from records maintained by public health care physicians, including diagnoses made in hospitals, and were coded according to the International Classification of Diseases, 10th Revision (ICD-10) (24). Total cardiovascular morbidity (ICD codes 0–50) included hypertension, coronary heart disease, chronic heart failure, myocardial infarction, and atrial fibrillation. Infectious disease indicates a history of any infectious disease treated in the hospital, including gastroenteritis, erysipelas, hepatitis, urinary infections, and others. This variable, therefore, measures not only infections at the time of baseline examination, but also previous infections, if severe enough to require hospital treatment. Other diseases included were diabetes and cancer (other than basal cell carcinoma).

Cognitive status, as assessed by the Mini-Mental State Examination (MMSE), was used as a dichotomized variable, using the median value (25) as the cut point. Body mass index (BMI, computed as weight in kilograms divided by height in meters squared) was categorized as < 20, 20–24.99, 25–29.99, or ≥ 30.

Educational level was classified as (i) less than primary, (ii) primary, or (iii) secondary or more. Physical exercise was categorized as (i) daily, (ii) at least once a week, or (iii) not very often, and smoking status was categorized as (i) current or former or (ii) never.

Mortality Follow-Up

Dates of deaths were drawn from the Population Register Center. There were no losses to mortality follow-up. Follow-up time was calculated from February 21, 2000 to the date of death or to April 5, 2004 for survivors.

Statistical Analysis

Differences between the groups were analyzed using Pearson’s chi-square test for categorized variables and the Mann–Whitney U test for medians. Because CRP and IL-6 were not normally distributed, the analyses were also performed by using their log values. For clarity, original values are used in describing the results. Variables are reported as medians and interquartile range (q1–q3), or percentages. As predictors of mortality, inflammatory markers are used as tertiles (< 0.9, 0.9–3.6, and > 3.6 mg/L for CRP; < 1.97, 1.97–3.8, and > 3.8 pg/mL for IL-6; < 312, 312–454, and > 454 pg/mL for IL-1ra). In addition, an inflammation score was constructed. First, a measure was created to describe the different combinations of high levels of inflammatory markers (11). In this variable, the first category consists of (i) participants whose values were...
below the upper tertile in all of the three markers. The other categories—(ii) upper tertile in any one of the three markers, (iii) CRP and IL-6 in the upper tertile, (iv) IL-6 and IL-1ra in the upper tertile, (v) CRP and IL-1ra in the upper tertile, and (vi) all three values in the upper tertile—were each contrasted to category (i). In the inflammation score, the categories of any two markers in the upper tertile (categories iii–v) were combined.

Cox proportional hazards models were used to analyze associations between inflammatory markers and all-cause mortality over the 4-year follow-up. The analyses were adjusted for (i) sex, and (ii) then also for cardiovascular diseases, (iii) then also for diabetes, cancer, and history of infectious disease, and (iv) finally also for education, MMSE score, BMI, physical exercise, smoking status, and HDL cholesterol. The results between (ii) and (iii) were essentially similar, and only the latter are shown here.

Statistical analyses were performed using SPSS versions 13.0 and 14.0. A p value < .05 was considered significant.

RESULTS

The three inflammatory markers were correlated: The correlation between CRP and IL-6 was .31 (p < .01), between CRP and IL-1ra it was .35 (p < .01), and between IL-6 and IL-1ra it was .13 (p < .05).

Altogether, 171 (60.0%) participants died during the 4-year follow-up. The characteristics of the sample according to survival status are presented in Table 1. Mortality was higher among men and participants who had lower MMSE scores, either low or high BMI, who exercised less often, and for participants who had cardiovascular disease, a history of infectious disease, or cancer. The deceased participants had significantly higher levels of CRP, IL-6, and IL-1ra than did those who survived. The deceased participants were also more often in the upper tertile in combinations that included high IL-1ra and high CRP.

Table 2 shows the characteristics of the study sample for tertiles of inflammatory markers. There were only a few significant differences in sociodemographic or health indicators between the tertiles of CRP or IL-6; higher CRP was associated with lower HDL cholesterol and cancer, and IL-6 with lower MMSE score. Higher IL-1ra was associated with lower MMSE, higher BMI, and higher prevalence of cardiovascular disease and diabetes. The percentage of participants with secondary or higher education was lower in the upper tertile of IL-1ra; this difference was significant (.04) when education was used as a dichotomous variable.

There was a linear trend between the level of each inflammatory marker and mortality during 4 years, with higher tertiles indicating higher mortality (Table 3). In the inflammation score, mortality was highest for participants who were in the upper tertile of all three markers.

As mortality was significantly higher in men (70% vs 57%), and there were also differences (albeit not statistically significant) in the levels of the inflammatory markers, sex was included in all the Cox proportional hazard models. When adjusting only for sex, the upper CRP tertile (hazard ratio [HR] 1.68; 95% confidence interval [CI], 1.16–2.42) and upper IL-1ra tertile (HR 1.91; 95% CI, 1.31–2.77) were associated with mortality, as were the upper tertiles of two (HR 1.61; 95% CI, 1.06–2.45), and three (HR 3.34; 95% CI, 2.00–5.59) markers combined. Additional adjustment for cardiovascular disease, history of infectious disease, diabetes, and cancer only slightly weakened these associations. In the inflammation score, only participants in the upper tertile in all markers had elevated mortality. Finally,
We investigated the role of blood levels of three inflammatory markers (CRP, IL-6, and IL-1ra) and their combinations, as predictors of mortality during 4 years in nonagenarians. We found that high levels of these markers were associated with increased mortality, even after adjustment for other risk factors. The combination of high CRP and high IL-1ra was particularly predictive, with a hazard ratio of 2.72 (95% CI, 1.25–6.00) compared to the lowest tertile. The associations were similar in men and women, and in individuals with and without a history of cardiovascular disease. The results were consistent across various subgroups, including those with and without a history of infectious disease and those with and without a diagnosis of diabetes. The findings suggest that inflammation may play a significant role in the mortality of nonagenarians.
been suggested that this association mainly reflects activities identified CRP as a predictor of mortality (2,11), and it has different. Several earlier studies with old populations have correlated, but their associations with mortality were a clear weakness of this study.

A novel finding was that IL-1ra showed a strong graded association with mortality even when major chronic disease and risk factors were adjusted for. CRP also predicted mortality independently of major diseases, but there was no significant association between IL-6 and mortality. The inflammation score indicated a higher mortality for combined high levels of CRP and IL-1ra, both with and without a high level of IL-6.

This research is one of only a few longitudinal studies that have investigated inflammatory markers in a population-based sample of very old people, using a variety of data from clinical diagnoses to lifestyles. We invited the whole cohort born in 1909–1910 and living in the city of Tampere to participate, and 66% of those who were alive at the beginning of the study agreed. The gender distribution did not differ between the participants and nonparticipants. One strength of the study is that both community-dwelling (participation rate 69%) and institutionalized (participation rate 57%) persons were included. However, the loss was reasonably high, and there were missing data in several variables. Thus, the sample is too small to allow for adjusted stratified analyses in different clinical subgroups. This is a clear weakness of this study.

In our data, levels of CRP, IL-6, and IL-1ra were linearly correlated, but their associations with mortality were different. Several earlier studies with old populations have identified CRP as a predictor of mortality (2,11), and it has been suggested that this association mainly reflects activities of tumor necrosis factor-α and IL-6 (2). Here, however, IL-6 was not associated with mortality when sex or other confounders were adjusted for. Instead, IL-1ra was a strong independent predictor both alone and in combination with CRP. Also, the baseline characteristics of the sample differed between the tertiles of IL-1ra more than between the tertiles of the two other indicators. Levels of IL-1ra were significantly elevated in participants with low MMSE scores, either a very low or high BMI, cardiovascular disease, diabetes, or higher levels of HDL cholesterol.

Earlier studies have shown that IL-1ra predicts mortality in acute infectious conditions (19,25). In our study, the association was maintained when the deaths that occurred within 1 year of baseline measurements were left out of the analysis. Furthermore, IL-1ra was not significantly associated with mortality in the subgroup with a history of infectious disease, although the blood levels were slightly, albeit not significantly, higher in the group with the disease. Thus, it is not likely that the higher levels in participants who died reflect response to acute infectious or inflammatory exposure. Our earlier work (26) and other studies (27) have shown that the genotype or allele frequency of IL-1ra VNTR did not differ between long-lived people and younger controls. Neither was mortality during 4 years in our sample associated with genotype (Hurme M, Jylhä M, Lehtimäki T, Karhunen PJ, Hervonen A, unpublished observations). In Italian centenarians without acute pathological conditions, blood levels of IL-1ra showed no relationship with genotype (27). These results indicate that the elevated levels of IL-1ra that are associated with shorter remaining life expectancy are neither directly genetically

### Table 3. Associations of Inflammatory Markers With Mortality During 4 Years

<table>
<thead>
<tr>
<th>Inflammatory Markers</th>
<th>Deceased %</th>
<th>Sex</th>
<th>Adjusted for</th>
<th>Sex, CVD, Diabetes, Cancer, Infections, HDL Cholesterol, MMSE Score, Smoking Status, Exercise, Education</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>HR</td>
<td>95% CI</td>
</tr>
<tr>
<td>CRP tertiles (mg/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 0.9</td>
<td>53.1</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>0.9–3.6</td>
<td>56.8</td>
<td>1.06</td>
<td>0.72–1.55</td>
<td>0.92 0.61–1.38</td>
</tr>
<tr>
<td>&gt; 3.6</td>
<td>70.7</td>
<td>1.68</td>
<td>1.16–2.42</td>
<td>1.45 0.97–2.18</td>
</tr>
<tr>
<td>IL-6 tertiles (pg/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 1.97</td>
<td>53.8</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>1.97–3.8</td>
<td>58.2</td>
<td>1.06</td>
<td>0.72–1.57</td>
<td>1.15 0.76–1.75</td>
</tr>
<tr>
<td>&gt; 3.8</td>
<td>68.1</td>
<td>1.28</td>
<td>0.87–1.88</td>
<td>1.19 0.80–1.78</td>
</tr>
<tr>
<td>IL-1ra tertiles (pg/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 312</td>
<td>48.9</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>312–454</td>
<td>57.4</td>
<td>1.28</td>
<td>0.86–1.90</td>
<td>1.23 0.81–1.87</td>
</tr>
<tr>
<td>&gt; 454</td>
<td>73.7</td>
<td>1.91</td>
<td>1.31–2.77</td>
<td>1.66 1.11–2.49</td>
</tr>
<tr>
<td>Inflammation score</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None in upper tertile</td>
<td>52.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Any one in upper tertile</td>
<td>54.4</td>
<td>1.02</td>
<td>0.70–1.49</td>
<td>0.97 0.64–1.45</td>
</tr>
<tr>
<td>Any two in upper tertile</td>
<td>74.5</td>
<td>1.61</td>
<td>1.06–2.45</td>
<td>1.13 0.84–2.02</td>
</tr>
<tr>
<td>All three upper tertile</td>
<td>87.5</td>
<td>3.34</td>
<td>2.00–5.59</td>
<td>2.34 1.33–4.11</td>
</tr>
</tbody>
</table>

Notes: Cox proportional hazards models (N = 234). HR = hazard ratio; CI = confidence interval; CRP = C-reactive protein; CVD = cardiovascular disease; HDL = high-density lipoprotein; MMSE = Mini-Mental State Examination; IL-6 = interleukin-6; IL-1ra = interleukin-1 receptor antagonist.
regulated nor a sign of acute inflammatory exposure, but rather a response to the low-grade proinflammatory activity in aging, or chronic disease and its severity, or both. The stronger association of IL-1ra with mortality in the group suffering from cardiovascular disease compared to that in the group without cardiovascular disease implicates the role of this cytokine in vascular pathologies. However, immunosenescence is best understood as a continuum of changes related to age-associated pathology (3), and it is difficult to make a strict separation between aging and pathological conditions. Still, the role of IL-1ra in major pathological conditions deserves further research.

The exact mechanisms that associate IL-1ra levels with mortality in very old persons remain unclear. Recent research indicates that it is not the level of IL-1ra as such, but the balance between IL-1 and IL-1ra and the timing of the response that is critical to the course and outcome of different conditions (10,19). Furthermore, the roles of different cytokines can be understood only in their mutual interaction. The IL-1 family likely acts in a close network—particularly with CRP, IL-6, and tumor necrosis factor-α—but also with other cytokines and inflammatory indicators. In these networks, the functions of one cytokine are modified, modulated, and substituted by another one (6). Thus, rather than being in a direct causal relationship with mortality, IL-1ra should be considered as a prognostic marker in the very old population, and as part of the dynamic reshaping of the cytokine network in the state of “inflamm-aging” (6).

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