Cytokines, Insulin-Like Growth Factor 1, Sarcopenia, and Mortality in Very Old Community-Dwelling Men and Women: The Framingham Heart Study

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BACKGROUND: Aging is associated with increased production of catabolic cytokines, reduced circulating levels of insulin-like growth factor 1 (IGF-1), and acceleration of sarcopenia (loss of muscle with age). We hypothesized that these factors are independently linked to mortality in community-dwelling older persons.

METHODS: We examined the relation of all-cause mortality to peripheral blood mononuclear cell production of inflammatory cytokines (tumor necrosis factor α [TNF-α], interleukin 1β, interleukin 6), serum interleukin 6 and IGF-1, and fat-free mass and clinical status in 525 ambulatory, free-living participants in the Framingham Heart Study.

RESULTS: Of the 525 subjects (aged 72 to 92 years at baseline), 122 (23%) died during 4 years of follow-up. After adjusting for age, sex, comorbid conditions, smoking, and body mass index, mortality was associated with greater cellular production of TNF-α (hazard ratio [HR] = 1.27 per $\log_{10}$ difference in ng/mL; 95% confidence interval [CI]: 1.00 to 1.61; $P = 0.05$) and higher serum interleukin 6 levels (HR = 1.30 per $\log_{10}$ difference in pg/mL; 95% CI: 1.04 to 1.63]; $P = 0.02$), but not with higher serum IGF-1 levels (HR = 0.70 per $\log_{10}$ difference in pg/mL; 95% CI: 0.49 to 0.99; $P = 0.04$). In a subset of 398 subjects (55 deaths) in whom change in fat-free mass index during the first 2 years was measured, less loss of fat-free mass and greater IGF-1 levels were associated with reduced mortality during the next 2 years.

CONCLUSION: Greater levels or production of the catabolic cytokines TNF-α and interleukin 6 are associated with increased mortality in community-dwelling elderly adults, whereas IGF-1 levels had the opposite effect. **Am J Med. 2003; 115:429–435.** ©2003 by Excerpta Medica Inc.

Aging is accompanied by many physiological changes, among them increased production of interleukin 6, reduced production of growth hormone and insulin-like growth factor 1 (IGF-1), and loss of fat-free mass (sarcopenia) (1–3). These changes, which occur even in the absence of overt disease, suggest that a subclinical inflammatory process may be part of normal aging, under the control of the inflammatory cytokines, such as interleukin 1β, tumor necrosis factor α (TNF-α), and interleukin 6 (4). We previously found that community-dwelling elderly men and women have higher levels of plasma and peripheral blood mononuclear cell production of interleukin 6, a pleiotropic cytokine that has both pro- and anti-inflammatory effects (3). Serum interleukin 6 has been associated with disability in elderly humans in some (5) but not other (6) studies, and with mortality in women with cardiovascular disease (7,8).

In previous studies involving patients from the Framingham Heart study, we found that IGF-1 levels and muscle mass decreased, whereas interleukin 6 levels increased, with age (2,3,9). We hypothesized that elevated levels of catabolic cytokines are associated with increased mortality in the elderly, and that this association functions partly via accelerated sarcopenia. We now report the association of cytokine and IGF-1 levels, and change in fat-free mass, with 2- and 4-year mortality in commu-

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nity-dwelling participants in the Framingham Heart Study who were aged 72 to 92 years at baseline.

METHODS

Subjects

The Framingham Heart Study, a population-based cohort examined biennially since 1948, originally comprised 5209 men and women aged 30 to 62 years. During 1992 to 1994, 1166 participants were still alive and 940 attended the 22nd clinic examination. Analyses of cytokines and IGF-1 were carried out in a subsample of 780 participants using blood collected at this examination. The 525 persons whose vital status was known at Examinations 22 through 24, and on whom there were complete data on all cytokines and IGF-1, were included in the statistical analyses. In addition, change in fat-free mass index from Examination 22 to Examination 23 was calculated, and the association of this variable with death by Examination 24 was tested in 398 subjects who survived to Examination 23, had fat-free mass measured at Examinations 22 and 23, and had IGF-1 and cytokine measurements.

Measurement of Cytokines and IGF-1

Twenty mL of blood was drawn from participants and shipped via courier to the United States Department of Agriculture Human Nutrition Research Center on Aging at Tufts University. Peripheral blood mononuclear cells were separated by Ficoll-Hypaque centrifugation, washed, and cultured at a final concentration of 5×10^6 cells/mL in ultrafiltered, pyrogen-free RPMI 1640 that had been supplemented with 100 μg/mL of streptomycin and 100 U/mL of penicillin (Sigma, St. Louis, Missouri), with 1% autologous heat-inactivated serum and 1% L-glutamine (Gibco, Grand Island, New York), for 24 hours in 96-well flat-bottom plates (3). Serum was stored at −80°C until assayed (3). Plates were thawed and frozen three times to lyse the cells.

For measurement of IGF-1, serum was shipped on dry ice to Endocrine Sciences (Calabasas Hills, California) for three batched measurements. Levels were measured by radioimmunoassay after acid ethanol extraction, with an intra-assay coefficient of variation <4% (10). No binding proteins were measured. For interleukin 1β and TNF-α, total peripheral blood mononuclear cell production (secreted and cell associated) was measured in unstimulated cells (spontaneous production) and also after stimulation with either 1 or 100 ng/mL of lipopolysaccharide (Sigma) (3). Interleukin 1 receptor antagonist was measured in unstimulated cells and after stimulation with 1 ng/mL of lipopolysaccharide. Interleukin 6 was measured in unstimulated cells and after stimulation with 100 μg/mL of phytohemagglutinin. Peripheral blood mononuclear cell production of interleukin 1β, interleukin 1 receptor antagonist, and TNF-α was measured using radioimmunoassays (3). The interassay variability for samples was <10%, and the intra-assay variability was <5% for all cytokines. Measurement of total interleukin 6 synthesis was carried out in duplicate by specific, non–cross-reacting enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, Minnesota). The interassay variability was <10%, and the intra-assay variability was <5%. Circulating interleukin 6 levels were measured in serum samples that had been collected at the same time as the peripheral blood mononuclear cells, and assayed using a high-sensitivity interleukin 6 ELISA (R&D Systems). C-reactive protein was measured using an immunoprecipitation assay (Incstar, Stillwater, Minnesota) (3).

Body Composition

Weight was measured to the nearest quarter pound and converted to kilograms, and height was measured to the nearest quarter inch and converted to meters (11). Imperial units were converted to metric by computer. Fat-free mass was measured by single-frequency (50-Hz) tetrapolar bioelectrical impedance (RJL 101; RJL Systems, Mt. Clemens, Michigan) (11). Fat-free mass was calculated as the fat-free mass index: fat-free mass (in kg) divided by height (in m^2) (11).

Other Variables

Information for each variable was obtained from Examination 22. Fat-free mass was measured at Examinations 22 and 23. With the exception of blood pressure, all measures were self-reported using an interviewer-administered questionnaire. Variables were sex, age, marital status, current smoking status, and comorbidity, as assessed by the presence or absence of chronic disease from the examiners' clinical diagnosis. Comorbid conditions included cardiovascular disease (heart disease, stroke, transient ischemic attack), diabetes mellitus, dementia, Parkinson's disease, pulmonary diseases, cancer, degenerative joint disease, and rheumatoid arthritis. Hypertension was defined as blood pressure ≥160/95 mm Hg on two subsequent measurements or report of treatment for hypertension. Smoking status was categorized as current smoker, ever-smoker, or nonsmoker.

Statistical Analysis

Logarithmic transformations were applied to the cytokine and IGF-1 values, as they were positively skewed. Kaplan-Meier analyses were performed using the log-rank test to assess the effect of quartiles of each cytokine on mortality. We assessed the associations of cytokines and other variables with mortality using proportional hazards models. The proportional hazard was checked for each cytokine by including an interaction with time variable. Models were adjusted for variables known to affect mortality (sex, body mass index, smoking, being

Table 1. Characteristics of the Study Subjects at Baseline (Examination 22)*

Characteristic	Alive at Examination 24 (n = 403)	Died before Examination 24 (n = 122)	*P* Value
	Number (%) or Mean ± SD		
Male sex	139 (35)	63 (52)	0.0002
Age (years)	78.0 ± 4.1	79.9 ± 5.2	0.0001
Body mass index (kg/m^2)	26.9 ± 4.5	27.0 ± 5.2	0.81
Fat-free mass index (kg/m^2)	16.9 ± 2.2	17.3 ± 2.2	0.89
Change in fat-free mass index from Examination 22 to 23†	−0.20 ± 0.70	−0.62 ± 1.03	0.0001
Cardiovascular disease	27 (6)	26 (21)	0.0001
Diabetes	42 (10)	22 (18)	0.003
In bed most of the day	81 (20)	40 (33)	0.007
Arthritis	105 (26)	39 (32)	0.07
Log_{10} serum interleukin 6 (pg/mL)	1.56 ± 0.64	1.89 ± 0.83	0.0001
Log_{10} IGF-1 (mg/dL)	4.92 ± 0.44	4.77 ± 0.62	0.03
C-reactive protein >3 mg/L	50 (12)	21 (25)	0.005
Unstimulated peripheral blood mononuclear cell log_{10} (ng/mL)			
Interleukin 1β	1.12 ± 0.95	1.17 ± 1.01	0.59
Interleukin 1 receptor antagonist	2.34 ± 0.65	2.36 ± 0.57	0.92
Interleukin 6	1.15 ± 0.89	1.25 ± 0.92	0.45
TNF-α	1.30 ± 0.81	1.43 ± 0.80	0.07
Peripheral blood mononuclear cells stimulated with lipopolysaccaride, 1 ng/mL (log_{10} ng/mL)			
Interleukin 1β	1.84 ± 0.89	1.88 ± 0.92	0.68
Interleukin 1 receptor antagonist	2.68 ± 0.60	2.71 ± 0.69	0.69
TNF-α	1.95 ± 0.80	1.96 ± 0.78	0.78
Peripheral blood mononuclear cells stimulated with lipopolysaccaride, 100 ng/mL (log_{10} ng/mL)			
Interleukin 1β	2.29 ± 0.84	2.36 ± 0.87	0.49
TNF-α	2.38 ± 0.73	2.38 ± 0.78	0.90
Peripheral blood mononuclear cells stimulated with phytohemagglutinin (log_{10} ng/mL)			
Interleukin 6	1.98 ± 0.68	1.98 ± 0.75	0.80

* Only diseases with a frequency greater than 5% are listed.
† n = 343 for those who were alive after 4 years; n = 55 for those who died during the 4 years.
IGF-1 = insulin-like growth factor 1; TNF-α = tumor necrosis factor α.

bedridden, an elevated C-reactive protein level, arthritis, cardiovascular disease, and diabetes), along with each cytokine of interest. Because of potentially high correlations among the cytokines, each was analyzed separately, and only those that were associated with the outcome at $P < 0.10$ were retained for further analysis (12). The data for C-reactive protein were very skewed and were therefore treated as tertiles (undetectable, 1 to 2 mg/dL, ≥3 mg/dL). These ranges are somewhat grosser than in current practice because of the limitations of the assay available at the time of sample analysis. To calculate change in fat-free mass index, a subgroup analysis was performed in the 398 subjects in whom follow-up data on body composition were available at 2 years after the cytokine measures. All analyses were performed using SAS, version 8.2 (Statistical Analysis Systems, Cary, North Carolina).

RESULTS

Of 732 possible observations, complete data were available in 525 participants (Table 1). All subjects were living in the community and participated in Examination 22. Although subjects who were excluded and those who were included were similar regarding potential confounding variables and mortality, there were differences

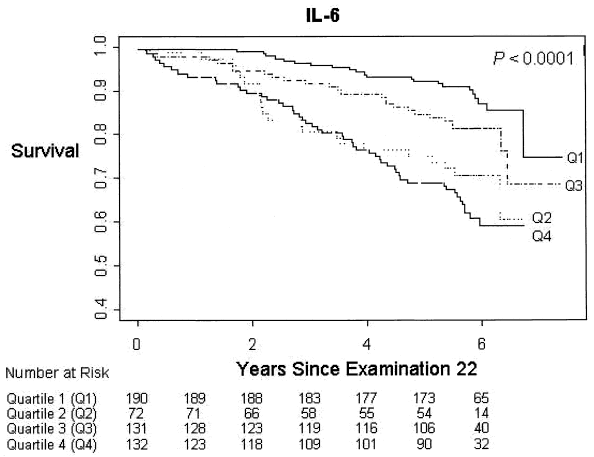

Figure 1. Survival curve for all-cause mortality by quartiles of serum interleukin 6 (IL-6).

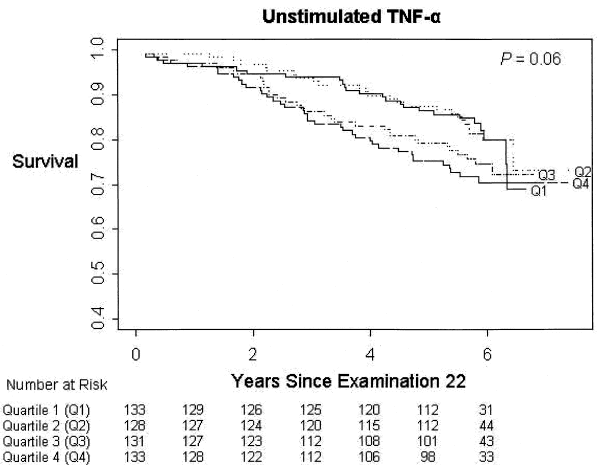

Figure 2. Survival curve for all-cause mortality by quartiles of cellular tumor necrosis factor α (TNF-α) production.

for log-stimulated TNF-α (using 1 ng/mL or 100 ng/mL of lipopolysaccharide) and log spontaneous interleukin 1 receptor antagonist. However, none of these variables were significant in subsequent analyses.

Of the 525 subjects, 122 died between Examinations 22 and 24, a period of 4 years. The leading causes of death were coronary heart disease (n = 25 deaths), stroke and other cardiovascular diseases (n = 30), and cancer (n = 22). As previously reported, correlations among peripheral blood mononuclear cell cytokines were high (interleukin 6 and interleukin 1β: $\rho = 0.69$, $P < 0.001$; interleukin 6 and TNF-α: $\rho = 0.78$, $P < 0.0001$), but there was little correlation between cellular interleukin 6 and interleukin 1 receptor antagonist production ($\rho = 0.07$, $P < 0.06$), or between serum IGF-1 and serum interleukin 6 ($\rho = -0.07$, $P < 0.10$). There was no association between serum IGF-1 and peripheral blood mononuclear cell TNF-α, or between serum interleukin 6 and peripheral blood mononuclear cell TNF-α.

In Kaplan-Meier analyses, serum interleukin 6 ($P < 0.0001$, Figure 1), spontaneous cellular TNF-α production ($P = 0.06$, Figure 2), and serum IGF-1 ($P = 0.04$, Figure 3) were associated with all-cause mortality. There were no associations between all-cause mortality and spontaneous cellular production of interleukin 1β, interleukin 1 receptor antagonist, or interleukin 6; or cellular production of any cytokine under stimulation with lipopolysaccharide (1 or 100 ng/mL) or phytohemagglutinin. These stimulated cell studies were used as an index of the ability to respond to a stress factor or infection. There also was no relation between survival and the ratio of production of interleukin 1β to interleukin 1 receptor antagonists.

After adjusting for potential confounders (staying in bed for most of the day, arthritis, cardiovascular disease, diabetes, elevated C-reactive protein level, and body mass index), cellular production of TNF-α, serum IGF-1, and serum interleukin 6 remained significantly associated with mortality at 4 years (Table 2). We also examined these variables in relation to deaths due to cancer versus all other causes, and found no differences in these associations after excluding subjects who died of cancer (n = 22).

In the subset of 398 subjects in whom change in fat-free mass index was available, 55 died during the 2 years following the second measurement of fat-free mass. After adjustment for age and sex, serum interleukin 6 (hazard ratio [HR] per $\log_{10}$ difference in pg/mL = 1.23; 95% confidence interval [CI]: 0.82 to 1.83; $P < 0.16$) and peripheral blood mononuclear cell TNF-α (HR per $\log_{10}$ difference in ng/mL = 1.5; 95% CI: 0.9 to 2.7; $P < 0.5$)

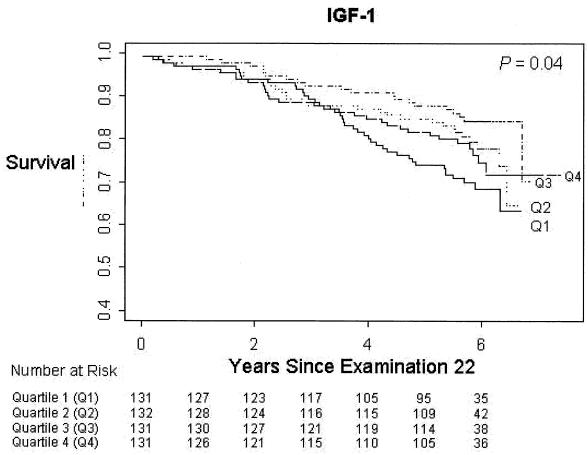

Figure 3. Survival curve for all-cause mortality by quartiles of serum insulin-like growth factor 1 (IGF-1).

Table 2. Associations between All-Cause Mortality and Serum Interleukin 6 and IGF-1 Levels, and Spontaneous Peripheral Blood Mononuclear Cell Production of TNF-α, at Examination 22 (n = 525)*

Cytokine	Hazard Ratio (95% Confidence Interval)	*P* Value
Log interleukin 6	1.30 (1.04–1.63)	0.02
Log IGF-1	0.70 (0.49–0.99)	0.04
Log peripheral blood mononuclear cell TNF-α	1.27 (1.00–1.61)	0.05

* Adjusted for age, sex, in bed most of day, arthritis, C-reactive protein level >3 mg/dL, cardiovascular disease, current smoking status, diabetes, and body mass index.
IGF-1 = insulin-like growth factor 1; TNF-α = tumor necrosis factor α.

were no longer significantly associated with mortality, although the point estimates of the associations were similar to those seen in the larger group. Interleukin 1β production, interleukin 1 receptor antagonist production, and the ratio of the two were not related to mortality. However, greater levels of IGF-1 were associated with reduced mortality (HR per $\log_{10}$ difference in pg/mL = 0.41; 95% CI: 0.24 to 0.70; $P < 0.0005$). Greater loss of fat-free mass was associated with increased mortality (HR per unit decline in fat-free mass [kg/m^2] = 1.9; 95% CI: 1.3 to 2.6; $P < 0.0001$). After adjustment for the same covariates as in the larger data set, these two factors were still associated with reduced mortality (both $P < 0.001$).

DISCUSSION

We hypothesized that subclinical inflammation, via higher levels of catabolic cytokines, especially interleukin 6, and lower levels of the anabolic factor IGF-1, would accelerate sarcopenia and mortality in the elderly. Our results indicate that cellular production of TNF-α, circulating interleukin 6, and circulating IGF-1 are associated with mortality in a cohort of community-dwelling elderly adults, after adjustment for important clinical conditions, body mass index, and smoking. Moreover, decline in fat-free mass (sarcopenia) was also associated with mortality, independently of the effects of the cytokines, suggesting that the effects of sarcopenia and cytokines, although they may share some mechanisms, also occur through independent pathways.

We chose the cytokines in this analysis based on their proven role as catabolic signals; a cross-sectional comparison with younger subjects in Framingham, which showed that interleukin 6 levels were increased in the elderly (3); a recent longitudinal analysis by Payette et al that showed that low IGF-1 and high interleukin 6 levels were associated with faster loss of fat-free mass index in the Framingham participants; and previous studies in which serum interleukin 6 was associated with disability and mortality in community-dwelling elderly adults (5,8,13). A substudy of the Women's Health and Aging Study found that interleukin 6 was associated with mortality in the presence of cardiovascular disease only (7). A similar result was recently reported in patients with unstable angina (14). In contrast, in extremely healthy elderly adults, interleukin 6 production may not differ from that in young persons (15). Hence, the cause of elevated interleukin 6 levels in the elderly remains unclear.

Overexpression of interleukin 6 in animals leads to muscle wasting and weight loss. In interleukin 6 knockout mice, body weight is normal but production of TNF-α, a much more catabolic cytokine, is increased (16–18). Serum interleukin 6 may represent a response to local TNF-α production because in experimental settings interleukin 6 reduces TNF-α production (17,19). We did not measure serum TNF-α because of sample limitations. Furthermore, we have found that the association between serum TNF-α and body composition is much weaker than between peripheral blood mononuclear cell TNF-α and body composition (12,20), largely because many subjects have undetectable serum TNF-α levels. There is evidence that interleukin 6 has anti-inflammatory as well as proinflammatory functions, so its effects on mortality in the elderly may be as a response to underlying inflammation, rather than as a cause (16).

We examined the effect of serum IGF-1 on survival because we hypothesized that this anabolic factor would be protective through its trophic effect on muscle, although the relation between serum and muscle IGF-1 is not necessarily strong, because there are two different forms of IGF-1 in the muscle and liver (21,22). Moreover, IGF-1 has been shown to be a negative acute phase protein, so its levels may fall in response to a person's cytokine status, and may also be affected by dietary intake and hepatic function (23,24). We found a weak inverse correlation between serum IGF-1 and serum interleukin 6, which supports this hypothesis. The results of this analysis, along with the decline in IGF-1 level with age, suggest that a decrease in IGF-1 level is a risk factor for mortality in the elderly.

We also examined the prognostic implication of change in fat-free mass over 2 years (between Examinations 22 and 23) on mortality between Examinations 23 and 24. Our hypothesis was that faster sarcopenia would be associated with higher cytokine levels and greater mortality. This analysis excluded all subjects who died between Examinations 22 and 23, reducing the sample size to 398 and the number of deaths to 55. The effects of cellular TNF-α and serum interleukin 6 (measured at Examination 22) were no longer significant in this subset, although the point estimates were similar to the larger

data set. However, greater IGF-1 levels (measured at Examination 22) and less loss of fat-free mass were associated with reduced mortality during the next 2 years. The lack of association with interleukin 6 or TNF-α could be due to lack of statistical power, or perhaps the biological effect of cytokine status could be more important over a short time. Because the point estimates for the associations between the cytokines and mortality did not vary much in the subset as compared with in the full data set, lack of power is a likely explanation.

Several limitations of this study must be considered. First, the participants were mostly white, elderly (mean age, 78 years), and mildly obese (mean body mass index, 26.9 kg/m^2). Second, we did not sample blood from participants who were institutionalized or unable to go to the study clinic, but this would have biased the study results toward the null, because it is likely that these subjects would have higher cytokine levels and higher mortality. Third, we measured body composition by bioelectrical impedance, which has limited precision and accuracy but is easy to perform in hundreds of persons and is relatively free of observer bias (25). However, imprecision would only have reduced the significance of change in fat-free mass in the statistical models. Fourth, because blood was transported from Framingham to downtown Boston, some activation of cytokine production occurred, probably because of shaking of white blood cells and platelets in the tubes (3). Thus, cytokine levels were somewhat higher than those collected directly in our laboratory (26). However, nonspecific activation of cytokine production would also bias our results toward the null, as the background variability of cytokine production would be expected to increase. Finally, we were not able to assess muscle IGF-1 directly, nor was there sufficient serum to measure the various IGF-1 binding proteins, which could have affected the assessment of IGF-1 function.

Our results support the hypothesis that aging is associated with subclinical inflammation, and that this inflammation affects mortality in the elderly via increased interleukin 6 and reduced IGF-1 levels, and perhaps also the development of sarcopenia near the end of life in ambulatory elderly adults. Further research is warranted to understand how the immune system modulates the aging process if we are to prevent or reverse unwanted immune activation and hypercytokinemia. It is not known whether dietary factors, such as higher antioxidant or protein intake, or lifestyle factors, such as avoidance of obesity, smoking, or a sedentary lifestyle, would reduce cytokine production or slow sarcopenia in the elderly, or indeed if such a reduction would improve survival.

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