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Vascular cell adhesion molecule 1, soluble Fas and hepatocyte growth factor as predictors of mortality in nonagenarians: The Vitality 90 + study

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ABSTRACT

Background: Ageing is characteristically accompanied by changes in vascular endothelial markers and growth factor as well as increased cellular death. We analysed the associations of the plasma levels of vascular cell adhesion molecule-1 (VCAM-1), hepatocyte growth factor (HGF) and soluble Fas (sFAS), and their combinations, with 4-year mortality to identify new biomarkers.
Methods: A total of 238 individuals, both community-dwelling and institutionalised, aged 89 91 years and participating in the Vitality 90+ study were included. Biomarkers of endothelial function (VCAM-1), growth factor (HGF) and a marker of apoptosis (sFAS) were determined from plasma using Luminex® technology. This newly-determined data was combined with earlier data, e.g., 4-year mortality and medical history.
Results: Subjects who died during the follow-up had higher baseline plasma levels of VCAM-1, sFas, and HGF. When other known risk factors were adjusted for, subjects in the highest concentration tertile for VCAM-1 (HR 1.85; 95% CI, 1.12–3.05) and HGF (HR 2.22; 95% CI, 1.33–3.71) had higher mortality compared to those in the lowest tertile. In the adjusted analyses, when compared to subjects with none of the biomarkers in the highest concentration tertile, mortality was also higher when sFas and VCAM-1 were simultaneously (HR 2.03; 95% CI, 1.13–3.64) or all three were simultaneously (HR 3.63; 95% CI, 1.65–7.97) in the highest concentration tertile.
Conclusions: Our results suggest that increased concentrations of these biomarkers, separately and in combination, associate with mortality among the aged and are prognostic markers of death.

1. Introduction

Ageing is a complex process connected with inflammation and degenerative processes. There are known risk factors that predict mortality among nonagenarians — for example, living in residential and nursing homes as well as having dementia and disability (Meller et al., 1999; Nybo et al., 2003). There are also biomarkers eligible for predicting mortality, such as the apoliporotein E genotype (Rontu et al., 2006), and some inflammatory markers, such as the interleukin (IL) IL-1Ra (Jylha et al., 2007) and C reactive protein (CRP) (Hurme et al., 2007) in nonagenarians.

In our study the levels of multiple cytokines were determined using multiplex assay kits. In order to analyse the data we first used a stepwise Cox regression model to identify cytokines with significant associations with mortality. Additional Cox proportional hazards models were performed individually for the cytokines indicated by the stepwise model and only vascular cell adhesion molecule-1 (VCAM-1), soluble Fas (sFas) and hepatocyte growth factor (HGF) showed an association with mortality. These three markers were then selected for further analyses. IL-6 and tumour necrosis factor-alpha (TNF-α) were also included in the assay kits but the results on their association with mortality have been published previously and were therefore not included in this study (Jylha et al., 2007 and Lisko et al., 2012).

According to Statistics Finland, the leading cause of death in nonagenarians is cardiovascular disease. VCAM-1, sFas and HGF have previously

Abbreviations: (BMI), body mass index; (CRP), C-reactive protein; (HGF), hepatocyte growth factor; (hs-CRP), high-sensitivity C-reactive protein; (IL-1Ra), interleukin 1 receptor antagonist; (sFas), soluble Fas; (TNF-α), tumour necrosis factor-alpha; (VCAM-1), vascular cell adhesion molecule 1.

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been connected with generalised degeneration of the cardiovascular system. Higher levels of VCAM-1 have been connected to low-grade inflammation in atherosclerosis (Davies et al., 1993) and found to have an association with higher mortality from cardiovascular causes among coronary artery disease patients (Blankenberg et al., 2001). There is evidence suggesting that elevated serum levels of sFas are connected with cardiovascular disease (Nishigaki et al., 1997; Ohtsuka et al., 1999; Toyozaki et al., 1998). Cardiovascular conditions with poor prognoses have been associated with elevated serum levels of HGF (Lamblin et al., 2005). Although these biomarkers and their association with cardiovascular disease and mortality have been widely investigated, studies focusing on nonagenarians are scarce. Only the association of VCAM-1 with mortality in nonagenarians has been previously established (Huffman et al., 2011). We wanted to explore whether or not these three biomarkers also have an independent connection to all-cause mortality in nonagenarians.

This is the first longitudinal study in which the association between mortality and the serum levels of these three markers, namely VCAM-1, sFas and HGF, as well as their combinations, are studied in nonagenarians. Our study brings new insight into the mechanisms behind the mortality of the very old. As life-expectancy keeps on rising, the amount of the elderly, especially in developed countries, keeps on growing. This results in a shift in the population structure of multiple countries (Ezeh et al., 2012). It is of economic and public health interest to seek new ways to keep the elderly healthy and keep up their quality of life. Although there is little clinical use for these cytokines at the moment, they might be used to guide clinical decision-making in the future.

2. Materials and methods

2.1. Participants and study design

This study is part of the Vitality 90+, a prospective multidisciplinary population-based study of people aged 90 years or older in the City of Tampere in southern Finland (Jylha and Hervonen, 1999). In this substudy, 535 residents of Tampere who were born between 1909 and 1910 were recruited from the local population register in January 2000. Both non-institutionalised and institutionalised individuals were included. During home visits, the participants were interviewed by study nurses, questionnaires were filled in with the nurses' assistance, and blood samples were taken. The medical history of each participant was collected from records maintained by health care centres. According to the Population Register Centre, 66 of the individuals originally enrolled had died before the beginning of data collection, leaving 469 persons eligible for the study. An additional 42 persons died during the study before they were examined, while 86 persons refused to participate, mostly due to poor physical or mental condition, and seven could not be contacted. Another 45 persons refused blood tests and only took part in the interviews. Data was missing for 26 participants and, therefore, the final sample for the present study consisted of 263 participants who agreed to have blood tests taken and most of whom (90%; n = 238) were also interviewed at home. A more detailed description of the study protocol can be found elsewhere (Jylha et al., 2007).

The study protocol was approved by the Ethics Committee of the Pirkanmaa Hospital District and the Ethics Committee of Tampere Health Centre, and written informed consent was obtained from all participants.

2.2. Biochemical analyses

The obtained fasting plasma samples were stored at −70 °C until biochemical analysis. The levels of total cholesterol, HDL and hs-CRP were determined using a Cobas Integra 700 automatic analyser with reagents and calibrators as recommended by the manufacturer (Hoffmann-La Roche Ltd., Basel, Switzerland).

The levels of VCAM-1, ICAM-1, sFas, sFasL, MIF, tPAI-1, IL-1, IL-6, IL-8, TNF-α, MCP-1, NGF, insulin, HGF and leptin were determined using the commercially available multiplex assay kits Human sepsis/apoptosis Milliplex kit #HSEP-63K and Human Serum Adiokine Panel B #HADK-61K-B according to the manufacturer's instructions (Millipore, United States) by using the Bio-Plex system (Bio-Plex 200, Bio-Rad Laboratories Inc., CA 94547, United States). Data was handled with Bio-Plex Manager software (Bio-Plex Manager Software 4.1, Bio-Rad Laboratories Inc., United States). For the cytokines selected for the final analyses the median CV% between runs was 3.3 6.8 for HGF, 2.5 4.8 for VCAM-1, and 3.1 5.0 for sFas. IL-1 and sFasL were left out of this study due to quality control issues. Control samples included in the kits were used as quality controls.

2.3. Clinical parameters

Medical diagnoses were available for 238 participants. They were collected from records maintained by public health care physicians, including diagnoses made in hospitals, and were coded according to the 10th Revision of the International Classification of Diseases (ICD-10) (Goebeler et al., 2003). Total cardiovascular morbidity (ICD codes I00-I99) included hypertension, coronary heart disease, chronic heart failure, myocardial infarction and atrial fibrillation. Infections were recorded and the infectious disease variable was determined to indicate a history of any infectious disease treated in a hospital, including gastroenteritis, erysipelas, hepatitis, pneumonia, urinary tract infections and other infections. This variable, therefore, measures infections at the time of the baseline examination as well as previous infections if severe enough to require hospitalisation. The other included diseases were diabetes, cancer (other than basal cell carcinoma) and dementia. In all examinations, body mass index (BMI) was calculated with the formula BMI = weight (kg) / height $(m)^2$. Other covariates included hs-CRP, high density lipoprotein (HDL) and housing (institution vs. home).

2.4. Mortality follow-up

Dates of death were drawn from the Population Register Centre. There were no losses to mortality follow-up. The follow-up time was calculated from 21 February 2000 to the date of death or to 21 February 2004 for survivors.

2.5. Statistical analysis

All statistical analyses were performed with PASW Statistics 18.0. We set out to find cytokines associated with mortality by banding all cytokines into tertiles and using a backward stepwise Cox regression model to identify cytokines that had a significant effect on mortality. The cytokines VCAM-1, sFas, HGF, NGF, ICAM-1 and IL-8 remained in the final step of the model. Cox proportional hazard models were used to analyse these markers' associations with mortality individually and in this analysis only VCAM-1, sFas and HGF showed a statistically significant association and were selected for further analysis (data not shown).

Plasma levels of VCAM-1, sFas and HGF were not normally distributed. Therefore, the variables are reported as medians and interquartile ranges (q1 q3) or as percentages, and the values of the biomarkers were divided into tertiles. The values of each marker were banded into tertiles I III as follows: <437 (I), 437–571 (II) and >571 ng/mL (III) for VCAM-1; <6.94 (I), 6.94–8.77 (II) and >8.77 (III) ng/mL for sFas; and <1.05 (I), 1.05–1.97 (II) and >1.97 (III) ng/mL for HGF.

In addition, a variable with different combinations of these markers was devised. In this variable, the first category consists of (i) participants whose values were below the highest tertile in all of the three markers. The other categories (ii) the combination of both sFas and HGF in the highest tertile, (iii) sFas and VCAM-1 in the highest tertile,