VERISUONISUONISOLUJEN ADHEESIOMOLEKYYLI 1 (VCAM-1), LIUKOINEN FAS (sFAS) JA MAKSASOLUJEN KASVUTEKIJÄ (HGF) YLI 90-VUOTIAIDEN KUOLLEISUUDEN ENNUSTAJINA

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SUOMENKIELINEN TIIVISTELMÄ

Verisuonisolujen adheesiomolekyyli 1 (VCAM-1), liukoinen Fas (sFas) ja maksasolujen kasvutekijä (HGF) yli 90-vuotiaiden kuolleisuuden ennustajina

TAUSTA JA TAVOITTEET: Lisääntynyt solukuolema ja muutokset verisuonien endoteelin merkkiaineiden ja kasvutekijöiden esiintymisessä elimistössä liittyy tyypillisesti ikääntymiseen. Vascular cell adhesion molecule (VCAM-1), hepatocyte growth factor (HGF) ja soluble Fas (sFas) osallistuvat kudosten kasvun, apoptoosin ja tulehdusreaktioiden säätelyyn monissa kudoksissa. Ne on yhdistetty mm. sydän-ja verisuonisairauksiin ja maligniteetteihin. Me analysoimme VCAM-1-, HGF- ja sFas-tasojen assosiaatioita yli 90-vuotiaiden kuolleisuuteen. Tarkoituksena oli tunnistaa uusia merkkiaineita iäkkäiden kuolleisuuden ennustamiseksi.

MENETELMÄT: Yhteensä 238:lta Tervaskanto 90+ tutkimukseen osallistuneelta henkilöltä määritettiin merkkiaineiden pitoisuudet plasmasta Luminex®-teknologialla. Merkkiainepitoisuusmääritykset yhdistettiin aiemmin kerättyihin tietoihin sairaushistoriasta ja kuolleisuudesta tutkimuksen nelivuotisen seuranta-ajan sisällä. Coxin suhteellisen vaaran mallia käytettiin näiden merkkiaineiden ja kuolleisuuden välisten assosiaatioiden analysoimiseksi.

TULOKSET: Seuranta-aikana menehtyneiden henkilöiden VCAM-1, sFas ja HGF plasmapitoisuudet olivat suurentuneet lähtötilanteessa. Analyyseissa, joissa useita muita tunnettuja riskitekijöitä otettiin huomioon, nähtiin, että kuolleisuus oli suurempi henkilöillä, joilla VCAM-1 (HR 1.85; 95% CI, 1.12-3.05) tai HGF (HR 2.22; 95% CI, 1.33-3.71) pitoisuudet olivat tutkimuspopulaation korkeimmassa pitoisuustertiilissä, kun heitä verrattiin matalimmassa pitoisuustertiilissä oleviin henkilöihin. Henkilöillä, joilla kaikki kolme merkkiainepitoisuutta olivat korkeimmassa tertiilissä, oli suurempi kuolleisuus, kuin niillä, joilla yhdenkään merkkiaineen pitoisuus ei yltänyt korkeimpaan tertiiliin (HR 3.63; 95% CI, 1.65-7.97).

PÄÄTELMÄT: Tutkittujen merkkiaineiden plasmapitoisuuksien nousu erillisenä ja yhdistelminä on yhteydessä hyvin iäkkäiden kuolleisuuteen.

SISÄLLYSLUETTELO

Avainsanat: vanheneminen, tervaskanto, merkkiaine, sydän- ja verisuonisairaus.

ABSTRACT

BACKGROUND: Aging 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

Aging 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 dementia and disability (Meller ym. 1999, Nybo ym. 2003). There are also biomarkers eligible for predicting mortality, such as the apoliporotein E genotype (Rontu ym. 2006), and some inflammatory markers, such as the interleukin (IL) IL-1Ra (Jylha ym. 2007) and C reactive protein (CRP) (Hurme ym. 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 tumor 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 ym. 2007) and (Lisko ym. 2012).

According to Statistics Finland, the leading cause of death in nonagenarians is cardiovascular disease. VCAM-1, sFas and HGF have previously been connected with generalised degeneration of the cardiovascular system. Higher levels of VCAM-1 have been connected to low-grade inflammation in atherosclerosis (Davies ym. 1993) and found to have an association with higher mortality from cardiovascular causes among coronary artery disease patients (Blankenberg ym. 2001). There is evidence suggesting that elevated serum levels of sFas are connected with cardiovascular disease (Nishigaki ym. 1997, Ohtsuka ym. 1999, Toyozaki ym. 1998). Cardiovascular conditions with poor prognoses have been associated with elevated serum levels of HGF (Lamblin ym. 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 ym. 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 ym. 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 ja Hervonen 1999). In this sub-study, 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 ym. 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 ym. 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, (iv) VCAM-1 and HGF in the highest tertile and (v) all three in the highest tertile – were each compared with category (i).

Differences between the groups were analysed using Pearson's chi-square test for categorised variables and both the Mann–Whitney U test and Kruskal Wallis test for medians. Spearman's correlation was used to analyse correlation between the plasma levels of VCAM-1, sFas and HGF.

Cox proportional hazards models were used to analyse associations between the markers and all-cause mortality over the 4-year follow-up. The analyses were made with no adjustments (i); with adjustment for sex, age and living at home or in an institution (ii); and with adjustment for hs-CRP, BMI, total cholesterol, HDL, cardiovascular disease, dementia, cancer, history of infectious disease and diabetes (iii). A two-tailed p value of <0.05 was considered statistically significant.

3 RESULTS

3.1 Characteristics of the Vitality 90+ Study sample

The characteristics of the study population according to survival status after 4-year follow-up are shown in Table 1. During the 4-year follow-up, 148 (56%) participants died. Mortality was higher among men, institutionalised participants and those with a lower BMI or higher hs-CRP. Levels of VCAM-1, sFas and HGF were significantly higher in those participants who had died as compared to those who were still alive at follow-up. It was more common for deceased participants to have all three markers in the highest tertile and on the other hand survivors were more likely to have none of the three markers in the highest tertile.

Table 1. Characteristics of the study population according to survival status after 4-year follow-up.

Characteristics	Survival status: Survivors (n = 115)	Deceased (n = 148)	p-value
Sex* (n = 263)			
Men, % (n = 65)	16.5	31.1	
Women, % (n = 198)	83.5	68.9	0.01
Live in community*, % (n = 263)	94.8	87.2	0.04
BMI, kg/m² (n = 236)			
Median	25	23	0.03
q1-q3	22–27	22–26	
hs-CRP, mg/L (n = 263)			
Median	1.30	2.25	< 0.01
q1-q3	0.40–3.50	0.60–5.68	
Cholesterol, mmol/L (n = 263)			
Median	5.56	5.39	0.24
q1-q3	4.92–6.38	4.77–6.12	
HDL, mmol/L (n = 263)			
Median	1.43	1.35	0.12
q1-q3	1.12–1.71	1.11–1.63	
VCAM-1, ng/mL (n = 263)			
Median	456	541	< 0.01
q1-q3	403–547	438–680	
sFas, ng/mL (n = 263)			
Median	7.40	7.94	0.01
q1-q3	6.38–8.71	6.74–10.13	
HGF, ng/mL (n = 263)			
Median	1.37	1.65	0.04
q1-q3	0.76–2.04	0.983–2.49	
Combi*, % (n = 263)			
None in highest tertile	48.7	25.7	<0.01
Anyone in highest tertile	32.2	36.5	0.47
Any two in highest tertile	16.5	28.4	0.02
All three in highest tertile	2.6	9.5	<0.01

Statistics: p-values calculated with the Mann Whitney or chi-square test (*).
Abbreviations: BMI=body mass index, CRP=C-reactive protein, HDL=high-density lipoprotein, VCAM-1=vascular cell adhesion molecule 1, sFas=soluble Fas, HGF=hepatocyte growth factor, combi=combination of markers.

In Spearman's correlation, VCAM-1 correlated positively with sFas (r =0.16, p = 0.01) and HGF (r =0.16, p = 0.01). There was also a positive but not statistically significant correlation between sFas and HGF (r=0.11, p = 0.07).

Table 2 shows the characteristics of the study population according to the tertile of the markers VCAM-1, sFas and HGF. Higher VCAM-1 was associated with lower total cholesterol. Cardiovascular disease was more common in participants in the highest VCAM-1 tertile than in those falling in the lowest or middle tertile. The highest and lowest tertiles of sFas had a stronger association with the prevalence of cardiovascular disease than the middle tertile. Furthermore, cardiovascular morbidity was higher in the highest and middle HGF tertile than in the lowest tertile. None of the three markers were associated with sex, BMI, hs-CRP, HDL, diabetes, infectious diseases, cancer, dementia or age.

Table 2. Characteristics of study population according to the tertile of the markers

	VCAM-1 (n=263)				sFas (n=263)				HGF (n=263)			
Characteristics	Lowest	Middle	Highest	p value	Lowest	Middle	Highest	p value	Lowest	Middle	Highest	p value
Sex*, men %	23.0	30.7	20.5	0.26, n=263	25.0	23.0	26.1	0.89, n=263	19.5	25.0	29.5	0.31, n=263
Live in community*, %	88.5	93.2	89.8	0.55, n=263	86.4	92.0	93.2	0.26, n=263	85.1	93.2	93.2	0.11, n=263
BMI kg/m², median	24.0	25.0	23.0	0.12, n=236	23.0	24.0	25.0	0.20, n=236	24.0	24.0	24.0	0.89, n=236
hs-CRP, mg/L, median	1.8	1.4	2.0	0.63, n=263	1.4	1.4	2.3	0.17, n=263	1.6	1.4	2.2	0.47, n=263
Cholesterol, mmol/L, median	6.0	5.4	5.2	<0.01, n=263	5.3	5.6	5.6	0.62, n=263	5.3	5.5	5.6	0.77, n=263
HDL, mmol/L, median	1.4	1.3	1.4	0.45, n=263	1.4	1.5	1.4	0.19, n=263	1.3	1.4	1.4	0.60, n=263
Cardiovascular disease*, %	71.6	74.7	90.2	0.01, n=238	83.8	69.1	84.4	0.03, n=238	69.1	80.8	87.3	0.02, n=238
Diabetes*, %	7.4	6.7	15.9	0.10, n=238	10.0	4.9	15.6	0.09, n=238	13.6	3.8	12.7	0.08, n=238
Infectious diseases*, %	34.6	49.3	50.0	0.08, n=238	41.3	39.5	53.2	0.17, n=238	46.9	34.6	51.9	0.08, n=238
Cancer*, %	15.0	13.5	9.9	0.61, n=235	11.5	9.9	17.1	0.37, n=235	13.6	14.1	10.5	0.77, n=235
Dementia*, %	13.6	14.7	13.4	0.97, n=238	18.8	12.3	10.4	0.28, n=238	18.5	12.8	10.1	0.29, n=238
Age, years, median	90.0	89.9	90.0	0.67, n=263	89.9	90.0	90.0	0.07, n=263	89.9	90.1	89.9	0.22, n=263

Statistics: p-values calculated with the Kruskal-Wallis or chi-square test (*).

Abbreviations: VCAM-1=vascular cell adhesion molecule 1, sFas=soluble Fas, HGF=hepatocyte growth factor, BMI=body mass index, hs-CRP=high-sensitivity C-reactive protein, HDL=high-density lipoprotein.

3.2 Cox proportional hazards models

Table 3 demonstrates the associations of the tertiles with all-cause mortality during the 4-year follow up. In the Cox proportional hazards model with no adjustments, the highest tertiles of all three markers, VCAM-1, sFas and HGF, were associated with higher mortality. The combination of sFas and HGF in the highest tertile was not associated with higher mortality. The combination of VCAM-

1 and sFas in the highest tertile resulted in higher hazard ratios as did the combination of VCAM-1 and HGF. If all three markers fell into the highest tertile, mortality was more likely.

After adjustment for sex, age and home/institutional dwelling, the results remained unchanged. Even after further adjustment for hs-CRP, BMI, total cholesterol, HDL, cardiovascular disease, dementia, cancer, history of infectious disease and diabetes, the associations were only slightly altered. The highest tertiles of VCAM-1, HGF and both the combination of VCAM-1 and sFas as well as the combination of all three values in the highest tertiles were still associated with higher mortality. Statistical significance for the association obetween the combination of VCAM-1 and HGF in the highest tertile and mortality was lost after the adjustment. After the adjustments, the data indicates that there is also an association between the highest tertile of sFas and mortality – however, the results were statistically borderline (p=0.06). When HGF and sFas were further adjusted for VCAM-1, the association of the highest tertile of HGF and mortality remained (HR 2.01; 95% CI, 1.25-3.54, $p < 0.01$). sFas did not show a statistically significant association with mortality after the adjustment for VCAM-1. When VCAM-1 was adjusted for TNF-α, the results did not differ from those found earlier (data not shown).

To determine whether the studied markers are also long-term indicators of mortality, deaths that occurred within 1 year of baseline data collection (n = 30) were excluded from the analysis. The results remained similar to the main analyses: the highest tertile of VCAM-1 (HR 2.20; 95% CI, 1.40–3.46, $p < 0.01$), the combination of HGF and VCAM-1 (HR 1.82; 95% CI, 1.01–3.27, $p = 0.05$), the combination of VCAM-1 and sFas (HR 1.97; 95% CI, 1.21-3.23, $p = 0.01$) and the combination of all three markers in the highest tertile (HR 2.56; 95% CI, 1.22–5.37, $p = 0.01$) were still associated with higher mortality. The combination of HGF and sFas in the highest tertile did not have an effect on mortality (HR 1.02; 95% CI, 0.47–2.21, $p = 0.96$). The trends were similar in the highest tertiles of sFas (HR 1.34; 95% CI, 0.87–2.06, $p = 0.18$) and HGF (HR 1.43; 95% CI, 0.92–2.21, $p = 0.11$), but they were not statistically significant.

Additionally, we analysed men and women separately with no adjustments. In the small subpopulation of men, only the highest sFas tertile was associated with higher mortality (n = 65, HR 2.49; 95% CI, 1.23–5.04, $p = 0.01$). Although statistical significance was not found, there seemed to be a trend of higher mortality among participants in the highest tertiles of VCAM-1 and HGF as well as among the participants who had the combination of sFas and HGF in the highest tertile or all three markers in the highest tertile. In women (n = 198), the middle (HR 1.79; 95% CI, 1.03–3.10, $p = 0.04$) and highest (HR 3.15; 95% CI, 1.90–5.20, $p < 0.01$) tertiles of VCAM-1 as well as having the

combination of HGF and VCAM-1 (HR 2.42; 95% CI, 1.33-4.40, $p < 0.01$), the combination of sFas and VCAM-1 (HR 2.59; 95% CI, 1.59–4.22, $p < 0.01$) or all three markers (HR 3.29; 95% CI, 1.58–6.87, $p < 0.01$) in the highest tertile were associated with higher mortality. The highest tertiles of HGF and sFas also seemed to be associated with higher mortality, but statistical significance was not achieved.

Table 3. Associations of tertiles with all-cause mortality during 4-year follow-up.

						Adjusted for					
						Sex, age, home/institution			Sex, age, home/institution, hs-CRP, BMI, cholesterol, HDL, CVD, dementia, cancer, infections, diabetes		
		Deceased (%)	HR	95% CI	p	HR	95% CI	p	HR	95% CI	p
VCAM-1 (ng/ml)	<437	41.4	1	(n = 263)		1	(n = 263)		1	(n = 208)	
	437–571	54.5	1.53	1.00–2.36	0.05	1.48	0.96–2.30	0.08	1.25	0.74–2.10	0.41
	>571	72.7	2.37	1.57–3.57	<0.01	2.35	1.56–3.54	<0.01	1.85	1.12–3.05	0.02
sFas (ng/ml)	<6.94	51.1	1	(n = 263)		1	(n = 263)		1	(n = 208)	
	6.94–8.77	49.4	0.95	0.62–1.44	0.80	1.01	0.66–1.54	0.97	1.06	0.65–1.73	0.82
	>8.77	68.2	1.58	1.08–2.33	0.02	1.70	1.15–2.53	0.01	1.59	0.98–2.61	0.06
HGF (ng/ml)	<1.047	48.3	1	(n = 263)		1	(n = 263)		1	(n = 208)	
	1.047–1.973	56.8	1.33	0.89–2.01	0.17	1.38	0.91–2.09	0.13	1.40	0.84–2.34	0.20
	>1.973	63.6	1.58	1.06–2.36	0.03	1.65	1.10–2.48	0.02	2.22	1.33–3.71	<0.01
Combi	None in highest tertile	40.4	1	(n = 263)		1	(n = 263)		1	(n = 212)	
	sFas and HGF in highest tertile	52.6	1.21	0.63-2.32	0.57	1.34	0.70-2.59	0.38	1.94	0.92-4.10	0.08
	VCAM-1 and sFas in highest tertile	81.1	2.34	1.55-3.53	<0.01	2.24	1.48-3.39	<0.01	2.03	1.13-3.64	0.02
	VCAM-1 and HGF in highest tertile	72.7	1.84	1.06-3.07	0.03	1.78	1.04-3.05	0.04	1.74	0.92-3.28	0.09
	All three in highest tertile	82.4	3.09	1.67–5.71	<0.01	3.16	1.71–5.85	<0.01	3.63	1.65–7.97	<0.01

Statistics: Cox proportional hazard models.
Abbreviations: HR=hazard ratio, CI=confidence interval, hs-CRP=high-sensitivity C-reactive protein, BMI=body-mass index, HDL=high-density lipoprotein, n=number of subjects, CVD=cardiovascular disease, VCAM-1=vascular cell adhesion molecule 1, sFas=soluble Fas, HGF=hepatocyte growth factor, Combi=combination of markers.

As cardiovascular disease is one of the leading causes of death among the aged, we carried out separate analyses for different subgroups as shown in Table 4. Mortality among those with a known

history of cardiovascular disease (n=188) associated with the highest VCAM-1 tertile. Among those with no known history of cardiovascular disease, we found no statistically significant correlation between any of the markers and mortality. However, the size of the population is fairly small (n=50). In the community-dwelling group (n=238), higher mortality was associated with the highest VCAM-1, sFas and HGF tertiles. In the very small subgroup of institutionalised participants (n = 25), the highest HGF tertile was associated with mortality. We were not able to carry out these separate analyses using the different combinations of the three markers due to the very small subgroups.

Table 4. Associations of different subgroups with all-cause mortality during follow-up.

		CVD (n=188)			non-CVD (n=50)			Home (n=238)			Institution (n=25)		
		HR	95% CI	p	HR	95% CI	p	HR	95% CI	p	HR	95% CI	p
VCAM-1	middle tertile vs lowest	1.38	0.83–2.28	0.22	1.09	0.40–3.01	0.87	1.52	0.95–2.43	0.08	2.82	0.85–9.41	0.09
	highest tertile vs lowest	1.95	1.24–3.09	<0.01	1.67	0.50–5.56	0.40	2.51	1.61–3.92	<0.01	1.70	0.57–5.06	0.34
sFas	middle tertile vs lowest	1.03	0.64–1.64	0.91	0.89	0.26–3.04	0.85	0.94	0.60–1.48	0.79	1.60	0.55–4.67	0.39
	highest tertile vs lowest	1.38	0.90–2.14	0.14	2.66	0.80–8.86	0.11	1.71	1.12–2.59	0.01	0.93	0.30–2.85	0.90
HGF	middle tertile vs lowest	1.20	0.74–1.93	0.47	1.18	0.42–3.32	0.75	1.44	0.92–2.25	0.11	1.24	0.38–4.03	0.72
	highest tertile vs lowest	1.55	0.98–2.45	0.06	1.31	0.40–4.25	0.66	1.62	1.04–2.51	0.03	2.97	1.04–8.48	0.04

Statistics: Cox proportional hazards models.
Abbreviations: CVD=cardiovascular disease, n=number of subjects, HR=hazard ratio, CI=confidence interval, VCAM-1=vascular cell adhesion molecule 1, sFas=soluble Fas, HGF=hepatocyte growth factor.

4 DISCUSSION

We found that the baseline concentrations of all three markers were significantly higher among those who had died during the 4-year follow-up as compared to survivors. In fact, VCAM-1 and HGF had a strong association with mortality even after adjustment for the major risk factors (e.g., sex, CVD, cancer, dementia and institutionalisation) and high levels of sFas seemed to slightly amplify the association of VCAM-1 and mortality. A combination of elevated levels of all three markers also indicated greater mortality.

The studied markers had only few associations with other known risk factors of cardiovascular disease. Although it has been previously shown that C-reactive protein causes the induction of VCAM-1 we did not find an association between hs-CRP levels and VCAM-1 levels (Kawanami ym. 2006). Low VCAM-1 levels were associated with higher total cholesterol levels but HDL levels were not affected by VCAM-1 levels. VCAM-1 levels did not show an association with diabetes or BMI. sFas and HGF levels did not show any association with BMI, hs-CRP levels, total cholesterol, HDL or diabetes. This seems to supports our hypothesis of the markers being independent predictors of cardiovascular disease related mortality in nonagenarians.

VCAM-1 mediates leukocyte cell adhesion and signal transduction on the endothelium (Cook-Mills ym. 2011). Higher levels of VCAM-1 have been connected to low-grade inflammation in atherosclerosis, and VCAM-1 expression is upregulated in atherosclerotic plaques (Davies ym. 1993). Therefore, increased plasma levels of VCAM-1 may indicate an early event in the pathogenesis of atherosclerosis and alternation in the functional state of endothelial cells. Higher serum levels of VCAM-1 have been found to be significantly related to future death from cardiovascular causes among coronary artery disease patients (Blankenberg ym. 2001). Some evidence also suggests that increased serum levels of VCAM-1 are associated with cardiovascular disease in a previously healthy population (Schmidt ym. 2009). However, contrary results regarding the previously healthy population have also been published (Malik ym. 2001). We found that participants with higher plasma levels of VCAM-1 did, indeed, have more cardiovascular disease than did those with lower levels at baseline. High levels of VCAM-1 were associated with higher mortality. The association persisted after adjusting for known risk factors, including cardiovascular disease, suggesting that high levels of VCAM-1 are predictive of higher mortality rates even in previously healthy nonagenarians. As VCAM-1 levels increase in inflammation, we hypothesised that nonagenarians with major inflammatory conditions did not survive, causing an increase in mortality.

sFas is cleaved from the Fas ligand by a matrix metalloprotease. Only the membrane-bound Fas ligand triggers apoptosis through the Fas receptor, whereas sFas counteracts it (Lettau ym. 2011). Many studies have demonstrated that serum levels of sFas are elevated in patients with myocarditis, chronic congestive heart failure and coronary artery disease and thus indicate a possible connection between sFas and cardiovascular disease (Nishigaki ym. 1997, Ohtsuka ym. 1999, Toyozaki ym. 1998). We found that participants with elevated sFas levels had more cardiovascular disease than those with moderate levels, which would support previous findings. Additionally we found that participants with low sFas levels also had more cardiovascular disease than those with moderate

levels. sFas levels are also elevated in neoplastic pathologies (Abbasova ym. 2009). We did not find a statistically significant difference in cancer prevalence as associated with sFas levels. In our population, higher serum levels of sFas seemed to slightly amplify the already strong association of high levels of VCAM-1 and mortality, but sFas did not show a statistically significant independent association with mortality after adjustment. We hypothesise that very low or elevated sFas levels can cause problems in the homeostasis between cell renewal and death. Deceased participants possibly had major disturbances in this delicate balance making them more susceptible to cardiovascular disease and less likely to survive major inflammatory conditions associated with elevated VCAM-1 levels.

HGF was originally discovered to be a mitogen for hepatocytes and a motility factor for epithelial cells. HGF is a heterodimeric glycoprotein of mesenchymal origin. HGF has mitogenic, antiapoptotic and angiogenic effects on various cell types (Maulik ym. 2002). Elevated serum levels of HGF have been connected with conditions with poor prognoses, such as heart failure (Lamblin ym. 2005). In our study we found that participants with high HGF-levels had more cardiovascular disease. HGF levels are elevated in various diseases (Funakoshi ja Nakamura 2003), and in our study population, the prevalence of different diseases was high. We found that higher levels of HGF were associated with higher mortality. The higher serum levels of HGF in the deceased participants may have been caused by attempts to repair failures in organ systems.

4.1 Limitations and strengths of the study

One strength of the study is that both community-dwelling and institutionalised persons were included. We acknowledge that there are limitations to our study. Data was missing for several variables, the loss of participants was reasonably high, and the relatively small population did not allow adjusted stratified analyses in different clinical subgroups. The small sample size and the lack of blind replication data might limit the ability of our data to decidedly indicate the studied markers as definitive predictors of mortality.

4.2 Conclusions

We found that elevated concentrations of HGF and VCAM-1 were strongly associated with higher mortality among nonagenarians. High levels of sFas seemed to slightly amplify the association of VCAM-1 and mortality. Moreover, the combination of simultaneously elevated concentrations of all

three markers predicted the risk of death in nonagenarians. Especially HGF and VCAM-1 seem to be independent prognostic markers, and their possible clinical uses should be studied further.

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REFERENCES

Abbasova SG, Vysotskii MM, Ovchinnikova LK, ym. Cancer and soluble FAS. Bulletin of Experimental Biology & Medicine 2009;148:638-42.

Blankenberg S, Rupprecht HJ, Bickel C, ym. Circulating cell adhesion molecules and death in patients with coronary artery disease. Circulation 2001;104:1336-42.

Cook-Mills JM, Marchese ME ja Abdala-Valencia H. Vascular cell adhesion molecule-1 expression and signaling during disease: regulation by reactive oxygen species and antioxidants. Antioxidants & Redox Signaling 2011;15:1607-38.

Davies MJ, Gordon JL, Gearing AJ, ym. The expression of the adhesion molecules ICAM-1, VCAM-1, PECAM, and E-selectin in human atherosclerosis. The Journal of Pathology 1993;171:223-9.

Ezeh AC, Bongaarts J ja Mberu B. Global population trends and policy options. Lancet 2012;380:142-8.

Funakoshi H ja Nakamura T. Hepatocyte growth factor: from diagnosis to clinical applications. Clinica Chimica Acta 2003;327:1-23.

Goebeler S, Jylha M ja Hervonen A. Medical history, cognitive status and mobility at the age of 90. A population-based study in Tampere, Finland. Aging-Clinical & Experimental Research 2003;15:154-61.

Huffman KM, Pieper CF, Kraus VB, Kraus WE, Fillenbaum GG ja Cohen HJ. Relations of a marker of endothelial activation (s-VCAM) to function and mortality in community-dwelling older adults. Journals of Gerontology Series A-Biological Sciences & Medical Sciences 2011;66:1369-75.

Hurme M, Kivimaki M, Pertovaara M, ym. CRP gene is involved in the regulation of human longevity: a follow-up study in Finnish nonagenarians. Mechanisms of Ageing & Development 2007;128:574-6.

Jylha M ja Hervonen A. Functional status and need of help among people aged 90 or over: a mailed survey with a total home-dwelling population. Scandinavian Journal of Public Health 1999;27:106-11.

Jylha M, Paavilainen P, Lehtimaki T, ym. Interleukin-1 receptor antagonist, interleukin-6, and C-reactive protein as predictors of mortality in nonagenarians: the vitality 90+ study. Journals of Gerontology Series A: Biological Sciences and Medical Sciences 2007;62:1016-21.

Kawanami D, Maemura K, Takeda N, ym. C-reactive protein induces VCAM-1 gene expression through NF-kappaB activation in vascular endothelial cells. Atherosclerosis 2006;185:39-46.

Lamblin N, Susen S, Dagorn J, ym. Prognostic significance of circulating levels of angiogenic cytokines in patients with congestive heart failure. American Heart Journal 2005;150:137-43.

Lettau M, Paulsen M, Schmidt H ja Janssen O. Insights into the molecular regulation of FasL (CD178) biology. European Journal of Cell Biology 2011;90:456-66.

Lisko I, Tiainen K, Stenholm S, ym. Inflammation, adiposity, and mortality in the oldest old. Rejuvenation Research 2012;15:445-52.

Malik I, Danesh J, Whincup P, ym. Soluble adhesion molecules and prediction of coronary heart disease: a prospective study and meta-analysis. Lancet 2001;358:971-6.

Maulik G, Shrikhande A, Kijima T, Ma PC, Morrison PT ja Salgia R. Role of the hepatocyte growth factor receptor, c-Met, in oncogenesis and potential for therapeutic inhibition. Cytokine & Growth Factor Reviews 2002;13:41-59.

Meller I, Fichter MM ja Schroppel H. Mortality risk in the octo- and nonagenerians: longitudinal results of an epidemiological follow-up community study. European Archives of Psychiatry and Clinical Neuroscience 1999;249:180-9.

Nishigaki K, Minatoguchi S, Seishima M, ym. Plasma Fas ligand, an inducer of apoptosis, and plasma soluble Fas, an inhibitor of apoptosis, in patients with chronic congestive heart failure. Journal of the American College of Cardiology 1997;29:1214-20.

Nybo H, Petersen HC, Gaist D, ym. Predictors of mortality in 2,249 nonagenarians--the Danish 1905-Cohort Survey. Journal of the American Geriatric Society 2003;51:1365-73.

Ohtsuka T, Hamada M, Sasaki O, ym. Clinical implications of circulating soluble Fas and Fas ligand in patients with acute myocardial infarction. Coronary Artery Disease 1999;10:221-5.

Rontu R, Ojala P, Hervonen A, ym. Apolipoprotein E genotype is related to plasma levels of C-reactive protein and lipids and to longevity in nonagenarians. Clinical Endocrinology (Oxf) 2006;64:265-70.

Schmidt C, Hulthe J ja Fagerberg B. Baseline ICAM-1 and VCAM-1 are increased in initially healthy middle-aged men who develop cardiovascular disease during 6.6 years of follow-up. Angiology 2009;60:108-14.

Toyozaki T, Hiroe M, Saito T, ym. Levels of soluble Fas in patients with myocarditis, heart failure of unknown origin, and in healthy volunteers. American Journal of Cardiology 1998;81:798-800.