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Cystatin C and NT-proBNP: a powerful combination of biomarkers for predicting cardiovascular mortality in elderly patients with heart failure. Results from a 10 year study in primary care

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Abstract

Aims: Heart failure is common among the elderly. It is essential to identify those at high risk in order to optimize the use of resources. We aimed to evaluate whether a combination of two biomarkers might give better prognostic information about the risk of cardiovascular mortality in patients with symptoms associated with heart failure, compared with only one biomarker.

Methods and results: Four hundred and sixty four primary health-care patients (mean age 73 years, range 65 – 87) with symptoms of heart failure were examined. All patients were evaluated using Doppler echocardiography and blood samples, including measurement of cystatin C and NT-proBNP. The patients were followed over a 10-year period. Patients with serum cystatin C levels within the highest quartile had almost three times the risk (HR: 2.92, CI 95%:1.23-4.90) of cardiovascular mortality compared with those patients who had levels within the first, second or third quartiles. If, at the same time, the patient had a plasma concentration of NT-proBNP within the highest quartile, the risk increased to more than 13 times (HR: 13.61;CI 95%: 2.56-72.24) during 10 years of follow-up, or more than 17 times (HR:17.04; CI 95%: 1.80-163.39) after 5 years of follow-up.

Conclusion: Combined analysis of cystatin C and NT-proBNP could provide important prognostic information among elderly patients in the community with symptoms of heart failure.

Keywords: Elderly patients, heart failure, NT-proBNP, Cystatin C, biomarkers

Introduction

In recent years, awareness of the problems of elderly patients with heart failure (HF) and patients with renal failure has increased [1]. The problems are more apparent in patients with both HF and renal insufficiency, which is not unusual among the elderly. Van Kimmenade et al [2] suggested that this condition – combined HF and renal insufficiency - might best be regarded as a separate condition, the “cardio-renal syndrome”, however, as yet there is no generally agreed definition for this “cardio-renal syndrome”.. The effective measurement of BNP or NT-proBNP, even in patients with renal failure, has already been demonstrated in the literature [3, 4]. However, in patients with both cardiac and renal insufficiency it is not known which biomarker should be used in addition to BNP or NT-proBNP, this issue was discussed by van Kimmenade et al in an editorial published in 2007 [2]. This question is particularly relevant where resources are limited and where it is therefore important to identify high-risk patients. In the present study we evaluated use of the combination of NT-proBNP and cystatin C as biomarkers in elderly patients with HF.

Cystatin C is a small serine protease inhibitor that is secreted from almost all active functional cells in the body. Its molecular mass of 13kD allows it to be freely filtered by the renal glomerulus. The secretion of cystatin C by the kidneys is regarded as being independent of the nutritional status of the patient and of the body mass index [5, 6]. It is also considered to be independent of gender among older patients [7] and, according to most reports, is also independent of the age of the patient [6]. Cystatin C is increasingly being considered as a better indicator of renal function than creatinine or estimated glomerular filtration rate [8, 9,

10, 11] . Therefore, cystatin C is deemed a useful biomarker of renal function [9, 12, 13]. Recent reports have indicated that cystatin C might also be a prognostic indicator of cardiovascular (CV) mortality. Lassus et al reported interesting data concerning the relationships between cystatin C and NT-proBNP in patients with acute HF[14].

The plasma concentration of the N-terminal fragment of proBNP (NT-proBNP) is a well-known indicator of myocardial wall tension, as it is secreted as wall tension increases [15]. Thus, the correlation between plasma concentration of NT-proBNP and the severity of cardiac impairment is well established. Published data show that the peptide has the prognostic power to indicate the risk of CV mortality [16, 17, 18], which we have reported earlier [19].

The aim of this study was to evaluate whether the combined use of these two potent biomarkers (cystatin C and NT-proBNP) could provide better prognostic information about the risk of CV mortality in elderly patients with symptoms of heart failure compared to a single biomarker.

Methods

The Ethics Committee of the University Hospital of Linköping approved the study protocol. All patients provided written informed consent given before inclusion into the study..

Patients

The study population has previously been described in detail [20]. All patients aged 65 – 87 years, with symptoms of and/or signs that might be attributed to HF and who attended primary health care during 1996, were evaluated. 1,168 patient records were identified, of these 548 patients in whom HF could not be excluded on the basis of the patients' records

were invited to participate. The symptoms and/or signs that indicated possible HF included shortness of breath and/or peripheral oedema and/or fatigue. Of these 548 patients, 510 agreed to participate in the study, 38 patients refused to participate either due to long travelling distances or because of severe illness or incapacity. Of the 510 patients, 464 consented to having blood samples drawn and constituted the final study population.

All patients were assessed by the same cardiologist (UA), who recorded patient history, performed a clinical examination and assessed New York Heart Association functional class (NYHA class). Dyspnoea was defined from the patient history, whereas the presence of peripheral oedema was defined from the patient history and/or by clinical examination. Twelve-lead electrocardiography was also performed, as was echocardiographic assessment of systolic and diastolic function, and blood sampling.

Echocardiography

Doppler echocardiographic examinations (Accuson *XP-128c*) were performed with the patient in the supine left position. Both M-mode and 2D methodology was used. Values for systolic function [21], expressed as ejection fraction (EF) [22, 23], were categorized into four classes with interclass limits of 30%, 40% and 50%. A semi-quantitative method of assessment was used. Normal systolic function was defined as $EF \geq 50\%$, whereas severely impaired systolic function was defined as $EF < 30\%$. For assessment of diastolic function, mitral early wave/atrial contraction ratio and pulmonary venous flow pattern were analyzed and compared with age-adjusted decision limits.

Blood sampling

Blood samples were drawn while the patients were fasting and in a sitting position after resting for 30 minutes. The samples were collected in pre-chilled plastic tubes containing EDTA (Terumo EDTA K-3), placed on ice and centrifuged at 3000 g for 10 min at $+4^{\circ}\text{C}$.

Plasma was then immediately transferred to a freezer with ambient temperature -70°C and stored at this temperature until analysis.

Analysis of Cystatin C

Cystatin C was measured by end-point turbidometry with antibodies from Dako (Glostrup, Denmark) utilizing an Advia 1650 clinical chemistry analyzer from Bayer (Gothenburg, Sweden). The between assay variation coefficient was 9.5% for 1.38 mg/L, (n=98) and 9.5% for 4.93 mg/L. The reference range for age >50 years is 0.63 – 1.44 mg/L.

Analysis of NT-proBNP

The Elecsys 2010 (Roche Diagnostics, Mannheim, Germany) NT-proBNP assay, first described by Karl, was used [24]. This assay utilizes two polyclonal antibodies directed against amino acids, 1-21 and 39-50, and electrochemiluminescence detection. The analytical range was 0.6 – 4130 pmol/L (5 – 35.000 ng/L). The total variation coefficient was 4.8% at the level of 26 pmol/L (217 ng/L) (n=70) and 2.1% at the level of 503 pmol/L (4261 ng/L) at our laboratory.

Comorbidity

Hypertension was defined as a blood pressure of more than 140/90 mm Hg measured in the right arm with the patient in the supine position after at least 30 minutes rest. Patients were defined as hypertensive if they had previously been diagnosed with hypertension and were receiving antihypertensive medication. Ischaemic heart disease was defined as a history of angina pectoris, treatment for angina and/or a previous myocardial infarction. Diabetes was defined as a fasting blood glucose concentration ≥ 7.0 mmol/L or ongoing treatment for diabetes.

Statistical methods

Descriptive data are presented as percentage or mean and standard deviation (SD). In the case of continuous variables, analyses were performed using Student's unpaired two-sided T-test, whereas the chi-square test was used for discrete variables. In the multivariate analysis of nominal variables the chi-square test for trend was used, and in the multivariate analysis of continuous variables ANOVA analysis for trend was used. NT-proBNP was $^{10}\log$ transformed before analysis, as there was a skewed distribution of values.

Cox proportional hazard regression analyses as well as a Kaplan-Meier analysis were used to analyze the risk of mortality during the follow-up period. In the analysis clinically well-known variables that were found to be significant in an elderly population were used [25].

A p value less than .05 was considered statistically significant.

All data were analyzed using generally available statistical analysis software packages (Statistica v. 8.0, Statsoft Inc, Tulsa, OK, USA; Analyse-it v. 2.04, Analyse-it Software Ltd. Leeds, United Kingdom).

Results

Follow-up period

Of the 464 patients, 294 were still alive after 3650 days (10 years). Of those who died during the follow-up period (n=170) 113 deaths were due to CV mortality. In the non-survivor group, the median follow-up period was 6.2 years (range 242 – 3,852 days). No patient dropped out of the study during follow-up. All mortality data was registered during the follow-up period and was collected from the National Board of Health and Welfare, Stockholm, Sweden or from autopsy records.

Relation of Cystatin C to clinical variables

The clinical characteristics of the study population according to quartiles of cystatin C are presented in Table I. Patients in the 4th quartile of cystatin C were older than those in the 1st quartile, and had greater impairment of functional capacity (NYHA functional class III). The patients in the higher quartiles of cystatin C also received more intensive pharmacological treatment. The proportion of patients with impaired systolic function increased as the quartile concentration of cystatin C increased. The median serum concentration of cystatin C in patients who survived to 10 years of follow-up was 1.39 mg/L (range: 0.72-3.31), compared with 1.53 mg/L (range: 0.88-3.61) in those who suffered CV mortality at some time during the 10-year follow-up period. The difference in serum concentration of cystatin C between survivors and non-survivors was significant ($t=6.00$; $p<0.0001$).

Relation of Cystatin C to renal function

Cystatin C was evaluated against creatinine levels and the estimated glomerular filtration rate (GFR) according to the MDRD formula [26]. The correlation was significant both between serum cystatin C and plasma creatinine ($r=0.63$), and between cystatin C and estimated GFR ($r=-0.70$). To analyze these two alternative markers for renal function (creatinine and estimated GFR) as indicators of the risk of CV mortality, a Cox proportional hazard regression analysis was performed to analyze the 1st quartile of the biomarker against quartiles 2, 3 and 4 (Table II). From the analysis, it was found that cystatin C was a better indicator than creatinine or eGFR of an increased risk of CV mortality. There was a higher hazard ratio in all three quartiles than in the 1st quartile, which was used as reference compared with creatinine or estimated GFR.

Table 1. Characteristics of the study population divided into quartiles of serum concentration of cystatin C.

Variable	Cystatin C Q1 <1.22 mg/L	Cystatin C Q2 1.22-1.42 mg/L	Cystatin C Q3 1.43-1.66 mg/L	Cystatin C Q4 >1.66 mg/L	p value
n	109	120	117	118	
Age mean (SD)	70 (5.1)	71 (5.3)	73 (5.3)	76 (4.8)	p<0.0001
Males/Females n	56/53	58/62	66/51	60/58	ns
BMI mean (SD)	27 (4.2)	28 (4.8)	27 (4.0)	27 (4.6)	ns
Smokers n (%)	36 (33)	37 (31)	43 (38)	35 (30)	ns
Diabetes n (%)	20 (18)	27 (22)	18 (15)	25 (21)	ns
Hypertension n (%)	97 (89)	106 (88)	100 (85)	103 (87)	ns
NYHA class I n (%)	65 (60)	58 (43)	50 (43)	39 (33)	p<0.0001
NYHA class II n (%)	38 (35)	54 (45)	59 (50)	49 (42)	ns
NYHA class III n (%)	6 (6)	8 (7)	8 (7)	30 (25)	p<0.0001
ACEI n (%)	27 (25)	36 (30)	35 (30)	53 (45)	p=0.002
BB n (%)	48 (44)	48 (40)	45 (38)	49 (42)	ns
Digitalis n (%)	10 (9)	8 (7)	5 (4)	23 (19)	p=0.006
Diuretics n (%)	17 (16)	43 (36)	49 (42)	82 (69)	p<0.0001
Enlargement of heart on chest x-ray n (%)	43 (39)	52 (43)	37 (32)	74 (63)	p=0.002
EF<40% n (%)	7 (6)	4 (3)	16 (14)	25 (21)	p<0.0001
NT-proBNP ng/L median	303	327	406	1144	p<0.0001
All cause mort n (%)	24 (23)	38 (32)	46 (39)	60 (51)	p<0.0001
CV mort n (%)	10 (9)	27 (23)	32 (27)	44 (37)	p<0.0001

Note: ACEI: ACE inhibitors; BB: Beta blockers; BMI: Body Mass Index; CV: Cardiovascular; EF: Ejection fraction; IHD: Ischaemic heart disease; NYHA: New York Heart Association functional class

We also analyzed the risk of CV mortality in those with the lowest quartile of serum cystatin C (n=107; CV mort: n=10) compared with those with the highest quartile (n=69; CV mort: n=23), but with normal renal function (defined as serum creatinine < 115 μ mol/L). The univariate Cox proportional hazard regression indicated that in this group the risk of CV

mortality among those within the 4th quartile of serum cystatin C was four times higher during 10 years of follow-up (HR: 3.61; 95% CI: 1.81-7.14) than those in the 1st quartile.

Table II. Univariate Cox proportional hazard regression analysis of three different biomarkers for renal function concerning risk of cardiovascular mortality during 10 years of follow-up.

Variable	HR	95% CI	p value
Creatinine Q2	1.71	0.99-2.97	0.05
Creatinine Q3	1.16	0.68-1.97	0.6
Creatinine Q4	4.42	2.46-7.99	<0.0001
eGFR Q2	0.90	0.58-1.38	0.6
eGFR Q3	2.12	1.30-3.46	0.003
eGFR Q4	1.24	0.70-2.18	0.5
Cystatin C Q2	2.51	1.27-4.98	0.009
Cystatin C Q3	3.20	1.61-6.33	0.0008
Cystatin C Q4	5.31	2.72-10.34	<0.0001

Note: All analyses have been performed against Q1 as reference.

CI: Confidence interval; eGFR: estimated GFR according to MDRD formula; HR: Hazard ratio; Q: Quartile

Prognostic information from cystatin C

A multivariate Cox proportional hazard regression was performed, in which some well-known risk factors for CV mortality were added to the 2nd, 3rd and 4th quartiles of cystatin C and analyzed against the 1st quartile with respect to risk of CV death during 10 years of follow-up (Table III). It was found that patients with a serum concentration of cystatin C in the 4th quartile had a three times greater risk of CV mortality than those in the 1st quartile even when combined with well-known variables that influence the risk of CV mortality and together with the 2nd, 3rd and 4th quartiles of NT-proBNP in a multivariate analysis.

Table III. Cox proportional hazard regression analysis evaluating risk for cardiovascular death analyzing 2nd, 3rd and 4th quartiles against the 1st quartile of plasma concentration of cystatin-C and NT-proBNP together with some well-known factors for cardiovascular death during a follow-up period of 10 years.

Variable	HR	95%CI	p value
Male gender	1.26	0.82-1.93	0.30
Age>75 years	1.49	1.96-2.27	0.07
Smoker	1.25	0.79-1.95	0.33
NYHA class III	1.71	1.05-2.80	0.03
Hypertension	1.67	0.62-2.18	0.63
Diabetes	1.90	1.42-2.53	<0.0001
IHD	0.81	0.54-1.22	0.31
BMI>27	0.91	0.61-1.38	0.65
Hb<120 g/L	1.70	0.90-3.19	0.10
EF<40%	1.33	0.98-1.82	0.27
Cystatin C Q2	1.27	1.07-2.16	0.019
Cystatin C Q3	1.36	1.07-1.73	0.008
Cystatin C Q4	1.52	1.10-2.23	0.005
NT-proBNP Q2	1.27	0.86-1.88	0.23
NT-proBNP Q3	1.31	1.02-1.68	0.04
NT-proBNP Q4	1.53	1.28-1.84	<0.0001

Note: BMI: Body mass index; EF: Ejection fraction;
 HR: Hazard ratio; IHD: Ischaemic heart disease;
 NYHA: New York Heart Association functional class;
 Q: Quartile

Prognostic information from NT-proBNP

The prognostic information from NT-proBNP concerning risk of CV mortality was analyzed in a multivariate Cox proportional hazard regression over a follow-up period of 10 years (Table III). We found that those patients with NT-proBNP levels in the 3rd and the 4th quartiles had an increased risk of CV mortality even when other well-known risk factors were taken into account.

Prognostic information from Cystatin C and NT-proBNP combined

Both cystatin C and NT-proBNP provided significant, independent prognostic information concerning the risk of CV mortality when analyzed as continuous variables in a multivariate analysis and when they were adjusted for each other as well as for the other background

variables (cystatin C: $p=0.0085$; NT-proBNP: $p<0.0001$). The two biomarkers were then classified into quartiles and the 4th and 1st quartiles were evaluated in different combinations. This is illustrated in a Kaplan-Meier analysis over the 10 years of follow-up (Fig 1). Of those patients with a plasma concentration in the 1st quartile for both biomarkers, more than 90% were still alive after 10 years, as compared with only 30% of those with a plasma concentration in the 4th quartile for both biomarkers combined.

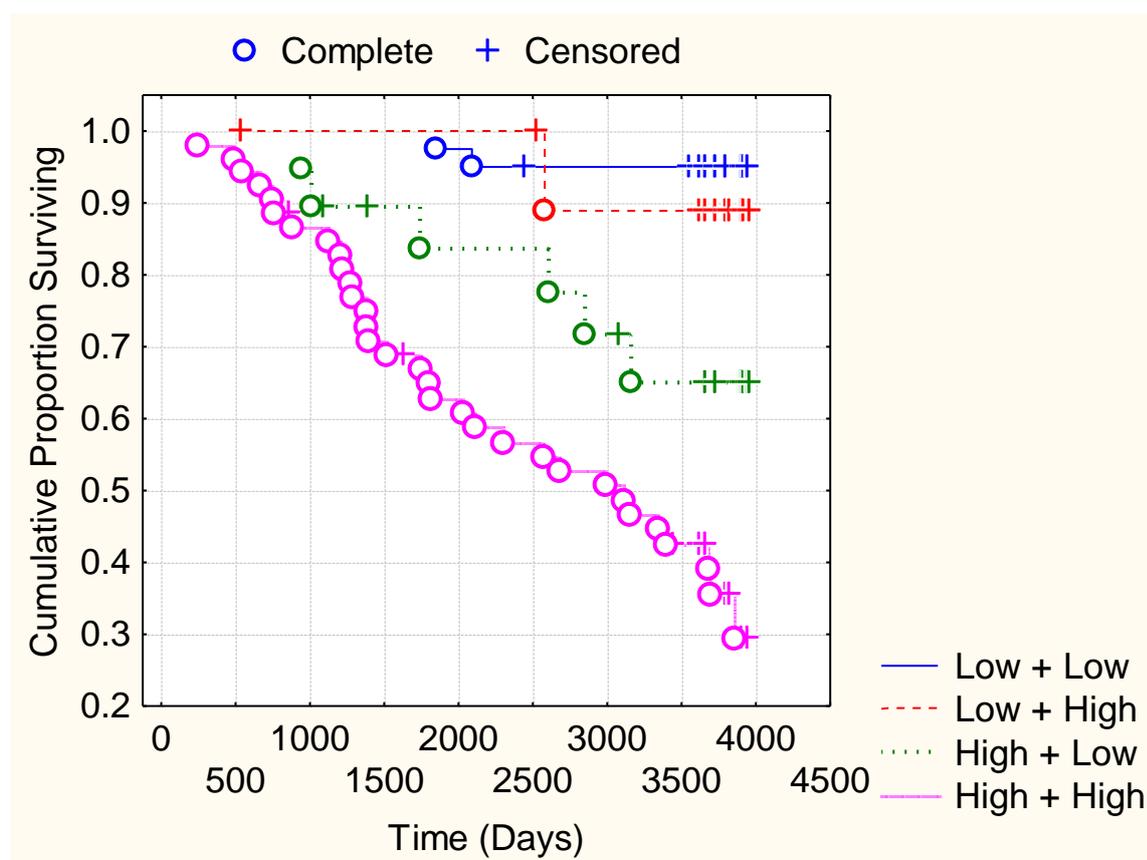


Fig. 1: Cumulative proportion surviving from CV mortality during 10 years of follow-up. Population divided into "high" and "low" plasma concentration of NT-proBNP and cystatin C
Note:

Censored = patients who were still alive at the end of the follow-up period, or who died from non-cardiac causes.

Complete = Patients who died from cardiovascular causes during the follow-up period.

Note: Low + Low: 1st quartile of NT-proBNP + 1st quartile of cystatin C; Low + high: 1st quartile of NT-proBNP + 4th quartile of cystatin C; High+low: 4th quartile of NT-proBNP + 1st quartile of cystatin C; High+high: 4th quartile of NT-proBNP + 4th quartile of cystatin C

In a multivariate Cox proportional hazard regression analysis, the group with a serum concentration of cystatin C in the 4th quartile and a plasma concentration of NT-proBNP in

the 4th quartile (n=61; CV mort: n=28) was compared with those in the 1st quartile of cystatin C and the 1st quartile of NT-proBNP (n=65; CV mort n=3). During a follow-up period of 5 years, use of the combined variable of cystatin C and NT-proBNP in the 4th quartile showed that the risk of CV mortality for these patients was more than 16 times higher (HR: 16.39; 95% CI: 1.77-66.70; p=.01) than for patients in the 1st quartiles of NT-proBNP and cystatin C combined. Use of this combined variable in estimating the risk of CV mortality showed that the hazard ratio decreased to almost 13 times increased risk of CV mortality for the combined variable if the study population was followed for 10 years (HR: 12.98; 95% CI: 2.48-67.36; p=.002). The patients with a serum concentration of cystatin C in the 1st quartile and a plasma concentration of NT-proBNP in the 1st quartile had a CV mortality of 5% during 10 years of follow-up whereas those having both biomarker concentrations in the 4th quartile had a cardiovascular mortality of 60% (table IV).

Table IV. Cox proportional hazard regression analysis evaluating risk of cardiovascular death comparing the 4th quartiles of cystatin-C and NT-proBNP against 1st quartiles of the two biomarkers, using some well-known risk factors for cardiovascular death during follow-up period of 5 and 10 years.

Variable	5 years			10 years		
	HR	95% CI	p value	HR	95% CI	p value
Male gender	2.35	0.80-6.92	0.12	1.21	0.53-2.75	0.65
Age >75 years	0.50	0.17-1.47	0.21	0.75	0.55-3.22	0.52
Smoker	0.89	0.30-2.66	0.83	1.28	0.55-2.95	0.57
NYHA class III	3.53	1.18-10.57	0.02	3.43	1.36-8.59	0.0009
Hypertension	0.83	0.22-3.22	0.79	1.17	0.38-3.67	0.78
Diabetes	1.10	0.32-3.21	0.88	1.79	0.70-4.57	0.23
IHD	0.98	0.48-1.99	0.95	1.18	0.70-1.99	0.53
BMI >27	0.47	0.16-1.37	0.17	0.48	0.89-4.95	0.09
EF<40%	3.37	1.19-9.48	0.02	2.56	1.13-5.81	0.03
Cystatin-C Q4+NT-proBNP Q4	16.39	1.77-66.70	0.01	12.98	2.48-67.36	0.002

Note: BMI: Body mass index; EF: Ejection fraction; IHD: Ischaemic heart disease; NYHA: New York Heart Association functional class

Discussion

Cystatin C provides important prognostic information about the risk of heart failure and CV mortality [27, 28]. However, we now report the possible benefits of the combined use of two biomarkers, NT-proBNP and cystatin C, to evaluate the risk of cardiovascular mortality in elderly patients with chronic HF.

In the present study, a high cystatin C concentration indicated a highly significant increase in CV mortality. This result concurs with findings in the literature [14, 29]. In addition, the follow-up period of 10 years was longer than that used in previous studies. During the follow-up period, the probability that a patient might change from one cystatin C quartile to another increases with time. However, if a shorter follow-up period such as 5 years is used, the hazard ratio obtained in multivariate analysis is almost the same (HR: 3.38; 95%CI: 1.23-9.30; $p=.02$) as in the 10 year follow-up (HR: 2.63; 95CI: 1.38-5.02; $p=.004$). This illustrates the robustness of the prognostic information.

NT-proBNP, another well-known biomarker related to the risk of CV mortality, has provided similar results, as reported in the literature [30, 31]. This biomarker is robust and has a documented ability to indicate the risk of CV mortality both for patients with HF and for those with acute coronary syndrome.

The two biomarkers chosen for evaluation in this study, cystatin C and NT-proBNP, were both independent predictors of CV mortality when analyzed as continuous variables. This provides the basis for an extended analysis of the two biomarkers.

By combining these two biomarkers in the risk evaluation of CV mortality, we obtained results that indicate a highly increased risk for those with a high concentration of both biomarkers. This was found in both the univariate and the multivariate analyses, analyses in which other well known risk factors for CV death were included.

The combined analysis of two biomarkers for evaluating the risk of cardiovascular mortality has been previously reported in the literature [4]. It has also been shown that a combination of two biomarkers can give a better risk prediction than a single biomarker. However, in the present study we chose a biomarker of renal function, i.e. cystatin C, which is more sensitive in patients with slightly impaired renal function. We applied it in an elderly outpatient population, the majority of whom did not have severe impairment of either cardiac or renal function. Despite this important prerequisite, the risk of CV mortality was increased in the subgroup of patients with increased plasma concentrations of both biomarkers. The group of patients with a serum concentration of cystatin C in the 4th quartile and a plasma concentration of NT-proBNP in the 4th quartile had a risk of CV mortality that was 16 and 13 times higher, respectively, than for those in the 1st quartiles of both cystatin C and NT-proBNP during 5 and 10 years of follow-up, respectively. Thus, in patients with less impaired cardiac and renal function the combined use of the two biomarkers still gave important prognostic information. In the multivariate analyses, where the 2nd, 3rd and 4th quartiles of the two biomarkers were analyzed in addition to well known risk factors for CV death, the 3rd and 4th quartiles of NT-proBNP and the 2nd, 3rd and 4th quartiles of cystatin C were found to be independent predictors of CV mortality (Table III). Thus, this combined biomarker analysis could be an alternative for identifying patients at increased risk, even though they only have slightly impaired cardiac or renal function, but may be said to have cardio-renal syndrome.

An important question is whether the increased serum concentration of cystatin C is merely an indicator of impaired renal function or something else as well. This discussion is still ongoing in the literature [32]. In a study of patients with chronic renal disease, and with cystatin C as prognostic indicator, Menon et al reported that cystatin C might provide prognostic information beyond information about renal function [33]. Therefore, an interesting aspect of

the prognostic information concerning CV mortality is use of dual biomarkers in patients with normal renal function according to serum creatinine, but with increased serum concentration of cystatin C. Arimoto et al found an increased risk of CV mortality in this group [8]. The present study confirms this result. Francis reports that almost two thirds of the patients admitted for acute HF at the Cleveland Clinic had normal levels of serum creatinine despite reduced creatinine clearance [34], indicating that serum creatinine might be insufficiently sensitive for detecting decreased renal function, compared with other markers of renal function, such as cystatin C [35].

We used 115 $\mu\text{mol/L}$ as a cut-off value, which was the upper reference value for men (for women, 100 $\mu\text{mol/L}$) at the Department of Clinical Chemistry at Linköping University, Sweden, at the time of the study. This was only slightly lower than the value (120 $\mu\text{mol/L}$) used by the Finnish group in the FINN-AKVA study [14]. Lassus et al reported that 18% of the study population in FINN-AKVA had normal serum creatinine but elevated serum cystatin C. They found an increase in mortality in the group, which is similar to our findings.

Limitations

This study was conducted in elderly patients with symptoms of HF. One limitation is the size of the study population. A larger sample of patients would have made the results from the subgroup analyses more robust. However, we believe that the results obtained are accurate and informative, and that the conclusions drawn are correct.

A second limitation is the restricted age range (70-80 years) of the study population. Inclusion of younger patients would have increased the information provided by the study and thus the possibility to extrapolate the findings to the HF population in general.

A third limitation is that only some patients had impaired systolic function according to Doppler echocardiography. However, recent guidelines from the European Society of Cardiology state that increased plasma concentrations of natriuretic peptides may be regarded as an objective sign of impaired cardiac function [36]. In accordance with this definition, the study population were examined using increased natriuretic peptide levels as an objective assessment of cardiac function. Using the 4th quartile of NT-proBNP (>400 pg/mL) as a cut-off for possible HF in patients with signs/symptoms of HF, 31% of the study population had increased plasma concentrations of NT-proBNP. It would have been preferable if all patients included in the study had HF. However, in this study we used a primary health care population that was well-known to the clinician and included patients with different plasma concentrations of NT-proBNP, who were evaluated according to these specific plasma concentrations.

If, as an alternative, we used a definition of HF based on plasma concentrations of NT-proBNP above the median concentration from a reference population of a corresponding age and in the same geographical area, we found that 372 patients out of 474 (78%) had a plasma concentration above the cut-off level. The reference population appeared free from heart disease and was not being treated with substances that influenced the plasma concentration of NT-proBNP [37].

The choice of reference values in an elderly population is certainly controversial, but as the chosen population was interviewed, underwent a clinical examination and Doppler echocardiography, and had blood samples drawn, we were able to exclude all known potential factors that influence NT-proBNP levels in this unselected elderly population. For this reason, we argue that the reference population is representative as a reference population and that the findings can be extrapolated to elderly HF patients in the community.

Conclusion

The use of biomarkers for identifying those at risk of CV mortality is central to the management of patients with heart failure. In the present study, the combination of cystatin C and NT-proBNP has been used to identify those at risk of CV mortality. A multivariate analysis showed that the risk of cardiovascular mortality in patients with serum concentrations of both cystatin C and NT-proBNP in the 4th quartile was 16 times that of a plasma concentration in the 1st quartile of both biomarkers combined during the first 5 years of follow-up and was almost 13 times that of the 1st quartile of both biomarkers combined during the full 10 years of follow-up.

The size of the study group is limited and there is therefore a need for additional evaluations of the biomarkers used. However, we suggest that use of the combined analysis among elderly patients with symptoms of heart failure is indeed useful for identifying those at high risk.

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