ESC HEART FAILURE
ESC Heart Failure 2020; **7**: 434–444
Published online 22 January 2020 in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/ehf2.12590

ORIGINAL RESEARCH ARTICLE

CT-IGFBP-4 as a novel prognostic biomarker in acute heart failure

Alexey A. Konev[1], Alexey V. Kharitonov[1,2], Fedor N. Rozov[1], Evgeny P. Altshuler[1,2], Daria V. Serebryanaya[1,2], Johan Lassus[3], Veli-Pekka Harjola[4], Alexey G. Katrukha[1,2] and Alexander B. Postnikov[1,2]*

[1]*HyTest Ltd, Intelligate 1, Joukahaisenkatu 6, Turku20520, Finland;* [2]*Faculty of Biology, Moscow State University, Moscow, Russian Federation;* [3]*Cardiology, University of Helsinki, Heart and Lung Center, Helsinki University Hospital, Finland;* [4]*Emergency Medicine, University of Helsinki, Department of Emergency Medicine and Services, Helsinki University Hospital, Finland*

Abstract

Aims Insulin-like growth factor binding protein-4 (IGFBP-4) fragments have been shown to predict the risk of major adverse cardiovascular events, including segment-elevation myocardial infarction, in patients with acute coronary syndrome. We evaluated the prognostic value of the carboxy-terminal fragment of IGFBP-4 (CT-IGFBP-4) for all-cause mortality in emergency room patients with acute heart failure (AHF).

Methods and results CT-IGFBP-4, N-terminal pro brain natriuretic peptide (NT-proBNP), and C-reactive protein (CRP) were measured at admission from the lithium-heparin plasma of 156 patients with AHF. All-cause mortality was recorded for 1 year. Receiver operator characteristic (ROC) curves, Kaplan–Meier, and Cox proportional hazard ratio analyses were performed to evaluate the prognostic value of the various clinical variables, CT-IGFBP-4, NT-proBNP, CRP, and their combinations. During 1 year of follow-up, 52 (33.3%) patients died. CT-IGFBP-4 only weakly correlated with NT-proBNP (Pearson correlation coefficient $r = 0.16$, $P = 0.044$) and did not correlate with CRP ($r = 0.08$, $P = 0.35$), emphasizing the different nature of these biomarkers. The receiver operator characteristic area under the curve (ROC AUC) of CT-IGFBP-4 for the prediction of all-cause mortality (0.727) was significantly higher than that of NT-proBNP (0.680, $P = 0.045$) and CRP (0.669, $P = 0.016$). The combination of CT-IGFBP-4, NT-proBNP, and CRP predicted mortality significantly better (ROC AUC = 0.788) than any of the biomarkers alone ($P < 0.01$ for all). The addition of CT-IGFBP-4 to a clinical prediction model that included age, gender, systolic blood pressure, creatinine, and sodium levels, as well as the history of previous heart failure, coronary artery disease, and hypertension significantly improved the mortality risk prediction (ROC AUC 0.774 vs. 0.699, $P = 0.025$). Cox hazard analysis indicated that elevated CT-IGFBP-4 was independently associated with 1 year mortality (hazard ratio 3.26, $P = 0.0008$) after adjustment for age, gender, history of previous heart failure, coronary artery disease, hypertension, chronic kidney failure, history of diabetes, heart rate, haemoglobin, plasma sodium, NT-proBNP, CRP, cystatin C, and elevated cardiac troponin I or T. Patients with increased levels of either two or three of the biomarkers CT-IGFBP-4, NT-proBNP, and CRP had significantly higher mortality risk (adjusted hazard ratio 10.04, $P < 0.0001$) than patients with increased levels of one or none of the biomarkers.

Conclusions CT-IGFBP-4 was independently associated with all-cause mortality in patients with AHF. Compared with single biomarkers, the combination of CT-IGFBP-4, NT-proBNP, and CRP improved the prediction of all-cause mortality in patients with AHF.

Keywords Heart failure; Biomarker; Prognostic value; Insulin-like growth factor binding protein 4 (IGFBP-4) fragments; Pregnancy-associated plasma protein-A (PAPP-A); N-terminal pro brain natriuretic peptide (NT-proBNP)

Received: 17 July 2019; Revised: 5 November 2019; Accepted: 25 November 2019
**Correspondence to: Alexander B. Postnikov, HyTest Ltd, Intelligate 1, Joukahaisenkatu 6, Turku 20520, Finland.*
Email: alexander.postnikov@hytest.fi

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Introduction

Acute heart failure (AHF) is defined as new-onset or worsening of symptoms and signs of heart failure (HF)[1] and often requires rapid hospital admission and intensification of therapy. In the USA and Europe, AHF is the principal cause of unplanned hospital admissions in patients aged 65 years or older and a leading contributor to health care costs.[2,3] Several epidemiological studies have shown high in-hospital mortality rates (from 4% to 28%), as well as poor short-term and medium-term prognoses for patients with AHF.[2,4]

Despite major achievements in the treatment of chronic HF in recent decades, achievements which led to a marked improvement in long-term survival,[5] the outcomes of AHF remain poor, with 1 year all-cause mortality and rehospitalization rates reaching 17.4% and 43.9%, respectively.[6] In the recent European Society of Cardiology Heart Failure Long-Term registry, the 1 year mortality rate was 23.6% for AHF.[7]

Biomarkers play a key role in the diagnosis of HF. Brain natriuretic peptide (BNP) and its amino-terminal precursor fragment (NT-proBNP) are diagnostic HF biomarkers produced by myocardial cells in response to myocardial wall stress. NT-proBNP can also be used for HF outcome prediction, along with other clinical factors, such as blood pressure and kidney function.[8–10] Nevertheless, mortality risk prediction in AHF is challenging, and accurate prognostication is the key for selecting the appropriate intensity of therapeutic interventions and follow-up.

The search for prognostic biomarkers of HF, which could be used alone or in combination with NT-proBNP, is an urgent task of clinical chemistry. Various circulating biomarkers, including C-reactive protein (CRP), cardiac troponins, galectin-3, mid-regional proadrenomedullin, growth differentiation factor-15, and soluble suppression of tumorigenicity-2, have been investigated for outcome prediction of HF.[11–13]

The carboxy-terminal fragment of insulin-like growth factor binding protein-4 (CT-IGFBP-4) is one of the products of proteolytic cleavage of IGFBP-4 mediated by pregnancy-associated plasma protein-A (PAPP-A).[14] In turn, PAPP-A is known to be involved in insulin-like growth factor (IGF)-dependent activation of the proliferation of smooth muscle cells within atherosclerotic plaques and the resultant destabilization of the plaques.[15,16] Recently, CT-IGFBP-4 was shown to provide incremental prognostic information on cardiovascular events and mortality in patients with diagnosed segment (ST)-elevation myocardial infarction.[17] As progressive HF is often caused by ischaemic heart disease and often follows acute myocardial infarction (AMI), we hypothesized that CT-IGFBP-4 could also be utilized as a biomarker for the prognosis of AHF outcomes. Thus, the goal of this study was to assess CT-IGFBP-4 for the prognosis of outcomes of patients with AHF.

Materials and methods

Design of the immunoassay for CT-IGFBP-4 measurements

The monoclonal antibodies IBP163, IBP182 conjugated with horseradish peroxidase ($IBP182^{HRP}$), recombinant human CT-IGFBP-4, amino-terminal fragment of IGFBP-4 (NT-IGFBP-4), and IGFBP-4 were obtained from HyTest Ltd, Turku, Finland. In the sandwich immunoassay for CT-IGFBP-4 measurement, mAb IBP163 (specific to the proteolytic neoepitope of CT-IGFBP-4) was used as a capture antibody, and mAb $IBP182^{HRP}$ (recognizing both full-length IGFBP-4 and CT-IGFBP-4) was used as a detection antibody.

Briefly, the immunoassay method was performed as follows: 10 mg/L of the capture monoclonal antibodies (IBP163) were absorbed on the high-binding polystyrene 96-well plate (Corning, New York, USA) in phosphate buffered saline (PBS; 20 mM potassium phosphate, 150 mM NaCl, pH 7.4), 100 μL/well (60 min at room temperature with constant shaking). The plates were washed with PBS with 0.1% Tween 20 (PBST). The calibrators or analysed samples were diluted in PBST that contained 225 mmol/L NaCl (50 μL/well), and mAb $IBP182^{HRP}$ in PBST that contained 225 mmol/L NaCl (2 mg/L, 50 μL/well) was subsequently added. The plates were then incubated for 30 min at room temperature with constant shaking. The plates were washed with PBST, and the substrate solution (0.4 mmol/L 3,3′,5,5′-tetramethylbenzidine in 100 mmol/L Na-acetate buffer, pH 4.5, with 0.01% H_2O_2; 100 μL/well) was added. The reaction was stopped with 2 mol/L H_3PO_4, and the absorbance (λ = 450 nm) was measured.

Validation of CT-IGFBP-4 assay

The analytical detection limit, within-assay imprecision coefficient of variation (CV), total imprecision CV, and linear range were defined for the IBP163-$IBP182^{HRP}$ CT-IGFBP-4 assay. In recovery studies, three concentrations of the calibrator (30, 100, and 300 ng/mL) were spiked into pooled normal lithium-heparin plasma free of the analyte. The concentrations of IGFBP-4 and NT-IGFBP-4 in the cross-reaction studies were 1000 ng/mL. The sandwich immunoassay IBP163-$IBP182^{HRP}$ had the following characteristics: cross-reactivity to IGFBP-4 and NT-IGFBP-4 $< 2\%$, linear range 0.3–6 ng/mL, analytical limit of detection 0.15 ng/mL, within-assay imprecision CV $< 6.0\%$, and total imprecision CV $< 9.7\%$. Recovery of the assay was 82%, 86%, and 87% for 300, 100, and 30 ng/mL CT-IGFBP-4, respectively.

DOI: 10.1002/ehf2.12590

Patients and study design

The study was performed in accordance with the current revision of the Helsinki Declaration. The original study population consisted of 620 consecutive patients hospitalized with AHF. Patients were enrolled at 14 hospitals in Finland between February and May 2004, as previously described.[10,18] Mortality data were obtained from the Finnish National Population Register. Follow-up was completed for all 156 patients. After collection, the lithium-heparin plasma samples were aliquoted and stored at −80 °C until analysis. Long-term stability of IGFBP-4 fragments at −80 °C was previously demonstrated.[19] For the purposes of this study, lithium-heparin plasma samples of 156 patients were randomly selected. At baseline, demographic and past medical history data were collected. Cardiac troponin T, NT-proBNP, plasma creatinine (all from Roche Diagnostics, Basel, Switzerland), cardiac troponin I (Abbott Diagnostics, Abbott Park, IL, USA), and cystatin C were analysed in a central laboratory. CRP, haemoglobin, and sodium levels were analysed locally with standard methods. Admission samples were used for the analysis.

Patients were followed for 1 year. One month and 1 year all-cause mortality were the endpoints of the study. All patients provided written informed consent.

Statistical analysis

We used the Student's *t*-test and Mann–Whitney *U* test to assess group-specific differences in the continuous and categorical variables, respectively. Clinical prognostic variables (age, gender, systolic blood pressure, creatinine, and sodium levels, as well as the history of previous HF, coronary artery disease, and hypertension) were used to build a baseline model for mortality risk prediction in the patient cohort (the 'clinical prediction model'). We performed receiver operator characteristic (ROC) curve analysis to investigate the predictive value of clinical variables, NT-proBNP, CT-IGFBP-4, CRP, and their combinations in the clinical prediction model. Log-transformation and subsequent logistic regression were performed to evaluate the analyte combinations in the ROC curve analysis. The cut-off values for NT-proBNP, CT-IGFBP-4, and CRP were derived from the ROC curves and were defined as the values that provided the maximal sum of the sensitivity and specificity.

We used the Cox proportional hazards model to estimate the hazard ratios (HRs) of all-cause mortality in relation to NT-proBNP, CT-IGFBP-4, CRP, and other variables. The values below the cut-off levels were accepted as the reference groups in these models. To identify independent predictors, a forward and backward stepwise procedure was used to choose the final model; variables retained in the model were considered significant at $P < 0.10$. The multivariate model included NT-proBNP, CT-IGFBP-4, CRP, cystatin C, age, gender, previous diagnosis of HF, coronary artery disease, hypertension, chronic kidney failure, history of diabetes, heart rate, haemoglobin, plasma sodium, and elevated cardiac troponin T or cardiac troponin I.

Cumulative event rates were estimated using the Kaplan–Meier method and were compared using the log-rank test.

Pearson's correlation coefficient (*r*) was used to assess the interrelationships of NT-proBNP, CT-IGFBP-4, and CRP. Values of $P < 0.05$ were considered statistically significant, and all tests were two sided. The software package XLSTAT (Addinsoft SARL, Paris, France) was used to perform statistical analyses.

Results

The baseline characteristics of the study population are shown in *Table 1*. Fifty-two patients (33.3%) met the endpoint (all-cause mortality) during the 1 year follow-up.

The NT-proBNP, CT-IGFBP-4, and CRP concentration ranges of the study cohort were 69–52 484 pg/mL, 9.4–1121 ng/mL, and 0–257 mg/L, respectively. NT-proBNP only weakly correlated with CT-IGFBP-4 (Pearson correlation coefficient $r = 0.16$, $P = 0.044$; *Figure 1A*), which emphasizes the different nature of these biomarkers. No correlation was identified between CT-IGFBP-4 and CRP ($r = 0.08$, $P = 0.35$; *Figure 1B*) or NT-proBNP and CRP ($r = 0.06$, $P = 0.45$; *Figure 1C*).

Both NT-proBNP and CT-IGFBP-4 were significantly elevated in the non-survivors compared with those in the survivors (*Table 1* and *Figure 2*). NT-proBNP and CT-IGFBP-4 were also significantly elevated in the patients who died within 1 month ($P = 0.022$ and $P = 0.0003$, respectively). CRP was not significantly elevated in the non-survivors ($P = 0.077$ and $P = 0.076$ for mortality at 1 month and 1 year, respectively).

The abilities of NT-proBNP, CT-IGFBP-4, and CRP, as well as clinical variables to predict all-cause mortality, both at 1 month and 1 year, were investigated via receiver operator characteristic area under the curve (AUC) analysis (*Figure 3* and *Table 2*).

CT-IGFBP-4 and NT-proBNP had similar properties for the prediction of short-term (1 month) mortality; however, CT-IGFBP-4 was better at the prediction of 1 year mortality. Both CT-IGFBP-4 and NT-proBNP were significantly better than CRP. Single NT-proBNP and CT-IGFBP-4 demonstrated higher ROC AUC values for 1 month than for the 1 year period. Combining CT-IGFBP-4 with NT-proBNP improved both 1 month and 1 year mortality prediction compared with NT-proBNP alone. Similarly, the combination of NT-proBNP and CRP predicted mortality better than NT-proBNP alone. However, the addition of CRP to the combination of

Table 1 Baseline demographic and clinical characteristics as a function of all-cause mortality during 1 year of follow-up

	Total (n = 156)	Non-survivors (all-cause mortality) (n = 52)	Survivors (n = 104)	P value
Age; mean (SD)	76.7 (9.9)	79.1 (9.8)	75.5 (9.7)	0.032
Mean; n = (%)	73 (47)	22 (42)	51 (49)	0.43
Underlying diseases; ***n*** = (%)				
Previous diagnosis of HF	100 (64)	37 (71)	63 (61)	0.20
Coronary artery disease	97 (62)	32 (62)	65 (63)	0.91
AMI, history	45 (29)	16 (31)	29 (28)	0.71
Hypertension	87 (56)	30 (58)	57 (55)	0.73
Stroke, cerebral infarction	24 (15)	11 (21)	13 (13)	0.16
Diabetes (type I or II)	52 (33)	19 (37)	33 (32)	0.55
Chronic obstructive pulmonary disease	24 (15)	9 (17)	15 (14)	0.64
Peripheral arterial disease	13 (8)	4 (8)	9 (9)	0.84
Hypercholesterolemia	31 (20)	8 (15)	23 (22)	0.32
Smoking	21 (13)	7 (13)	14 (13)	1
Ex-smoker	17 (11)	5 (10)	12 (12)	0.72
Medication at admission; ***n*** = (%)				
ß-blocker	97 (62)	35 (67)	62 (60)	0.35
ACEI/ARB	84 (54)	26 (50)	58 (56)	0.50
Furosemide	86 (55)	32 (62)	54 (52)	0.26
Dihydropyridine Ca blocker	21 (13)	6 (12)	15 (14)	0.62
ASA	63 (40)	21 (40)	42 (40)	1
Warfarin	41 (26)	18 (35)	23 (22)	0.10
Lipid lowering	45 (29)	13 (25)	32 (31)	0.46
Spironolactone	16 (10)	9 (17)	7 (7)	0.041
ICD; ***n*** = (%)	8 (5)	1 (2)	7 (7)	0.20
Clinical presentation				
Systolic blood pressure,[1] mmHg; mean (SD); N = 152	149 (36)	139 (34)	154 (36)	0.014
Diastolic blood pressure,[1] mmHg; mean (SD); N = 152	83 (20)	77 (19)	86 (20)	0.009
LVEF[1] (%); mean (SD); N = 79	42 (16)	43 (19)	42 (14)	0.78
Heart rate,[1] beats/min; mean (SD); N = 151	93 (29)	97 (36)	90 (25)	0.21
Na,[1] mmol/L; median (IQR); N = 149	139 (135–141)	138 (134–141)	139 (136–141)	0.061
Haemoglobin,[1] g/L, median (IQR); N = 147	128 (115–139)	125 (115–135)	130 (114–142)	0.157
Cystatin C, mg/L, median (IQR)	1.33 (111–1.64)	1.47 (1.33–1.79)	1.21 (0.96–1.46)	0.0001
Creatinine, μmol/L, median (IQR)	87.0 (73.0–118.0)	106.0 (81.5–125.8)	84.5 (71.8–109.3)	0.032
CRP,[1] mg/L; median (IQR); N = 150	9.0 (3.6–20.4)	15.0 (6.9–27.5)	7.0 (3.0–15.6)	0.076
Elevated cTn[1,2]; n = (%); N = 126	52/126 (41)	20/42 (48)	32/84 (38)	0.36
CT-IGFBP-4, ng/mL; median (IQR)	106 (67–160)	136 (104–203)	88 (47–133)	0.0018
NT-proBNP, pg/mL; median (IQR)	4282 (2223–7397)	5490 (3604–14 575)	3581 (1568–6172)	0.007

ACEI/ARB, angiotensin-converting enzyme inhibitor/angiotensin receptor blocker; AMI, acute myocardial infarction; ASA, acetylsalicylic acid; CRP, C-reactive protein; cTn, cardiac troponin; ICD, implantable cardioverter defibrillator; IQR, interquartile range; LVEF, left ventricular ejection fraction; NT-proBNP, N terminal pro brain natriuretic peptide; SD, standard deviation.
[1]Some data are missing; available number of patients (N) is indicated; for CRP, 142 samples were available at admission and eight (5.3%) samples were obtained during hospitalization.
[2]Elevated cTn corresponds to cTnT ≥ 0.03 ng/mL or cTnI ≥ 0.035 ng/mL.

NT-proBNP and CT-IGFBP-4 further increased the prognosis of both 1 month and 1 year mortality compared with NT-proBNP or CT-IGFBP-4.

The addition of CT-IGFBP-4 to the clinical prediction model improved the mortality risk prediction for both the 1 month and 1 year follow-up periods (*Table 2*). Although NT-proBNP and CRP individually did not significantly add to the clinical prediction model, combining the clinical model with all three biomarkers (CT-IGFBP-4, NT-proBNP, and CRP) provided the best prognostic value.

The optimal cut-off values of NT-proBNP, CT-IGFBP-4, and CRP for predicting all-cause mortality at 1 year in patients with AHF were 3078 pg/mL, 92.5 ng/mL, and 8.9 mg/L, respectively. These cut-off values corresponded to 85% sensitivity and 47% specificity for NT-proBNP, 81% sensitivity and 58% specificity for CT-IGFBP-4, and 70% sensitivity and 60% specificity for CRP.

Table 2 presents the HRs for all-cause mortality by applying the cut-off values previously defined of circulating NT-proBNP, CT-IGFBP-4, and CRP concentrations and their combinations. In the unadjusted univariate model, increased NT-proBNP, CT-IGFBP-4, and CRP were statistically significant predictors of all-cause mortality. Simultaneous inclusion of NT-proBNP, CT-IGFBP-4, and CRP in the multivariate model, along with standard clinical variables, attenuated the HRs to a certain degree; however, all three biomarkers remained independent predictors of mortality at both 1 month and 1 year.

The combination of NT-proBNP, CT-IGFBP-4, and CRP stratified patients so that two or three elevated biomarkers

Figure 1 Correlation of N-terminal pro brain natriuretic peptide (NT-proBNP), CT-IGFBP-4, and C-reactive protein (CRP) in a study cohort of patients with acute heart failure.

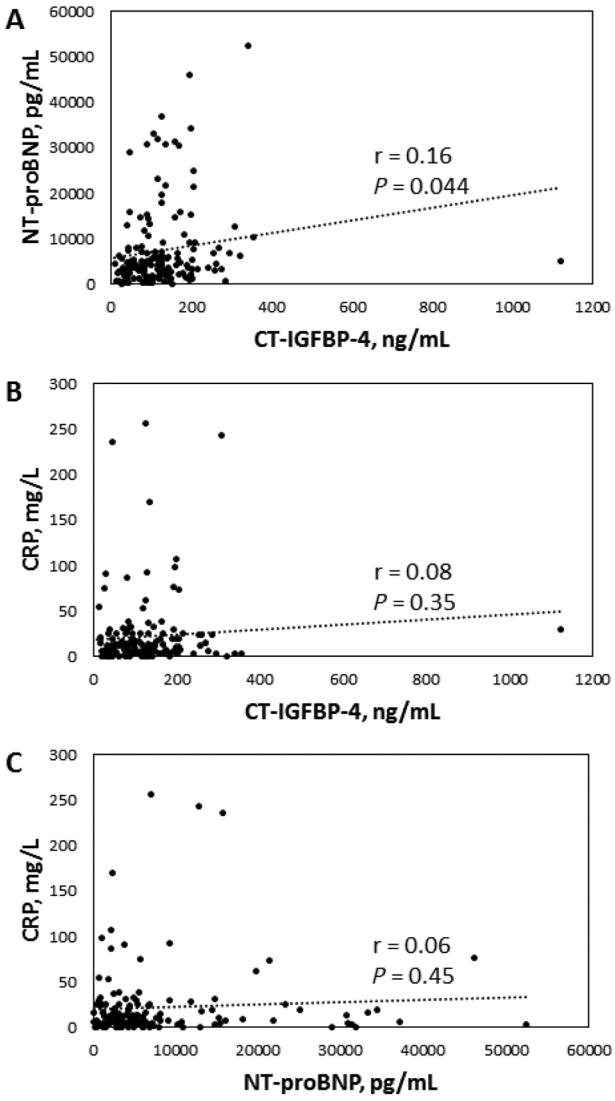
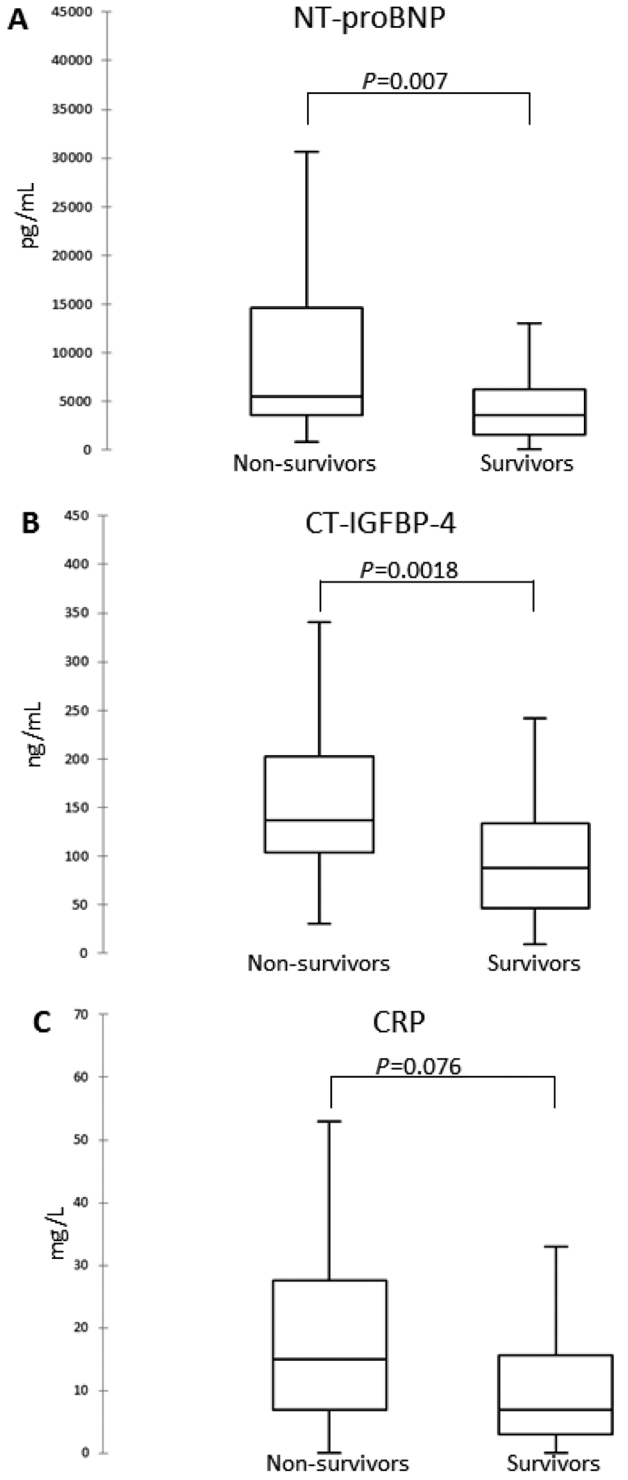


Figure 2 N-terminal pro brain natriuretic peptide (NT-proBNP) (A), CT-IGFBP-4 (B), and C-reactive protein (CRP) (C) concentrations at admission in 1 year survivors and non-survivors with acute heart failure. The central line represents median, box represents interquartile range, and whiskers represent 5th and 95th percentiles.

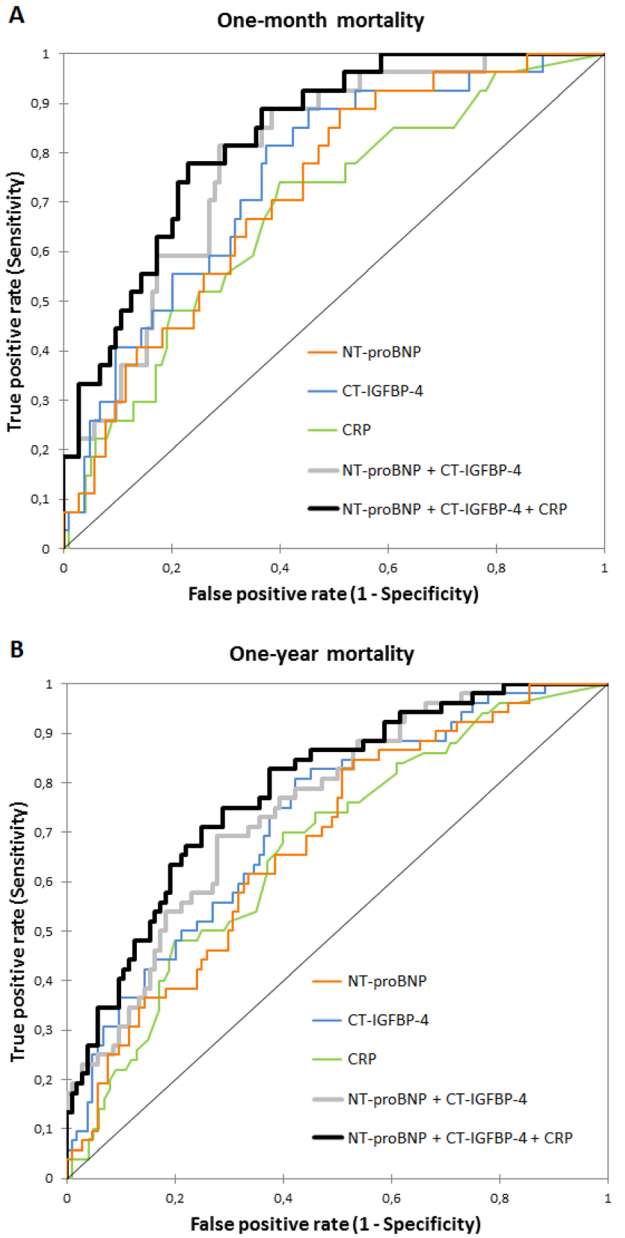


identified a group with a particularly high risk of death (with the group in which no biomarker or only one biomarker was above the cut-off as a reference). After adjustment for standard clinical variables, the combination of the biomarkers remained independent predictors of mortality (HR 10.04 for 1 year mortality).

Based on the defined cut-off values, a Kaplan–Meier analysis of survival was performed. In the Kaplan–Meier analysis, we compared the mortality in three or two patient groups divided based on the CT-IGFBP-4, NT-proBNP, and CRP levels (*Figure 4*).

The stratification of the patients into two groups according to the NT-proBNP, CT-IGFBP-4, and CRP cut-off levels demonstrated a significant improvement in the risk stratification

Figure 3 Receiver operator characteristic analysis of the clinical prediction model, N-terminal pro brain natriuretic peptide (NT-proBNP), CT-IGFBP-4, C-reactive protein (CRP), and their combinations. Prediction of all-cause mortality at 1 month (A) and 1 year (B) by NT-proBNP, CT-IGFBP-4, CRP, and their combinations. $P < 0.001$ for all ROC curves compared with 0.5 curves.



with the combined biomarkers. If the groups were divided based on a single biomarker (NT-proBNP, CT-IGFBP-4, or CRP) value, the subgroup of the patients who died in the groups of elevated biomarkers ranged from 44.4% to 48.8% compared with 14.0–20.0% in the groups of low biomarkers. However, the stratification of the patients into two groups according to the combination of NT-proBNP, CT-IGFBP-4, and CRP indicated 51.0% mortality in the group in which two or three biomarkers increased and only 5.0% in the group in which one biomarker or no biomarkers increased (*Figure 4*; Supporting Information, *Table* S1, Supplementary materials).

The stratification of the patients into three groups was more flat and informative as it enabled defining low, intermediate, and high risk groups of patients. While 54.8% died in the 'high risk' group (high NT-proBNP and high CT-IGFBP-4), no patient died in the 'low risk' group (low NT-proBNP and low CT-IGFBP-4) during the 1 year follow-up period. The addition of CRP to NT-proBNP and CT-IGFBP-4 in the combined analysis enabled defining a 'very high risk' group (all three biomarkers increased) in which 76.9% of the patients died during the 1 year follow-up period (*Figure 4*; Supporting Information, *Table* S1).

Discussion

In the present study, we demonstrated that elevated CT-IGFBP-4 was a strong predictor of all-cause mortality in patients with AHF at both the 1 month and 1 year follow-ups. CT-IGFBP-4 showed a weak or no correlation with NT-proBNP and CRP, which likely reflected the different molecular mechanisms involved in the elevation of these biomarkers in AHF.

NT-proBNP is currently part of the recommended diagnostic workup of suspected AHF. NT-proBNP is also widely investigated as the biomarker for the prognosis of mortality in patients with AHF.[9,18,20] Although NT-proBNP independently predicted mortality in patients with AHF after adjustment for standard clinical variables, such as age, gender, systolic or diastolic blood pressure, heart rate, impaired renal function, glomerular filtration rate, sodium, and haemoglobin,[12] NT-proBNP in its prognostic application is clearly far from ideal. In most clinical studies, the ROC AUC values for short-term mortality for NT-proBNP in patients with AHF are typically within 0.67–0.77.[20,21] The use of additional biomarkers in combination with NT-proBNP demonstrated a significant increase in the prognostic value compared with NT-proBNP alone.[11–13] The search for novel biomarkers that maximally contribute to the known prognostic biomarkers and clinical variables is of substantial importance for early and effective AHF management, which has progressed significantly in recent years.[22,23]

IGFBP-4 fragments were initially established as prognostic biomarkers of major adverse cardiac events (MACE) and mortality in patients with cardiac ischaemia and type 1 diabetes.[19,24] Hjortebjerg *et al.*[17] recently showed that NT-IGFBP-4 and CT-IGFBP-4 predict cardiac and all-cause mortality in a group of 656 patients with ST-elevation myocardial infarction. CT-IGFBP-4 demonstrates the best prognostic value in the

DOI: 10.1002/ehf2.12590

Table 2 ROC curve and Cox proportional hazard ratio analyses of all-cause mortality within 1 year and 1 month follow-up.

ROC curve analysis		
	One month mortality; ROC AUC (95% CI)	One year mortality; ROC AUC (95% CI)
Comparison of biomarkers and their combinations		
NT-proBNP	**0.722 (0.624–0.820)** $P = 0.2563^{2}$	**0.680 (0.595–0.765)** $P = 0.0454^{2}$
CT-IGFBP-4	**0.753 (0.657–0.850)** $P = 0.0144^{3}$	**0.727 (0.646–0.807)** $P = 0.0159^{3}$
CRP	**0.683 (0.575–0.791)** $P = 0.1749^{1}$	**0.669 (0.583–0.755)** $P = 0.6627^{1}$
NT-proBNP combined with CT-IGFBP-4	**0.793 (0.709–0.877)** $P = 0.0079^{1}$ $P = 0.1337^{2}$	**0.752 (0.676–0.829)** $P = 0.0018^{1}$ $P = 0.2575^{2}$
NT-proBNP combined with CRP	**0.776 (0.682–0.871)** $P = 0.0475^{1}$ $P = 0.0011^{3}$	**0.748 (0.669-0.827)** $P = 0.0035^{1}$ $P = 0.0009^{3}$
CT-IGFBP-4 combined with CRP	**0.801 (0.719–0.883)** $P = 0.0720^{2}$ $P < 0.0001^{3}$	**0.763 (0.688–0.839)** $P = 0.1048^{2}$ $P = 0.0001^{3}$
NT-proBNP combined with CT-IGFBP-4 and CRP	**0.833 (0.759–0.907)** $P < 0.0001^{1}$ $P = 0.0022^{2}$ $P < 0.0001^{3}$	**0.788 (0.716–0.860)** $P < 0.0001^{1}$ $P = 0.0064^{2}$ $P < 0.0001^{3}$
Comparison of clinical models with different biomarkers included		
Clinical prediction model	**0.710 (0.594–0.825)**	**0.699 (0.610–0.789)**
Clinical prediction model combined with NT-proBNP	**0.770 (0.675–0.865)** $P = 0.1062^{4}$	**0.732 (0.648–0.816)** $P = 0.1679^{4}$
Clinical prediction model combined with CT-IGFBP-4	**0.798 (0.700–0.89)** $P = 0.0357^{4}$	**0.774 (0.696-0.853)** $P = 0.0254^{4}$
Clinical prediction model combined with CRP	**0.784 (0.681–0.886)** $P = 0.0671^{4}$	**0.747 (0.662–0.832)** $P = 0.0643^{4}$
Clinical prediction model combined with CT-IGFBP-4, NT-proBNP, and CRP	**0.870 (0.802–0.938)** $P = 0.0009^{4}$	**0.817 (0.747–0.887)** $P = 0.0020^{4}$
Cox proportional hazard ratio analysis		
	One month mortality; Hazard ratio (95% CI)	One year mortality; Hazard ratio (95% CI)
Univariate analysis		
NT-proBNP	**5.83 (1.76–19.39)** $P = 0.004$	**3.89 (1.83–8.26)** $P = 0.0004$
CT-IGFBP-4	**6.15 (2.12–17.79)** $P = 0.0008$	**4.20 (2.11–8.39)** $P < 0.0001$
CRP	**2.86 (1.48–5.53)** $P = 0.0018$	**2.42 (1.46–3.99)** $P = 0.0006$
Combination NT-proBNP, CT-IGFBP-4, and CRP (Two or three biomarkers above cut-off[6])	Not applicable as no endpoint occurred within 1 month in the reference group	**13.66 (4.25–43.87)** $P < 0.0001$
Multivariate analysis[5]		
NT-proBNP	**4.95 (1.85–13.27)** $P = 0.0015$	**3.29 (1.66–6.55)** $P = 0.0006$
CT-IGFBP-4	**5.39 (2.11–13.76)** $P = 0.0004$	**3.26 (1.63–6.51)** $P = 0.0008$
CRP	**2.54 (1.37–4.72)** $P = 0.0032$	**2.16 (1.34–3.49)** $P = 0.0017$
Combination NT-proBNP, CT-IGFBP-4, and CRP (two or three biomarkers above cut-off[6])	Not applicable as no endpoint occurred within 1 month in the reference group	**10.04 (4.62-21.86)** $P < 0.0001$

Clinical prediction model included age, gender, systolic blood pressure on admission, creatinine, sodium, previous history of HF, hypertension, and coronary artery disease. $P < 0.001$ for all ROC curves compared with 0.5 curves.

CRP, C-reactive protein; NT-proBNP, N terminal pro brain natriuretic peptide; ROC AUC, receiver operator characteristic area under the curve.

[1]*P*-values for comparison with NT-proBNP.

[2]*P*-values for comparison with CT-IGFBP-4.

[3]*P*-values for comparison with CRP.

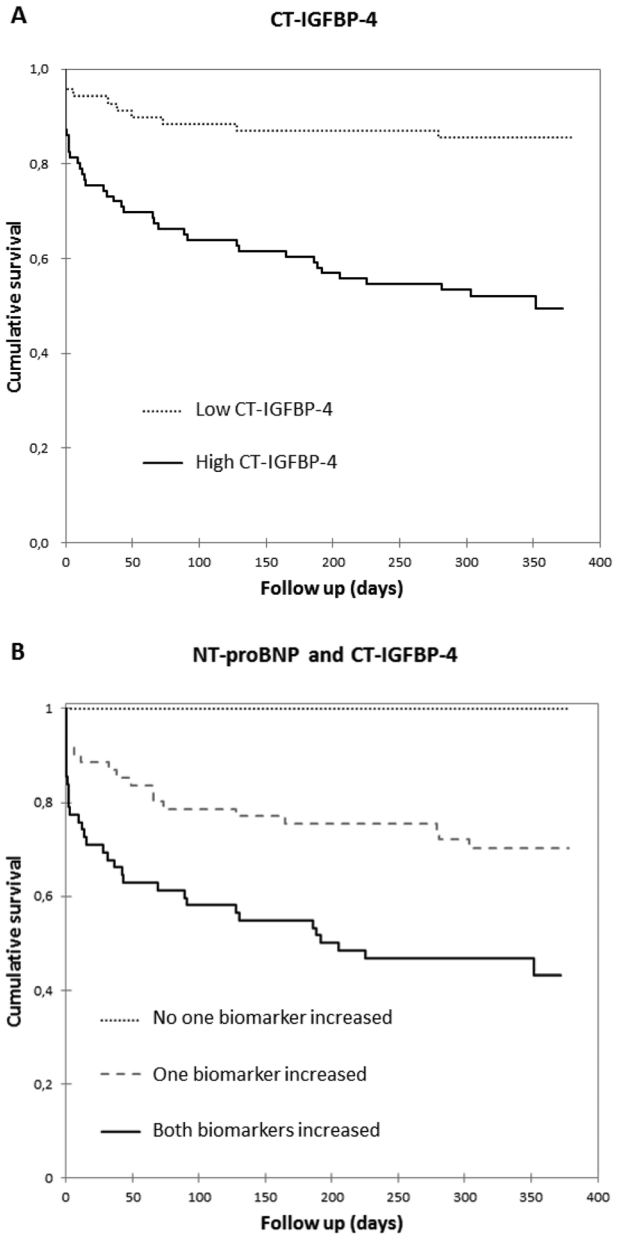
[4]*P*-values for comparison with clinical prediction model.

[5]Multivariate Cox proportional hazard ratio analysis included NT-proBNP, CT-IGFBP-4, CRP, cystatin C, cTnT or cTnI, age, gender, early diagnosed HF, coronary artery disease, hypertension, chronic kidney failure, history of diabetes, heart rate, haemoglobin, and plasma sodium.

[6]Combination NT-proBNP, CT-IGFBP-4, and CRP (two or three biomarkers above cut-off) compared with reference group: no biomarkers increased or one biomarker increased for NT-proBNP, CT-IGFBP-4, and CRP. CI, confidence interval.

DOI: 10.1002/ehf2.12590

Figure 4 Kaplan–Meier survival curve for patients according to N-terminal pro brain natriuretic peptide (NT-proBNP), CT-IGFBP-4, and C-reactive protein levels. The patients are divided into two groups (A) or three groups (B) according to the NT-proBNP ('Increased': ≥3078 pg/mL) and CT-IGFBP-4 ('Increased': ≥92.5 ng/mL) levels as indicated in the legends. Log-rank *P*-values were <0.001 for all figures.



subgroup of these patients with left ventricular ejection fraction (LVEF) 46 ± 9. García-Osuna *et al*. followed up 196 patients with ST-elevation myocardial infarction for 6 months. The authors found that high CT-IGFBP-4 is associated with an increased risk of future MACE.[25]

We hypothesized that CT-IGFBP-4 might be a prognostic biomarker in patients with AHF. The most important finding of the present study is that the combination of CT-IGFBP-4 and NT-proBNP provided a significant added value for the prediction of mortality over NT-proBNP alone, even in the limited number of patients (*n* = 156). CRP was also significantly predictive for all-cause mortality in the ROC curve analysis; that is why CRP, along with CT-IGFBP-4 and NT-proBNP, was included in the analysis of the prognostic value of combinations of the analytes. We subsequently used a clinical model that included standard variables predicting mortality in patients with AHF as a comparator for additional biomarkers. Other clinical variables such as New York Heart Association (NYHA) class, LVEF value, or medical therapy did not significantly add to the prognostic value of the model and were not included into the model. It was found that CT-IGFBP-4, in contrast to NT-proBNP and CRP, added significant prognostic value to the model. The inclusion of all three biomarkers into the basic clinical model made the model even stronger.

Kaplan–Meier and Cox proportional hazard regression analyses supported the advantage of the combination of CT-IGFBP-4, NT-proBNP, and CRP for all-cause mortality in patients with AHF. As mortality could be associated with many factors such as age, heart rate, and others, multivariate HRs models were adjusted for standard clinical variables. It was previously speculated that some of the differences in IGFBP-4 fragment concentrations in the circulation could be explained by distinct clearance rates.[19] However, in patients with AHF, CT-IGFBP-4 remained an independent predictor of mortality after the inclusion of cystatin C into the multivariate model (*Table 2*).

Notably, 52% of deaths occurred during the first month of observation. Thus, it is important that the improvement was demonstrated for the combination of NT-proBNP, CT-IGFBP-4, and CRP in the 1 month follow-up period. The combination of these three biomarkers enabled the selection of a group of 'high risk' patients, in which 50% patients died within 1 month, while no single patient died within 1 month in the group of 60 (38.5%) patients who had one biomarker or no biomarkers increased (*Figure 4*; Supporting Information, *Table* S1). It has previously been shown that the admission levels of NT-proBNP in patients with acute decompensated HF are poorer predictors of adverse outcomes than levels of this biomarker at 48 h or discharge (after a median 13 days of hospitalization).[21] Thus, the combination of the admission levels of NT-proBNP, CT-IGFBP-4, and CRP can be a valuable tool to guide immediate treatment decisions during the index AHF hospitalization.

The lack of dependency of the biomarkers may explain the significant added value of the combination of CT-IGFBP-4, NT-proBNP, and CRP. The absence of a correlation or a very weak correlation among all three biomarkers in the investigated cohort (*Figure 1*) may indicate the different natures and different pathophysiological functions of these proteins in the development of HF.

The ability of CT-IGFBP-4 to predict mortality in patients with AHF is rather surprising and currently does not fit well into the accepted role and place of the PAPP-A/IGFBP-4/IGF system in the pathogenesis of cardiovascular diseases. As the products of IGFBP-4 cleavage under the action of metalloproteinase PAPP-A, IGFBP-4 fragments can reflect the clinical value of PAPP-A. The majority of PAPP-A studies related to cardiovascular diseases were performed in patients with acute coronary syndrome or chest pain. The studies show that measurements of blood PAPP-A could be of clinical value for AMI diagnosis, as well as all-cause mortality, AMI, or MACE prediction.[26–28]

Few studies have examined the prognostic significance of PAPP-A in patients with established HF. In a recent study on a group of 683 patients with NYHA HF Classes III–IV, the ability of PAPP-A to predict 7 year mortality was investigated.[29] In a univariate Cox proportional hazard model, PAPP-A $>$ 10 mIU/L is a significant predictor of mortality. In a 2011 study, Funayama *et al*. examined mortality in 182 patients with HF with different NYHA classes during a mean follow-up period of 796 days. The authors show that the serum PAPP-A levels were related to the severity of HF and associated with a higher risk for adverse cardiac events. In a multivariate Cox proportional hazards regression model, both BNP and PAPP-A are independent predictors of cardiac events.[30] On the basis of their data, Funayama *et al*. suggest that PAPP-A might be involved in the pathogenesis of HF.

Thus, it can be assumed that the role of PAPP-A in the pathogenesis of cardiovascular diseases is not limited to the activation of proliferation of smooth muscle cells within atherosclerotic plaques and destabilization of the plaques; it may also be associated with myocardial remodelling that occurs after AMI. Further research is clearly needed to clarify the role of the PAPP-A/IGFBP-4/IGF regulatory system in myocardial remodelling.

As a cardiovascular biomarker, IGFBP-4 fragments have several advantages over PAPP-A, including several orders of magnitude higher levels in circulation, a lack of interfering complexes with other circulating proteins, and the independence of the presence of heparin in the blood.[31,32] The current findings suggest that CT-IGFBP-4 could become an important component of the prognostic assessment of patients with AHF, along with other biomarkers and clinical variables.

At present, it seems that the best risk stratification of adverse outcomes in patients with AHF can be obtained by a combination of several circulating biomarkers and clinical variables but not by any single biomarker. The advantage of the multimarker approach in the prediction of AHF adverse outcomes seems to be related to the variety of different processes that occur during myocardial remodelling. The mechanisms of pathological remodelling of the myocardium of ischaemic and other origins have been actively studied in recent years. Changes in the phenotypes of cardiomyocytes, inflammation, cardiac fibrosis, and specific immune responses related to HF may all provide a certain contribution to the pathological changes of the heart. Each facet of the process of myocardial remodelling can be reflected by certain circulating biomarkers. Further studies on larger samples of patients with HF at different stages will make it possible to refine the prognostic ability of IGFBP-4 fragments in this application and will also stimulate investigations of the role of the PAPP-A/IGFBP-4/IGF axis in the pathophysiology of HF.

Conclusions

CT-IGFBP-4 independently predicted all-cause mortality in patients with AHF. CT-IGFBP-4, together with NT-proBNP and CRP, significantly improved the prognostic risk stratification for all-cause mortality in patients with AHF.

Limitations

Additional clinical studies on larger cohorts of patients with a larger number of different types of endpoints are needed to confirm the value of IGFBP-4 fragments as a prognostic biomarker in HF. Unfortunately, we did not have enough data to analyse cardiac mortality separately, as the reason for death is only known for limited number of non-survivors. We have data on eight patients having implantable cardioverter defibrillator. However, this number is not enough for reliable statistical analysis of the impact of implantable cardioverter defibrillator on the management of the patients within the investigated cohort. Some clinical characteristics of patients were lacking (*Table 1*), which limited detailed analysis of the influence of these factors to the prognostic value of CT-IGFBP-4. Management of patients with HF enrolled in the study in 2004 might not necessarily reflect modern clinical guidelines. Studies are needed to compare IGFBP-4 fragments with other prospective biomarkers of HF, such as galectin-3, growth differentiation factor-15, and soluble suppression of tumorigenicity-2.

Conflict of interest

Alexey Konev, Alexey Kharitonov, Fedor Rozov, Evgeny Altshuler, Daria Serebryanaya, Johan Lassus, Veli-Pekka Harjola, Alexey Katrukha, Alexander Postnikov have nothing to declare.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Distribution of patients according to NT-proBNP, CT-IGFBP-4, and CRP levels.

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