
SYMBIOSIS

Part II

THE ROLE OF THE KIDNEYS IN HEART FAILURE AND BEYOND

CHAPTER 7

Cardiometabolic Biomarkers and their Temporal Patterns Predict Poor Outcome in Chronic Heart Failure The Bio-SHiFT study

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ABSTRACT

Background

Multiple hormonal and metabolic alterations occur in chronic heart failure (CHF), but their proper monitoring during clinically silent progression of CHF remains challenging. Hence, our objective was to explore whether temporal patterns of six emerging cardiometabolic biomarkers predict future adverse clinical events in stable patients with CHF.

Methods

In 263 CHF patients, we determined the risk of a composite endpoint of HF-hospitalization, cardiac death, LVAD-implantation and heart transplantation in relation to serially assessed blood biomarker levels and slopes (i.e., rate of biomarker change per year). During 2.2 years of follow-up, we repeatedly measured insulin-like growth binding protein 1, 2, and 7 (IGFBP-1, IGFBP-2, IGFBP-7), adipose fatty acid-binding protein 4 (FABP-4), resistin, and chemerin (567 samples in total).

Results

Serially measured IGFBP-1, IGFBP-2, IGFBP-7, and FABP-4 levels predicted the endpoint (univariable HR [95% confidence interval] per 1SD increase: 3.34 [2.43–4.87], 2.86 [2.10–3.92], 2.45 [1.91–3.13], and 2.46 [1.88–3.24], respectively). Independently of the biomarkers' levels, their slopes were also strong clinical predictors (per 0.1SD increase/year: 1.20 [1.11–1.31], 1.27 [1.14–1.45], 1.23 [1.11–1.37], and 1.27 [1.12–1.48]). All associations persisted after multivariable adjustment for patient baseline characteristics, baseline NT-proBNP and cardiac troponin T, and pharmacological treatment during follow-up.

Conclusions

The temporal patterns of IGFBP-1, IGFBP-2, IGFBP-7, and adipose FABP-4 predict adverse clinical outcomes during outpatient follow-up of CHF patients, and may be clinically relevant as they could help detect more aggressive CHF forms and assess patient prognosis, and ultimately aid in designing more effective biomarker-guided therapy.

INTRODUCTION

Chronic heart failure (CHF) is a clinical syndrome characterized by recurrent episodes of decompensation that require constant therapeutic interventions.[1] After occurrence of initial cardiac alterations in heart failure, the failing heart also induces abnormalities in peripheral organs including the lungs, liver, kidneys, gastrointestinal tract, skeletal muscles, and endocrine system.[2] Together, these abnormalities cause the overall energy balance to shift towards a catabolic state, leading to exercise intolerance and weight loss, both of which strongly determine poor outcome.[3,4] In this context, circulating biomarkers could be an effective clinical tool, as these cellular signals naturally precede the patient's functional decline, and may therefore provide early tissue-specific information on CHF. Similarly, their temporal patterns could help in monitoring disease progression even in the presymptomatic phase, potentially enabling physicians to timely modify therapy to prevent impending decompensation.

Although it has long been known that multiple hormonal and metabolic alterations occur in CHF[5], the biomarkers that reflect these alterations have only recently received increasing attention with the upcoming use of modern -omics technologies that allow us to discover new highly sensitive proteins.[6] To date, ongoing controversy exists concerning the role of these cardiometabolic biomarkers in CHF. Studies have suggested that insulin-like growth factor binding proteins (IGFBPs) 1, 2, and 7 are increased in CHF and are associated with adverse outcomes after myocardial infraction.[7-9] IGFBPs regulate insulin-like growth factor (IGF) activity which is crucial for indirect effects of growth hormone (GH).[10] Of note is that IGFBPs also exhibit IGF-independent effects on the cardiovascular system.[11] In this way, the IGF-IGFBPs system has an important role in the regulation of cardiac remodeling, myocardial contractility, and vascular system function.[10] Similarly, the adipose tissue acts as an endocrine organ by secreting adipokines which are involved in a plethora of metabolic functions including glucose and lipid metabolism, inflammation, atherosclerosis, and cardiac remodeling.[12] Among secreted adipokines, fatty acid-binding protein (FABP)-4, resistin, and chemerin have recently been linked to CHF.[13-15] Nevertheless, the scientific evidence on these biomarkers in CHF is limited, and their potential utility remains undetermined.

Therefore, we investigated the associations of the temporal patterns of cardiometabolic biomarker levels and biomarker slopes (i.e., rates of biomarker change per year) with adverse clinical events in CHF patients who had undergone 3-monthly repeated blood sampling during their outpatient follow-up.

METHODS

CHF cohort

The Serial Biomarker Measurements and New Echocardiographic Techniques in Chronic Heart Failure Patients Result in Tailored Prediction of Prognosis (Bio-SHiFT) is a prospective cohort study of stable patients with CHF, conducted in Erasmus MC, Rotterdam, and Noordwest Ziekenhuisgroep, Alkmaar, the Netherlands.[16] Patients were included if aged ≥18 years, capable of understanding and signing informed consent, and if CHF had been diagnosed ≥3 months ago according to European Society of Cardiology guidelines.[1,17,18] Patients were ambulatory and stable, i.e., they had not been hospitalized for HF in the past three months. The study was approved by the medical ethics committees, conducted in accordance with the Declaration of Helsinki, and registered in ClinicalTrials.gov (NCT01851538). Written informed consent was obtained from all patients. This investigation comprised 263 CHF patients enrolled during the first inclusion round period (October 2011 until June 2013).

Baseline assessment

All patients were evaluated by research physicians, who collected information on HF-related symptoms, NYHA class, and performed a physical examination. Information on HF etiology, left ventricular ejection fraction, cardiovascular risk factors, medical history and treatment was retrieved primarily from hospital records and was checked in case of ambiguities. History of cardiovascular and other comorbidities was defined as their clinical diagnosis as recorded in the medical file.

Follow-up and study endpoints

During the study, all patients were routinely followed at the outpatient clinic by treating physicians who were blinded for biomarker sampling. Additionally, study follow-up visits were predefined and scheduled every 3 months (±1 month). This 3-month interval was chosen to ensure that blood sampling occurred as often as possible during a relatively long follow-up period (>2 years), while keeping the study burden acceptable for this CHF population. At each study follow-up visit, a short medical evaluation was performed and samples were collected. During follow-up, all medication changes and occurrence of hospitalizations for HF, MI, PCI, CABG, arrhythmias, and CVA, cardiac transplantation, left ventricular assist device (LVAD) implantation and mortality, were recorded in the electronic case

report forms, and associated hospital records and discharge letters were collected. Subsequently, a clinical event committee, blinded to the biomarker results, reviewed hospital records and discharge letters and adjudicated the study endpoints.

The composite endpoint comprised cardiac death, cardiac transplantation, LVAD implantation, and hospitalization for the management of acute or worsened HF, whichever occurred first. Cardiac death was defined as death from MI or other ischemic heart disease (ICD-10: I20-I25), death from other heart disease including HF (I30-I45 and I47-I52), sudden cardiac death (I46), sudden death undefined (R96) or unwitnessed or ill-described death (R98, R99). Hospitalization for acute or worsened HF was defined as a hospitalization for an exacerbation of HF symptoms, in combination with two of the following: BNP or NT-proBNP >3x upper limit of normal, signs of worsening HF, such as pulmonary rales, raised jugular venous pressure or peripheral edema, increased dose or intravenous administration of diuretics, or administration of positive inotropic agents.[1]

Study measurements and laboratory analysis

Blood samples were collected at baseline and at each study follow-up visit, and were processed and stored at -80°C within two hours after collection. Treating physicians were unaware of biomarker results as biomarkers were measured batch-wise after completion of follow-up. Thus, the biomarker measurements did not lead to drug adjustments. All patients received usual care. All laboratory personnel was blinded for clinical data and patient outcomes.

For efficiency, for the current investigation we selected all baseline samples, the two samples closest in time to the primary composite endpoint, and the last sample available for patients in whom the primary endpoint did not occur during follow-up.

Glomerular filtration rate (GFR) was determined by the Chronic Kidney Disease-Epidemiology Collaboration (CKD-EPI) equation validated in HF patients.[19]

The Olink multiplex PEA platform for new biomarkers

The Cardiovascular (CVD) panel III (Olink Proteomics AB, Uppsala, Sweden) was used for analysis of high-abundance proteins. The proteins present in this Olink panel were selected because either they have a proven pathophysiological role in cardiovascular disease, or because they are promising in this respect but yet unexplored. This assay is based on PEA (proximity extension assay) technology.[6] In brief, the assay uses two oligonucleotide-labeled antibodies to bind to their respec-

tive target proteins in the sample. When the two antibodies are in close proximity, a new PCR target sequence is formed by a proximity-dependent DNA polymerization event. The resulting sequence is subsequently detected and quantified using standard real-time PCR. Each sample includes two incubations, one extension, and one detection control to determine the lower limit of detection and normalize the measurements. The biomarkers are presented in normalized protein expression (NPX) units on a 2log scale. In a validation study, the mean intra-assay and inter-assay coefficients of variation were 8% and 12%, respectively.[6] For the current investigation, six emerging cardiometabolic biomarkers (IGFBP-1, IGFBP-2, IGFBP-7, FABP-4, resistin, and chemerin) were examined.

Statistical analysis

For the analysis, we used the Z-score (i.e., the standardized form) of the 2log-transformed biomarkers to allow for direct comparisons of different biomarkers. For the network analysis we used only the biomarkers that showed significant correlations based on Pearson's correlation coefficients ($p<0.05$). We assessed the clustering coefficient as a measure of the degree to which biomarkers tend to cluster together, where higher coefficients suggest a certain centrality of a biomarker within the network.[20]

To study the effect of baseline characteristics on repeatedly measured biomarkers, linear mixed-effects (LME) models were performed using biomarkers as the dependent variables and baseline characteristics as the independent variables (fixed part). The sampling time was entered into the fixed- and random parts of the models.

To estimate the associations between patient-specific biomarker levels and survival, we applied a joint modeling (JM) analysis that combines LME models for repeated measurements, and Cox survival analysis for time-to-event data.[21] For both the fixed- and random-effects parts of the LME models, linear terms were used for sampling times, and both intercepts and slopes were included in the random-effects design matrix. This allowed the markers' trajectories to differ at baseline and over time. We also estimated the time-dependent slope (i.e., rate of change) of each biomarker from these joint models, indicating whether and by how much the levels are increasing or decreasing and how they relate to patient prognosis. The slope mathematically corresponds to the first derivative of a marker's trajectory, and is presented as an annual change in Z-scores (i.e., delta Z-scores per year).

Besides sampling time, all markers were adjusted as follows: (1) clinical model: Cox and LME models were adjusted for age, sex, diabetes, atrial fibrillation, baseline NYHA class, diuretics, systolic blood pressure, and eGFR; (2) clinical & time-varying HF medication model: after adjusting for clinical characteristics, biomarker values were extracted from the joint models and entered simultaneously with equivalent doses of carvedilol, enalapril, furosemide, and spironolactone (repeatedly assessed during follow-up) into a time-dependent Cox analysis to examine the incremental value of the new biomarkers over clinical characteristics and medication during follow-up; (3) cardiac biomarker model: Cox and LME models were adjusted for biomarkers of myocardial stretch and damage (NT-proBNP and c-TnT). Data on all variables were complete, except for systolic blood pressure which was missing in <5% of patients and for which imputations were applied using the patients' clinical and outcome data. Results are given as hazard ratios (HR) and 95% confidence intervals (CI) per 1SD increase of the marker's level and per 0.1SD increase of the slope at any time-point during follow-up.

To correct for multiple testing, we performed matrix spectral decomposition which has previously been demonstrated to be more effective than Bonferroni correction.[22] Consequently, the corrected significance level was set at $p < 0.0127$ (0.05/4).

All analyses were performed with R Statistical Software using packages nlme and JMbayes.[21] The network analysis was performed using Gephi software (https://gephi.org) and the matSpD application (https://gump.qimr.edu.au/general/daleN/matSpD) available online.

RESULTS

Baseline characteristics

Table 1 displays the patients' baseline characteristics. Specifically, the patients who reached the composite endpoint were older, more frequently had diabetes, atrial fibrillation, lower systolic blood pressure, higher NYHA class, higher levels of NT-proBNP and cardiac troponin T, and were more frequently on loop diuretics.

TABLE 1 Baseline characteristics in relation to the occurrence of the composite endpoint.

Variable	Total	Composite endpoint		p-value
		Yes	No	
n (%)	263 (100)	70 (27)	193 (73)	
Demographics				
Age, years (mean ± SD)	67±13	69±13	66±12	0.05
Men, n (%)	189 (72)	53 (76)	136 (70)	0.41
Clinical characteristics				
BMI, kg/m^2	27.5±4.7	27.6±4.8	27.4±4.7	0.80
Heart rate, b.p.m.	67±12	69±13	67±11	0.31
SBP, mmHg	122±20	117±17	124±21	0.02
DBP, mmHg	72±11	70±10	73±11	0.06
Features of heart failure				
NYHA class III or IV, n (%)	69 (26)	31 (44)	38 (20)	< 0.001
HF-rEF n (%)	250 (95)	66 (94)	184 (95)	0.75
HF-pEF n (%)	13 (5)	4 (6)	9 (5)	
LVEF, %	32±11	30±11	33±10	0.18
NT pro-BNP (pmol/L) †	137.3 (51.7–272.6)	282.4 (176.4–517.4)	95.3 (31.72–207.7)	< 0.001
Hs-TnT (ng/L) †	18.0 (9.5–33.2)	31.9 (20.6–49.7)	13.9 (8.4–26.7)	< 0.001
Etiology of heart failure, n (%)				
Ischemic	117 (44)	36 (51)	81 (42)	0.17
Hypertension	34 (13)	10 (14)	24 (12)	0.70
Valvular disease	12 (5)	5 (7)	7 (4)	0.23
Cardiomyopathy	68 (26)	15 (21)	53 (28)	0.32
Unknown or Others	32 (12)	4 (6)	28 (15)	
Medical history, n (%)				
Prior MI	96 (36)	32 (46)	64 (33)	0.06
Prior PCI	82 (31)	27 (39)	55 (28)	0.12
Prior CABG	43 (16)	13 (19)	30 (15)	0.57
Atrial fibrillation	106 (40)	36 (51)	70 (36)	0.03
Diabetes	81 (31)	32 (46)	49 (25)	0.002
Hypercholesterolemia	96 (36)	30 (43)	66 (34)	0.20
Hypertension	120 (46)	38 (54)	82 (42)	0.09
COPD	31 (12)	12 (17)	19 (10)	0.10
Medication use, n (%)				
Beta-blocker	236 (90)	61 (87)	175 (91)	0.40
ACE-I or ARB	245 (93)	63 (90)	182 (94)	0.22

continued

Variable	Total	Composite endpoint		p-value
		Yes	No	
Diuretics	237 (90)	68 (97)	169 (88)	0.02
Loop diuretics	236 (90)	68 (97)	168 (87)	0.02
Thiazides	7 (3)	3 (4)	4 (2)	0.28
Aldosterone antagonist	179 (68)	53 (76)	126 (65)	0.11
Glomerular function				
Creatinine, mg/dl †	1.18 (0.99–1.49)	1.30(1.02–1.52)	1.17(0.98–1.45)	0.18
eGFR, mL/min/1.73m^2 †	58 (43–76)	53 (40–73)	59 (44–77)	0.16
KDOQI classification, n (%)				0.18
eGFR ≥90	28 (11)	7 (10)	21 (11)	
eGFR 60-89	95 (36)	20 (28)	75 (39)	
eGFR 30-59	119 (45)	37 (53)	82 (42)	
eGFR <30	21 (8)	6 (9)	15 (8)	

BMI, Body mass index; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; NYHA class, New York Heart Association class; HF-rEF, Heart failure with reduced ejection fraction; HF-pEF, heart failure with preserved ejection fraction; LVEF, left ventricular ejection fraction; MI, myocardial infarction; PCI, percutaneous coronary intervention; CABG, coronary artery bypass grafting; CVA, cerebrovascular accident; TIA, transitory ischemic attack; COPD, chronic obstructive pulmonary disease; ACE-I, angiotensin-converting enzyme inhibitors; ARB, angiotensin II receptor blockers; eGFR, estimated glomerular filtration rate.†Median with inter-quartile range (IQR).

Follow-up and study endpoints

During a median of 2.2 (IQR: 1.4–2.5) years of follow-up, we collected a total of 1984 blood samples at fixed 3-month intervals (per patient: 9 [IQR: 5–10] samples), and measured biomarkers in all samples collected at baseline, the two samples closest in time to the composite endpoint, and the last sample available for event-free patients (567 samples in total). During the follow-up, 70 (27%) patients experienced the composite endpoint. Specifically, 56 patients were re-hospitalized for acute or worsened HF, 3 patients underwent heart transplantation, 2 patients underwent LVAD placement, and 9 patients died of cardiovascular causes.

Network analysis

Figure 1 displays baseline inter-marker correlations and crude associations of their serially measured levels with the composite endpoint (for HRs see Table 3). Of note is that all biomarkers correlated with each other and no clustering was present. The strongest correlations were present within the IGFBP biomarker family,

and between these biomarkers and NT-proBNP. Similarly, adipokines correlated strongly with each other.

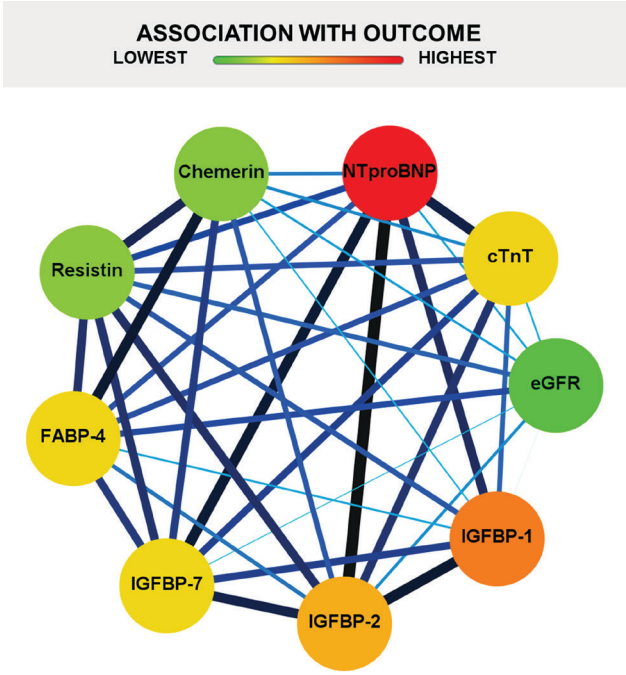


FIGURE 1 Network analysis of cardiometabolic biomarkers depicting inter-marker correlations and associations with the composite endpoint. Node color represents the crude association with the composite endpoint, and ranges from white (the weakest) to black (the strongest). Node size represents the clustering coefficient (a measure of the degree to which biomarkers tend to cluster together suggesting a certain centrality within the biomarker network). The thickness of the line between the biomarkers represents the correlation coefficient (presented only if $p<0.05$); a thicker line represents stronger coefficients.

Patients' clinical characteristics and cardiometabolic biomarkers during follow-up

Table 2 shows the associations between the patients' baseline characteristics and the biomarkers' temporal trends; the reported associations were independent of each other.

IGFBPs

Higher serially measured IGFBP-1 levels during follow-up were associated with increased baseline NT-proBNP and c-TnT, and lower baseline BMI values (per doubling of NT-proBNP: adjusted β [95% confidence interval CI] 0.15SD [0.09; 0.22], p<0.001; c-TnT: 0.13SD [0.02; 0.24], p=0.018; BMI: -0.95SD [-1.38; -0.53], <0.001). Likewise, patients with increased baseline cardiac markers and lower BMI had higher IGFBP-2 levels during follow-up (for regression coefficients see Table 2). The IGFBP-2 levels were also positively associated with older age, higher baseline NYHA class, and impaired baseline eGFR. Similarly, higher IGFBP-7 levels were found in patients with increased baseline cardiac markers and decreased eGFR, and in those who were on higher loop diuretic doses. Patients with atrial fibrillation (AF) also had markedly increased IGFBP-7 levels.

Adipokines

Higher serially measured FABP-4 levels were associated with increased baseline cardiac biomarkers, higher BMI, female sex, and impaired eGFR. Moreover, patients who were on higher baseline β-blockers and loop diuretic doses had higher FABP-4 levels during follow-up. Higher serially measured resistin levels were found in patients with AF, and in those with increased NT-proBNP and decreased eGFR levels. During follow-up, higher serially measured chemerin levels were associated with female gender, higher baseline BMI, decreased eGFR, and higher loop diuretic doses.

Temporal evolutions of IGFBPs and adipokines in relation to study endpoints

Average temporal trajectories of cardiometabolic biomarkers in patients who experienced an incident endpoint and those who did not are displayed in Figure 2. In patients with an incident endpoint, all biomarkers showed increasing patterns to various degrees, with the steepest rise seen in IGFBP-1, IGFBP -2, IGFBP-7, and FABP-4 levels prior to the occurrence of the endpoint.

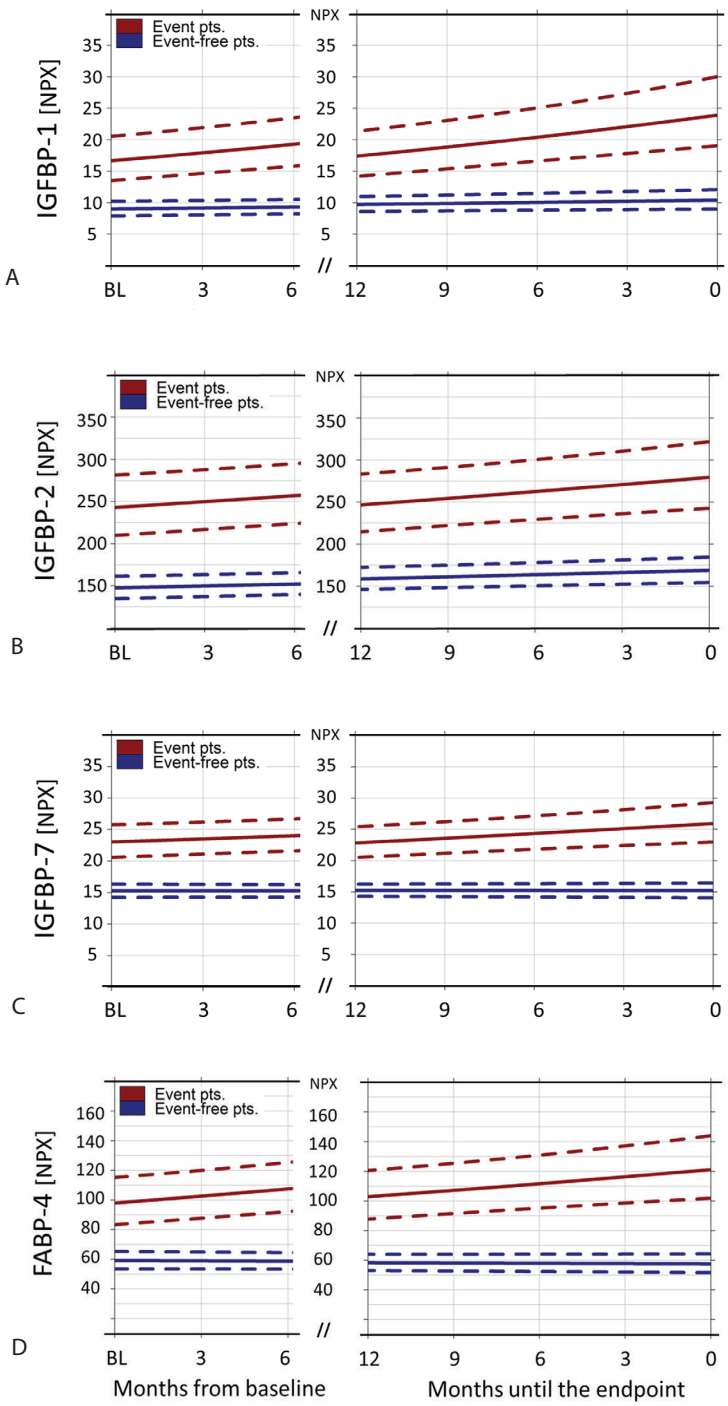
TABLE 2 **Association between baseline characteristics and levels of serially measured biomarkers during follow-up.**

	Dependent variable					
	IGFBP-1		IGFBP-2		IGFBP-7	
Independent variable	**β (95% CI)**	**p-value**	**β (95% CI)**	**p-value**	**β (95% CI)**	**p-value**
Age per 10 yrs.		ns	0.12 (0.01 to 0.20)	0.004		ns
Male sex		ns		ns		ns
BMI per doubling	-0.95 (-1.38 to -0.53)	<0.001	-0.70 (-1.07 to -0.34)	<0.001		ns
NYHA class		ns	0.11 (0.00 to 0.22)	0.05		ns
DM		ns		ns		ns
AF		ns		ns	0.42 (0.23 to 0.60)	<0.001
SBP per 10mmHg		ns		ns		ns
eGFR per 20 ml/min/1.73m^2		ns		ns	-0.10 (-0.19 to -0.01)	0.026
NT-proBNP per doubling	0.15 (0.09 to0.22)	<0.001	0.20 (0.14 to 0.25)	<0.001	0.18 (0.11 to 0.24)	<0.001
cTnT per doubling	0.13 (0.02 to 0.24)	0.018	0.16 (0.07 to 0.25)	<0.001	0.11 (0.00 to 0.21)	0.041
Carvedilol per 50 mg		ns		ns		ns
Enalapril per 40 mg		ns		ns		ns
Furosemide per 40 mg		ns		ns	0.06 (0.02 to 0.11)	0.005
Spironolactone per 25 mg		ns		ns		ns

continued

	Dependent variable					
	FABP-4		Resistin		Chemerin	
Independent variable	**β (95% CI)**	**p-value**	**β (95% CI)**	**p-value**	**β (95% CI)**	**p-value**
Age per 10 yrs.		ns		ns		ns
Male sex	-0.62 (-0.80 to -0.44)	<0.001		ns	-0.38 (-0.60 to -0.16)	<0.001
BMI per doubling	1.41 (1.05 to 1.76)	<0.001		ns	0.51 (0.07 to 0.94)	0.022
NYHA class		ns		ns		ns
DM		ns		ns		ns
AF		ns	0.34 (0.13 to 0.55)	0.001		ns
SBP per 10mmHg		ns		ns		ns
eGFR per 20 ml/min/1.73m^2	-0.23 (-0.31 to -0.15)	<0.001	-0.25 (-0.35 to -0.15)	<0.001	-0.20 (-0.30 to -0.10)	<0.001
NT-proBNP per doubling	0.11 (0.06 to 0.16)	<0.001	0.09 (0.02 to 0.16)	0.011		ns
cTnT per doubling	0.21 (0.12 to 0.30)	<0.001		ns		ns
Carvedilol per 50 mg	0.10 (0.01 to 0.20)	0.038		ns		ns
Enalapril per 40 mg		ns		ns		ns
Furosemide per 40 mg	0.06 (0.03 to 0.10)	0.001		ns	0.08 (0.04 to 0.13)	<0.001
Spironolactone per 25 mg		ns		ns		ns

IGFBP-1, insulin-like growth factor-binding protein 1; IGFBP-2, insulin-like growth factor-binding protein 2; IGFBP-7, insulin-like growth factor-binding protein 7; FABP-4, fatty acid-binding protein 4 (adipocytes); ns, not significant (only the associations with significance level of p-value <0.05 are presented). The effects of the patients' baseline characteristics are given as adjusted β (95% confidence interval) per 1SD differences of biomarkers as measured on the 2log scale. This method allows a direct comparison of the effects on different biomarkers. All βs are adjusted for patients' age, sex, body mass index (BMI), diabetes mellitus (DM), atrial fibrillation (AF), baseline NYHA class, systolic blood pressure (SBP), estimated glomerular filtration rate (eGFR), NT-proBNP levels, cardiac troponin T levels (c-TnT), and equivalent doses of carvedilol, enalapril, furosemide, and spironolactone. Only the associations with significance level of p-value <0.05 are presented.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Months from baseline
Months until the endpoint

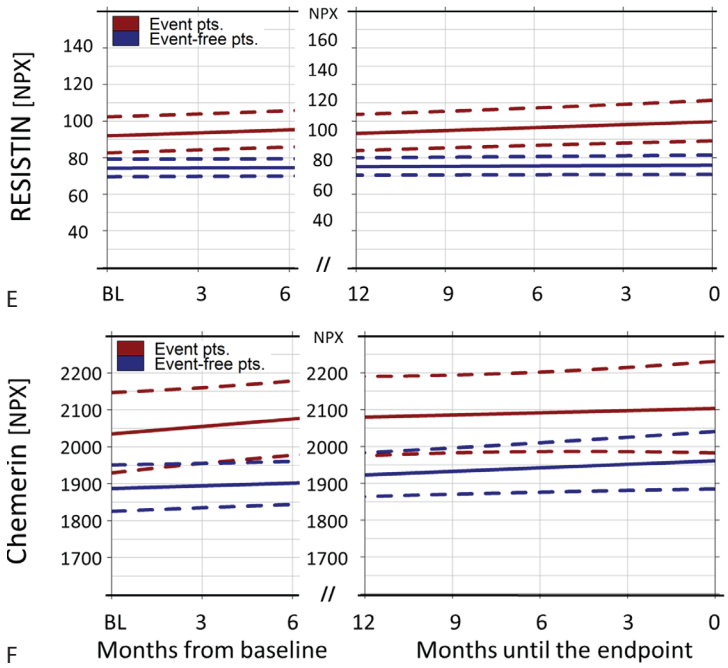

FIGURE 2 Average temporal evolution of IGFBP-1, IGFBP-2, IGFBP-7, FABP-4, resistin, chemerin during follow-up. Average evolution in patients who reached the composite endpoint (solid black line), and in endpoint-free patients (solid gra line). Dashed lines represent the 95% confidence interval. X-axis depicts the time from baseline (BL: left part of the x-axis), and time remaining to the event (patients who experienced incident events) or last sample moment (patients who remained event-free) (right part of the x-axis). Biomarker levels are presented on the y-axis. (a) IGFBP-1 (b) IGFBP-2, (c) IGFBP-7, (d) FABP-4, (e) resistin, (f) chemerin.

Table 3 summarizes the associations of the cardiometabolic biomarkers with the composite endpoint. After adjustment for baseline clinical characteristics and HF pharmacological treatment during follow-up, IGFBP-1, IGFBP-2, IGFBP-7, and FABP-4 independently predicted the endpoint (per 1SD increase of biomarker levels HR [95%CI]: 4.21 [2.96; 6.01], 2.93 [2.11; 4.08], 2.72 [2.06; 3.60], 3.15 [2.36; 4.21], respectively, each $p<0.001$). These biomarkers remained significant predictors even after controlling for baseline NT-proBNP and cTnT levels. Notably, their higher slopes were strong predictors independently of their absolute levels, baseline clinical characteristics and cardiac biomarkers, and HF medication during follow-up. Serially measured resistin predicted the endpoint independently of the patients' clinical characteristics and pharmacological treatment, but lost significance after controlling for cardiac markers. The chemerin did not show clear prognostic value after multivariable adjustments (Table 3).

TABLE 3 **Associations between serially measured cardiometabolic markers and the composite endpoint.**

	Crude model		Clinical model		Clinical & time-varying medication model		Cardiac biomarkers model	
	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Levels (per 1SD increase)								
IGFBP-1	3.34 (2.43–4.87)	<0.001*	3.10 (2.16–4.70)	<0.001*	4.21 (2.96–6.01)	<0.001*	2.65 (1.78–4.12)	<0.001*
IGFBP-2	2.86 (2.10–3.92)	<0.001*	2.71 (1.87–3.96)	<0.001*	2.93 (2.11–4.08)	<0.001*	2.02 (1.37–2.97)	<0.001*
IGFBP-7	2.45 (1.91–3.13)	<0.001*	2.44 (1.78–3.36)	<0.001*	2.72 (2.06–3.60)	<0.001*	1.96 (1.51–2.62)	<0.001*
FABP-4	2.46 (1.88–3.24)	<0.001*	3.94 (2.61–5.95)	<0.001*	3.15 (2.36–4.21)	<0.001*	1.97 (1.51–2.58)	<0.001*
Resistin	1.75 (1.37–2.21)	<0.001*	1.63 (1.18–2.25)	<0.001*	1.74 (1.34–2.26)	<0.001*	1.27 (0.97–1.68)	0.09
Chemerin	1.72 (1.22–2.50)	<0.001*	1.37 (0.91–2.10)	0.13	xx		1.31 (0.93–1.86)	0.12
Slope (per 0.1SD increase / year)[a]								
IGFBP-1	1.20 (1.11–1.31)	<0.001*	1.50 (1.25–1.86)	<0.001*	1.19 (1.13–1.25)	<0.001*	1.18 (1.10–1.28)	<0.001*
IGFBP-2	1.27 (1.14–1.45)	<0.001*	1.66 (1.27–2.33)	<0.001*	1.13 (1.05–1.22)	0.002*	1.21 (1.10–1.35)	<0.001*
IGFBP-7	1.23 (1.11–1.37)	<0.001*	1.43 (1.16–1.86)	<0.001*	1.18 (1.10–1.25)	<0.001*	1.14 (1.04–1.25)	<0.001*
FABP-4	1.27 (1.12–1.48)	<0.001*	1.57 (1.25–2.04)	<0.001*	1.19 (1.12–1.28)	<0.001*	1.16 (1.05–1.31)	0.002*
Resistin	1.35 (1.21–1.56)	<0.001*	1.51 (1.26–1.90)	<0.001*	1.11 (1.02–1.20)	0.018	1.25 (1.13–1.39)	<0.001*
Chemerin	1.30 (1.10–1.50)	<0.001*	1.67 (1.45–1.96)	<0.001*	1.02 (0.65–1.09)	0.58	1.09 (0.95–1.22)	0.22

Hazard ratios (HRs) and 95% confidence intervals (CIs) are given per 1SD increase of the level and per 0.1SD increase of the annual slope of a biomarker at any point in time during follow-up. **Crude model**: Cox model unadjusted, LME model adjusted for sampling time. **Clinical model**: Cox and LME models adjusted for age, sex, body mass index, diabetes, atrial fibrillation, baseline NYHA class, diuretics, systolic blood pressure, eGFR, and sampling time (LME); **Clinical & time-varying medication model**: time-dependent Cox model using a biomarker's fitted values from clinical model adjusted for total daily doses of equivalents of carvedilol, enalapril, furosemide, and spironolactone during follow-up. **Cardiac biomarkers model**: Cox and LME models adjusted for baseline NT-proBNP and c-TnT, and sampling time (LME).
[a] Annual slopes were additionally adjusted for the levels of serially measured biomarker during follow-up. * p value below the corrected significance level for multiple testing (p<0.0127); xx not performed because marker's levels were not significant in clinical model.

DISCUSSION

This study is the first to demonstrate that temporal patterns of IGFBP-1, IGFBP-2, IGFBP-7, and FABP-4 strongly predict adverse clinical outcomes in CHF. Independently of the absolute biomarker levels, their higher slopes (i.e., higher rates of change) were also strong clinical predictors. All aforementioned associations were robust to the multivariable adjustment for baseline clinical characteristics and cardiac natriuretic peptide and troponin levels, as well as CHF pharmacological treatment during follow-up.

IGFBPs in CHF

In CHF, an impaired anabolic drive with increased GH levels but peripheral GH resistance, and elevated IGFBPs, has been reported.[8,23,24] In this regard, studies have suggested that elevated IGFBPs, especially high-affinity IGF binders such as IGFBP-1 and IGFBP-2, indirectly control anabolic activity via their inhibitory function on the GH–IGF-1 axis.[11] The IGFBPs also directly control cell growth and survival which may also contribute to adverse CHF outcomes.[11] Our study confirms that patients who experienced adverse clinical events had higher levels of all three IGFBPs than event-free patients, which rose during the follow-up. These elevated IGFBPs might also explain the conflicting results of clinical trials on GH therapy in CHF.[25] A meta-analysis on GH treatment showed that CHF patients who had a reduced IGF-1 response to GH administration were less likely to benefit from this treatment. Thus, it may be speculated that elevated IGFBPs may be responsible for the "non-responsiveness" to GH treatment in these patients, but further investigations are needed to confirm this hypothesis.

Besides higher levels, the rise of IGFBP-1, IGFBP-2, and IGFBP-7 over the time-course of CHF strongly predicted adverse outcome. In other words, we found elevated risk in patients in whom the levels were increasing (i.e., a higher slope was present) compared to patients with similar IGFBP levels, but in whom levels remained constant (for details see Figure 3). These new insights into IGFBPs' temporal dynamics is important considering the dynamic nature of myocardial remodeling itself, which has a pivotal role in CHF progression. These findings are novel and carry clinical implications for the monitoring of CHF patients.

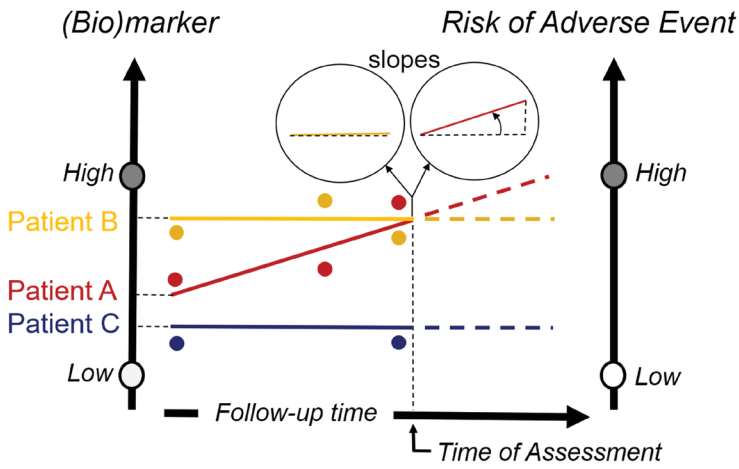

FIGURE 3 An illustration of different aspects of the underlying trajectory of a serially assessed biomarker that may be relevant for a patient's prognosis. Figure illustrates biomarker trajectories of three distinct patients. Their biomarker levels are measured on the y-axis and their follow-up time on the x-axis. At the time of assessment, patient C has lower biomarker levels than patients A and B. Corresponding risk in patient C is lower than in patients A and B. By comparing patients A and B, we observe that they have the same biomarker levels. Thus, if we would like to compare these two patients we have to look at their slopes and relate them to the risk of event. In other words, for two patients with the same biomarker levels, increase in slope provides us with risk estimates related to the adverse event independently of their levels. These risk estimates are provided in Table 3 under *"Slope (per 0.1SD increase / year)"*. The dots represent actual biomarker levels.

All three IGFBPs correlated independently with CHF severity as assessed by cardiac natriuretic peptide and troponin levels. However, IGFBPs differed with respect to their associations with other patient characteristics. Specifically, we found an independent inverse relation between the patients' BMI and IGFBP-1 and IGFBP-2. This has been consistently reported.[7,26] Thus, it may be speculated that the "obesity paradox", in which higher BMI is associated with better survival in CHF[27], might be (partially) mediated through the suppressed adverse effects of IGFBP-1 and IGFBP-2. Yet, this association could not be demonstrated between BMI and IGFBP-7. On its part, IGFBP-7 correlated independently with higher prevalence of atrial fibrillation. In this context, IGFBP-7 has previously been linked to left ventricular (LV) diastolic dysfunction[28] and to increased collagen deposition[29], both of which may contribute to atrial fibrillation. Taking these findings together with the fact that IGFBP-7 belongs to the category of low-affinity IGF binders[30], it appears that direct effects of IGFBP-7 on the myocardium may predominate in the setting of CHF.

Altogether, based on our findings we may tentatively hypothesize that preventing alterations in IGFBPs, by means of lowering IGFBP levels, might potentially help protect the myocardium from further damage.

Adipokines in CHF

Adipose tissue has been identified not only as an energy deposit, but also as a hormonally active organ that releases numerous bioactive molecules called adipokines.[12] Among them, adipose FABP-4 has been linked to metabolism-related cardiac alterations including LV hypertrophy and systolic dysfunction.[31,32] Animal models suggest that adipose FABP-4 causes these cardiac alterations by reducing shortening amplitude and the intracellular systolic Ca^{2+} peak in cardiomyocytes.[33] FABP-4 also predicted the onset of HF among elderly population within the Cardiovascular Health Study.[13] The present study extends these findings by showing that higher FABP-4 levels and higher rate of change in these levels predict adverse clinical events, also in patients with prevalent CHF.

Resistin and chemerin are two other adipokines which are mainly involved in the inflammatory activity underlying cardiovascular diseases.[12] Resistin is largely secreted by mononuclear cells in response to inflammatory stimuli.[34] Animal experiments have demonstrated that in the heart, resistin alters glucose handling, herewith leading to hypertrophy and impaired cardiomyocyte contractility.[35] In murine cardiomyocytes, chemerin was found to induce apoptosis through the activation of several apoptotic mediators.[36] In humans, both resistin and chemerin correlated with LV mass and systolic dysfunction[15,37] but their prognostic role in CHF is less firmly established. In this regard, we found increased levels of both biomarkers in CHF patients who reached adverse events compared to those who did not, but we could not demonstrate their independent prognostic value in CHF.

Cardiometabolic markers as a new link for cardio-renal interaction

We found that repeatedly measured IGFBP-7, FABP-4, resistin, and chemerin are associated with impaired baseline eGFR independently of the patients' characteristics, cardiac markers, and CHF pharmacological treatment. In this context, increased urinary IGFBP-7 has been identified in the settings of acute kidney injury[38], and FABP-4 has been correlated with renal dysfunction and progression of proteinuria.[39] Similarly, resistin and chemerin have been linked to impaired kidney functioning.[14,40] Altogether, this raises the question whether these cardiometabolic

biomarkers also represent another link underlying cardiorenal interaction responsible for worse CHF prognosis. However, additional studies including animal experiments exclusively focusing on this subject are needed to elucidate this promising concept.

Clinical implications

Our findings, together with previous reports, indicate that the use of cardiometabolic biomarkers for monitoring of CHF progression is a rapidly growing area of interest. The current study explores a potential clinical role of these biomarkers to assist in the care of CHF patients through better phenotyping of CHF. We found that CHF patients with higher levels and slopes of IGFBP-1, IGFBP-2, IGFBP-7, and FABP-4 had a more aggressive form of CHF ultimately leading to adverse outcomes such as re-hospitalization or death. This may be important in practice to enable timely adjustment of therapy in patients without clinically overt CHF. These results provide a basis for future studies to further explore this hypothesis. Besides their prognostic role, these biomarkers are bioactive proteins as they activate distinct cell signaling pathways within the IGF-IGFBPs system and adipose-related tissue. Therefore, targeting these biomarkers may be a promising approach in designing more effective biomarker-guided CHF therapy. Finally, future research should focus on better standardization of the assays, and combining the results with genetic analyses may further help to successfully translate these biomarkers into clinical practice.

Study limitations

Firstly, this cohort consisted mainly of HFrEF patients. The low number of HFpEF patients can most likely be attributed to the fact that in the Netherlands, most HFpEF patients are followed in secondary referral centres or by their primary care provider, while our study was performed in two tertiary referral centres. Potential selection bias is not a likely reason for the low rate of HpEF patients, because all consecutive patients were screened in both participating centres. Secondly, although we had trimonthly blood samples available for all patients, because of efficiency reasons only two sampling moments were selected for event-free patients, and three sampling moments for patients with a PE. In previous investigations within this cohort, we have used all available sampling moments to determine NT-proBNP, cTnT, CRP as well as glomerular and tubular renal biomarkers.[16] Those investigations demonstrated that most of these biomarkers show an increase shortly prior to the incident adverse event. Thus, we believe that by selecting baseline

samples, as well as the last two samples prior to the incident endpoint, we retain the most informative measurements while enhancing efficiency.

CONCLUSION

The temporal patterns of IGFBP-1, IGFBP-2, IGFBP-7, and adipose FABP-4 strongly predict adverse clinical outcomes during outpatient follow-up of CHF patients. These biomarkers may, therefore, be relevant for clinical practice as they could help detect more aggressive forms of CHF and assess patient prognosis, and ultimately aid in designing more effective biomarker-guided therapy.

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