Toward personalized risk assessment in patients with chronic heart failure: Detailed temporal patterns of NT-proBNP, troponin T, and CRP in the Bio-SHiFT study

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Background We examined the prognostic information of detailed temporal patterns of N-terminal proBNP (NT-proBNP), high-sensitive troponin T (HsTNT), and C-reactive protein (CRP) in patients with chronic heart failure.

Methods The first inclusion round (2011-2013, N = 263) from the ongoing Bio-SHiFT study was used. Biomarkers were measured at baseline and every 3 months. The primary end point (PE) comprised heart failure hospitalization, cardiovascular mortality, cardiac transplantation, and left ventricular assist device implantation. Associations between temporal biomarker patterns and the PE were investigated by joint modeling.

Results Mean age was 67 ± 12 years, 72% were men, 95% had systolic dysfunction, and 73% were in New York Heart Association class I or II. Median follow-up was 2.2 (interquartile range 1.4-2.5) years. We used 2,022 blood samples (median 9 [interquartile range 5-10] per patient), and 70 (27%) patients reached the PE. Temporal patterns of NT-proBNP, HsTNT, and CRP level were associated with the PE (multivariable-adjusted hazard ratio per doubling of biomarker: NT-proBNP 2.28 (95% CI 1.82-2.86), HsTNT 2.05 (1.63-2.58), and CRP 1.65 (1.30-2.08). A combined 3-biomarker model demonstrated independent associations for the temporal patterns of NT-proBNP and CRP level (hazard ratios 2.06 [1.53-2.79] and 1.38 [1.01-1.89], respectively). Instantaneous change in biomarker level was also independently associated with the PE for NT-proBNP and CRP. Long-term biomarker elevation showed an association for NT-proBNP.

Conclusions Temporal patterns representing evolution of level and rate of change in level of NT-proBNP and CRP, and long-term elevation of NT-proBNP were independently associated with adverse prognosis in patients with chronic heart failure. Individual patterns of change and combining multiple biomarkers could carry value for prognostication and for therapy guidance. (Am Heart J 2018;196:36-48.)

The diagnosis of progression of chronic heart failure (CHF) is primarily based on clinical signs and symptoms, and the decision to adjust therapy is usually made once symptoms of progression have become manifest. Blood biomarkers are capable of monitoring subtle (patho)-physiological processes that reflect and possibly predict adverse changes before they become clinically apparent.[1,2] B-type natriuretic peptides (BNP) and N-terminal proBNP (NT-proBNP), cardiac troponin T and I, and C-reactive protein (CRP) have been unequivocally related to adverse clinical outcomes in patients with heart failure (HF) in several large studies.[2-12]

Most of these studies have examined single, baseline measurements of these blood biomarkers. However, because patients with CHF display large biological heterogeneity, distinguishing patients at different levels of risk of adverse events based on single biomarker measurements only is challenging. Measuring biomarkers repeatedly could contribute to individualized risk

assessment. Studies that have assessed changing bio-marker patterns over time have mostly focused on natriuretic peptides, generally used only few repeated biomarker measurements, and have used simplified representations of temporal biomarker evolution, such as change between 2 time points.[3-6,12-14] Results of these studies strongly depend on the statistical approach that was used.[1] Subsequent trials on natri-uretic peptide-guided therapy of HF have provided inconsistent results.[8-10,15,16] Although such trials are promising for individual risk assessment and personal-ized treatment, most existing trials have applied uniform natriuretic peptide cutoff values for all patients and did not use individualized target levels, nor did they take into consideration multiple biomarkers. Moreover, they did not investigate optimal frequency of the measurements, nor did they adapt this frequency to the patient's individual situation.

The above illustrates that to properly install person-alized risk assessment that makes use of blood biomarkers, first, more detailed information is needed on temporal biomarker patterns in individual patients. Specifically, having measurements available that are performed closely in time to the moment that the end point of interest occurs would provide further insight into the biomarkers' behavior as this end point nearly approaches. This would enable an adequate investiga-tion of whether, and to which degree, increasing (or decreasing) biomarker levels contribute to an indivi-dual's risk regardless of whether his or her blood levels exceed classic, absolute cut points at any random point in time (such as "study baseline"). However, in practice, biomarker measurements performed shortly before the end point occurs are difficult to acquire because they require a high frequency of blood sampling during prolonged follow-up. Therefore, most studies on this topic have performed only 2 measure-ments over time and are thus not able to properly investigate the biomarker trajectory shortly before the end point occurs.

In the current study, we have performed frequent (up to 11), repeated measurements of multiple blood biomarkers (NT-proBNP, HsTNT, and CRP) in 263 patients with CHF and have investigated the associa-tions of the thus obtained temporal patterns with adverse clinical outcome. These 3 biomarkers were chosen because each of them represents different aspects of HF pathophysiology (wall stress, myocyte damage, and inflammation) and because a large body of evidence exists for the prognostic value of single measurements of these markers. By performing multi-ple, longitudinal measurements; assessing multiple biomarkers simultaneously; and using appropriate, modern statistical methods, we aimed to provide a basis for improved, personalized risk assessment in patients with CHF.

Methods

Patients

Bio-SHiFT is a prospective, observational study of stable outpatients with CHF conducted in Erasmus MC, Rotterdam, the Netherlands, and Noordwest Ziekenhuis-groep, Alkmaar, the Netherlands. Patients were recruited during their regular outpatient visits and were in clinically stable condition. Detailed inclusion and exclusion criteria are shown in Figure 1. Patients were eligible if CHF (including HF with preserved ejection fraction) was diagnosed ≥3 months ago according to the guidelines of the European Society of Cardiology.[17-19] This study was approved by the medical ethics committee of the Erasmus MC, Rotterdam, the Netherlands, and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients. The study is registered in ClinicalTrials.gov, number NCT01851538. Estimated enrolment is 400 patients. In this article, we have performed an interim analysis on the 263 patients who were enrolled during the first inclusion period between October 2011 and June 2013.

Baseline assessment

At baseline, patients were evaluated by trained research physicians, who collected information on HF-related symptoms, including New York Heart Association (NYHA) class,[17,18] and performed physical examination, including blood pressure, heart rate, and body mass index. Information on etiology of HF, presence of systolic dysfunction, cardiovascular risk factors, medical history, and medical treatment was retrieved primarily from hospital records. *History of chronic renal failure* was defined as glomerular filtration rate <60 mL/min/1.73 m^2. *Alcohol consumption* was defined as drinking ≥1 alcoholic consumption per day. Data were entered into electronic case report forms. Electrocardiography and echocardiography were performed. Nonfasting blood and urine were collected, as described below.

Follow-up visits

Routine outpatient follow-up by the treating physician continued for all patients during the study. Study follow-up visits were scheduled every 3 months (a window of ±1 month was allowed) to a maximum follow-up duration of 30 months. At each follow-up visit, a short medical evaluation was performed, and blood and urine samples were collected. Changes in medication as well as occurrence of adverse cardiovascular events since the previous visit were recorded.

Blood sampling and biomarker measurement

Blood samples were processed and stored at a temperature of −80°C within 2 hours after blood collection. When applicable, samples were transported

Figure 1
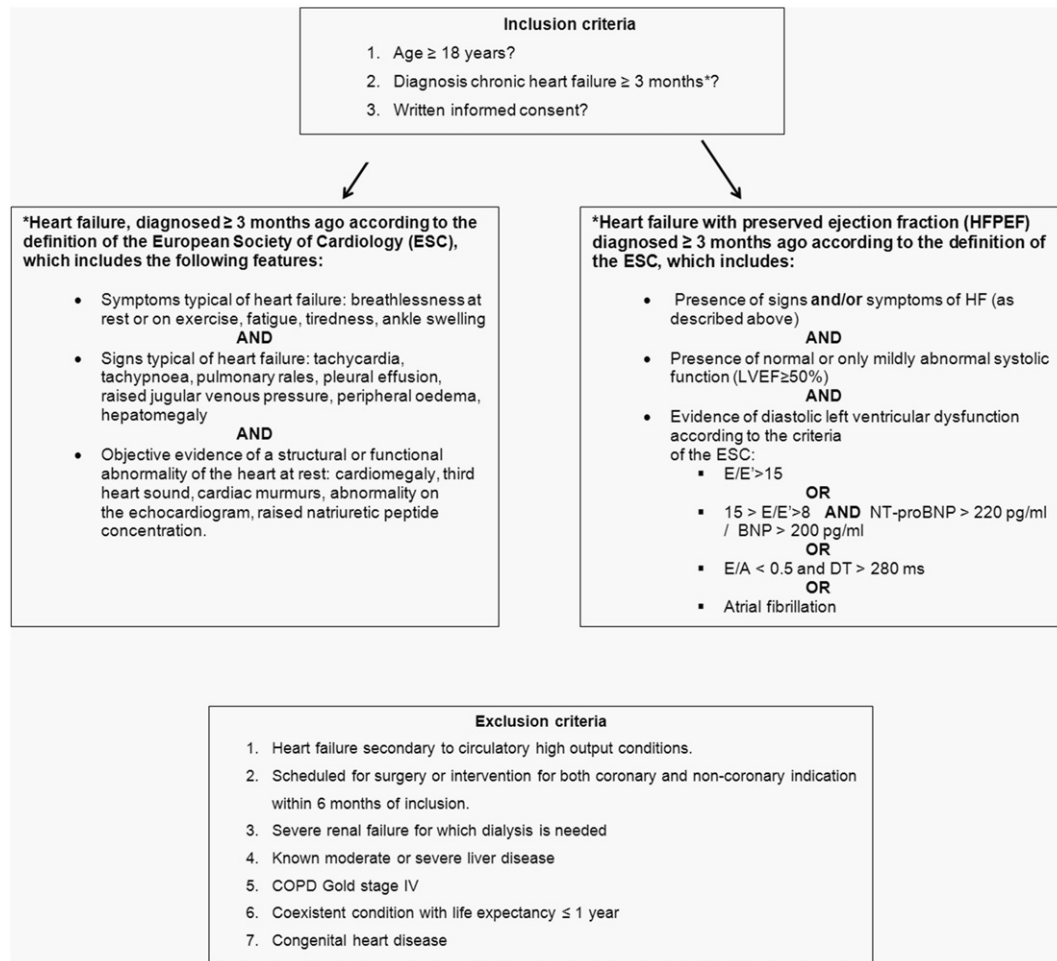
Inclusion and exclusion criteria.

to the central laboratory (Erasmus MC, Rotterdam, the Netherlands) under controlled conditions (at a temperature of −80°C) until batch analysis was performed. Accordingly, results of the biomarker assays were not available to treating physicians at the time of the outpatient visits. Thus, the biomarker measurements performed for this study did not lead to drug adjustments, and all patients received usual care. This concurs with Bio-SHiFT being a strictly observational study, as described above.

For the purpose of the current analysis, 3 biomarkers (NT-proBNP, HsTNT, and CRP) were measured in 1 batch in stored serum samples. Plasma NT-proBNP was analyzed using an electrochemiluminescence immunoassay (Elecsys 2010; Roche Diagnostics, Indianapolis, IN), which measures concentrations ranging from 5 to 35,000 ng/L. Cardiac troponin T was also measured using an electrochemiluminescence immunoassay (Elecsys 2010 immuno-

assay analyzer; Roche Diagnostics, Indianapolis, IN) which measures concentrations ranging from 3 to 10,000 ng/L. CRP was measured using an immunoturbidimetric assay (Roche Hitachi 912 chemistry analyzer; Roche, Basel, Switzerland). This system measures concentrations ranging from 0.3 to 350 mg/L. All coefficients of variation were <5%.

Clinical study end points

During follow-up, hospitalizations for HF, myocardial infarction (MI), percutaneous coronary interventions (PCIs), coronary artery bypass grafting (CABG), arrhythmias, and cerebrovascular accidents (CVAs), as well as cardiac transplantation, left ventricular assist device (LVAD) implantation, and mortality, were recorded in the electronic case report form by trained research physicians, and associated hospital records and discharge letters were collected.

Table I. Baseline characteristics

	Total (N = 263)
	n (%)/mean (±SD)/median (25th-75th percentile)
Demographics	
Age	67 (±13)
Male gender	189 (72)
Caucasian ethnicity	244 (94)
Clinical characteristics	
Body mass index, kg/m^2	28 (±5)
Heart rate, beat/min	67 (±12)
Systolic blood pressure, mm Hg	122 (±20)
Diastolic blood pressure, mm Hg	73 (±11)
Biomarker level	
NT-proBNP (ng/L)	1161 (439-2305)
HsTNT (ng/L)	18.0 (9.6-33.2)
CRP (mg/L)	2.2 (0.9-4.8)
Features of HF	
Duration of HF, y	4.6 (1.7-9.9)
NYHA class I or II	190 (73)
NYHA class III or IV	69 (27)
Left ventricular function	
Systolic dysfunction	250 (95)
HFPEF	13 (5)
LVEF*	32 (±10)
Etiology of HF	
Ischemic heart disease	117 (44)
Hypertension	34 (13)
Secondary to valvular heart disease	12 (5)
Cardiomyopathy	68 (26)
Dilated	49 (19)
Hypertrophic	12 (5)
Non compaction	4 (1)
Unclassified	3 (1)
Unknown	19 (7)
Other	13 (5)
Medical history	
Myocardial infarction	94 (36)
PCI	82 (31)
CABG	43 (16)
Valvular heart disease	136 (53)
Atrial fibrillation	105 (40)
Other arrhythmia	82 (32)
ICD	151 (59)
CRT	78 (30)
Pacemaker	38 (15)
CVA	41 (16)
Chronic renal failure	136 (53)
Diabetes mellitus	81 (31)
Known hypercholesterolemia	93 (35)
Hypertension	120 (46)
Sleep apnea	26 (10)
Intoxications	
Alcohol consumption (>1 U/d)	108 (42)
Smoking	185 (71)
Ever	186 (72)
Current	26 (10)
Medication use	
ACE-I	173 (67)
ARB	75 (29)
Aldosterone antagonist	178 (68)
Diuretic	237 (90)
β-Blocker	232 (88)
Aspirin	45 (17)

Table I (continued)

	Total (N = 263)
	n (%)/mean (±SD)/median (25th-75th percentile)
Vitamin K antagonist	200 (77)
Nitrates	44 (17)
Digoxin	59 (23)
Antiarrhythmics	39 (15)

Normally distributed continuous variables are presented as mean (± SD). Nonnormally distributed continuous variables are expressed as median (25th-75th percentile). Categorical variables are expressed as count (percentage). Valid percentages may vary for some counts because of missing values.
HFPEF, Heart failure with preserved ejection fraction; *LVEF,* left ventricular ejection fraction; *ICD,* implantable cardioverter/defibrillator; *CRT,* cardiac resynchronization therapy; *ACE-I,* angiotensin-converting enzyme inhibitor; *ARB,* angiotensin II receptor blocker.
* Baseline echocardiograms were available in 72% of all patients because of logistic reasons.

Subsequently, hospital records and discharge letters were reviewed by a clinical event committee blinded to the biomarker results, and primary and secondary end points were adjudicated. The primary end point comprised the composite of cardiac death, cardiac transplantation, LVAD implantation, and hospitalization for HF, whichever occurred first in time. Secondary end points included individual components of the primary end point and also MI, PCI, CABG, CVA, and all-cause mortality.

Cardiac death was defined as death from MI or other ischemic heart disease (*International Classification of Diseases, 10th Revision*: 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 primarily based on exacerbation of HF symptoms requiring hospitalization. On top of this, a combination of 2 of the following was required: BNP or NT-proBNP >3× 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.

Statistical analysis

Distributions of continuous variables, including biomarker concentrations, were tested for normality using the Kolmogorov-Smirnov test. Normally distributed continuous variables are presented as mean ± SD. Nonnormally distributed continuous variables are expressed as median and interquartile range (IQR). Categorical data are displayed as count and percentage.

In case of skewed distributions, continuous variables were logarithmically transformed (log base 2) for further analyses. Associations between patient characteristics and baseline biomarker levels were evaluated using univariable linear regression. Associations between

Table II. End points

End point	n (%)
Primary	
Combined primary end point*	70 (27)
Secondary	
Hospitalization for acute or worsening HF	56 (21)
All-cause mortality	32 (12)
Cardiovascular mortality	27 (10)
Heart transplantation	5 (1.9)
Left ventricular assist device implantation	3 (1.1)

Variables are displayed as count (percentage).
*The primary end point comprised HF hospitalization, cardiovascular mortality, cardiac transplantation, and LVAD implantation.

baseline patient characteristics, including baseline biomarker levels, and the primary end point were evaluated using Cox proportional-hazards models. These analyses were first performed univariably. Subsequently, to evaluate independent associations, all baseline characteristics that showed statistically significant associations (with P values < .05) were forced into a multivariable Cox model.

Associations between temporal biomarker patterns of each separate biomarker and the primary end point were assessed using a joint modeling approach, which combines a linear mixed-effects (longitudinal) submodel to assess the temporal evolution of the repeatedly measured marker with a Cox proportional-hazards submodel to analyze the association of this temporal evolution with the study end point. In line with the logarithmic (base 2) transformation of the biomarker concentrations, the results are presented as hazard ratios (HRs) per doubling of the biomarker concentration at any point in time, along with the corresponding 95% CIs. First, analyses were performed univariably. Subsequently, potential confounders were entered into the joint models. These included all variables that were significantly associated with the primary end point in the multivariable "baseline" Cox proportional hazards model (NYHA class and diabetes mellitus), as well as variables selected from existing literature (age, gender, renal function, body mass index). Covariates were missing in less than 3% of patients. Multiple imputations (5 times) of these covariates were performed in the multivariable analyses.

The above-described analysis assesses the predictive value of repeatedly measured biomarker levels; specifically, it provides HRs that estimate the risk of the end point associated with doubling of biomarker level at any point in time. However, in the context of serial marker measurements, there could be additional features of the marker's trajectory that better predict the primary end point.[20] Therefore, we investigated the predictive value of (1) the "instantaneous slope" of the marker's trajectory, indicating whether a marker is decreasing, is increasing, or remains stable, and (2) the area under the curve of the marker's trajectory, indicating the cumulative effect of all the values the marker has taken in the past (this area under the curve does not provide information on increasing or decreasing biomarker values, which should be derived from the slope).

We chose not to correct for multiple testing because the selection of the currently investigated 3 biomarkers was based on previous research and thus hypothesis driven.[2-12]

To simultaneously investigate the effect of all 3 biomarkers on the primary end point and thus to assess their independent predictive value, all individual temporal biomarker patterns derived from the adjusted joint models were saved and subsequently entered simultaneously as time-varying covariates into an extended Cox analysis. The same approach was used to investigate the independent predictive value of the slope and the area under the curve of the 3 temporal biomarker patterns. Adjustment for potential baseline confounders was performed as described above. Additionally, these extended Cox models were adjusted for temporally changing total daily doses of equivalents of carvedilol, enalapril, furosemide, and spironolactone, which were also entered into the models as time-varying covariates.

To illustrate how joint modeling can be applied to estimate prognosis of an individual patient based on his or her repeatedly assessed biomarker values, we plotted the temporal patterns of the biomarkers in several individual patients (ie, example patients drawn from our dataset) together with their corresponding dynamic, individual probabilities of survival as estimated by the joint model (which we developed on the total study population as described above). As such, we graphically demonstrated individual survival probabilities, which are updated each time that an additional measurement is performed in the patient as he or she visits the outpatient clinic.

Finally, to investigate the discriminative ability of models containing serial measurements and models containing baseline measurements only, we calculated C-indices based on extended Cox models containing temporal biomarker patterns derived from the adjusted joint models, as well as C-indices based on Cox models containing baseline biomarker values only.

All analyses were performed with R Statistical Software using package JM.[20] All statistical analyses were 2-sided, and P values < .05 were considered statistically significant.

Power calculation

The current investigation comprised 263 patients, of whom 70 reached the primary end point. For baseline measurements, these numbers are sufficient to detect odds ratios around 2 for the upper quintile of a biomarker associated with the end point (α error .05, power of 80%) when comparing cases with noncases. For repeated

Table III. Associations between baseline characteristics and the primary end point

Variable	Crude HR (CI)	P	Adjusted HR (CI)‡	P
NT-proBNP (pmol/L)*	1.02 (1.02-1.03)	<.001	1.02 (1.01-1.02)	<.001
HsTNT (pg/mL)*	1.12 (1.08-1.16)	<.001	1.08 (1.02-1.16)	.020
CRP (mg/L)*	1.26 (1.06-1.50)	.016	1.18 (0.96-1.45)	.12
Age†	1.02 (1.01-1.05)	.035	1.00 (0.98-1.02)	.86
Male gender	1.27 (0.80-2.19)	.40		
Systolic blood pressure†	0.99 (0.98-0.99)	.040	0.99 (0.98-1.01)	.26
Diastolic blood pressure†	0.98 (0.96-1.00)	.055		
Heart rate†	1.01 (0.99-1.03)	.24		
Body mass index (kg/m^2)†	1.00 (0.96-1.05)	.88		
NYHA class†	2.10 (1.56-2.54)	<.001	1.61 (1.14-2.26)	.006
Chronic renal failure	2.11 (1.28-3.50)	.004	1.25 (0.72-2.18)	.42
Diabetes mellitus	2.06 (1.29-3.29)	.003	1.91 (1.17-3.11)	.010
Hypercholesterolemia	1.37 (0.85-2.20)	.20		
Hypertension	1.31 (0.82-2.10)	.26		
Ever smoker	1.48 (0.84-2.62)	.18		
History of CAD	1.56 (0.96-2.53)	.074		
History of CVA	1.40 (0.78-2.51)	.26		
ICD	1.20 (0.74-1.95)	.47		
CRT	0.80 (0.47-1.36)	.42		

* HR per 10-unit increase.
† HR per unit increase.
‡ All characteristics univariably associated with the primary end point (P < .05) were entered into the multivariable Cox regression model.

measurements, power is further enhanced. A median of 9 samples per patient was available. We calculated power for repeated measurements by assuming a linear association and a continuous autoregressive correlation matrix. We used NT-proBNP to derive the measurement error standard deviation (σ, equal to 463) and the input parameter for the autoregressive correlation matrix (ρ, equal to 0.49). Based on these input parameters and using 1,000 simulations, we calculated that a difference in change of NT-proBNP level over time of 51 ng/L per month can be demonstrated between cases and noncases (α error .05, power of 80%). This difference is small in clinical terms, demonstrating that the study has high statistical power.

Results
Baseline findings

From October 2011 to August 2015, 263 patients were included. Baseline characteristics are shown in Table I. Mean age of the study population was 67 years (SD ±12). The majority were men (72%) in NYHA class I or II (73%). Median duration of HF was 4.6 years (IQR 1.7-9.9). Median baseline NT-proBNP was 1161 ng/L (IQR 439-2305), HsTNT was 18.0 ng/L (IQR 9.6-33.2), and CRP was 2.2 mg/L (IQR 0.9-4.8). Positive associations were found between baseline NT-proBNP level and age (P = .01), heart rate (P = .01), NYHA class (P < .001), and renal failure (P < .001). Inverse associations were found between NT-proBNP and diastolic blood pressure (P < .001) and BMI (P < .001). Baseline HsTNT level was positively associated with age (P < .001), NYHA class (P < .001), and renal failure (P < .001). Baseline CRP level showed positive associations with heart rate (P = .01) and renal failure (P = .045), and inverse associations with systolic (P = .046) and diastolic blood pressure (P < .001).

Clinical end points

During a median follow-up of 2.2 (IQR 1.4-2.5) years, 27 (10%) patients died from a cardiovascular cause, 56 (21%) patients were rehospitalized for worsened HF, 5 (1.9%) patients underwent heart transplantation, and 3 (1.1%) patients received LVAD implantation (Table II). Because 21 patients were rehospitalized for worsened HF before dying from cardiovascular causes eventually during further follow-up, 70 patients (27%) reached the composite primary end point. Overall all-cause mortality was 32 (12%).

Associations between baseline characteristics and the primary end point are shown in Table III. After multivariable adjustment, baseline NT-proBNP (HR 1.02, CI 1.01-1.02), baseline HsTNT (HR 1.08, CI 1.02-1.16), NYHA class (HR 1.61, CI 1.14-2.26), and diabetes mellitus type 2 (DM) (HR 1.91, CI 1.17-3.11) were independently associated with the primary end point (Table III).

Temporal biomarker patterns and the primary end point

During follow-up, we collected 2,193 blood samples, of which 2,022 were drawn before the occurrence of the primary end point (median of 9 [IQR 5-10] samples per

Table IV. Association between temporal patterns of logarithmically transformed NT-proBNP, HsTNT, and CRP and the primary end point

	NT-proBNP		HsTNT		CRP	
	HR* (95% CI)	P value	HR* (95% CI)	P value	HR* (95% CI)	P value
Temporal pattern of biomarker level						
Adjusted for age and gender	2.20 (1.83-2.65)	<.001	2.21 (1.79-2.72)	<.001	1.80 (1.43-2.26)	<.001
Multivariable adjusted†	2.28 (1.82-2.86)	<.001	2.05 (1.63-2.58)	<.001	1.65 (1.30-2.08)	<.001
Instantaneous slope of temporal pattern						
Adjusted for age and gender	2.16 (1.79-2.62)	<.001	2.14 (1.71-2.66)	<.001	1.85 (1.43-2.41)	<.001
Multivariable adjusted†	2.15 (1.71-2.68)	<.001	2.02 (1.58-2.58)	<.001	1.69 (1.32-2.18)	<.001
Area under the curve of temporal pattern						
Adjusted for age and gender	1.68 (1.45-1.96)	<.001	1.74 (1.46-2.07)	<.001	1.32 (1.13-1.55)	<.001
Multivariable adjusted†	1.54 (1.30-1.83)	<.001	1.55 (1.29-1.86)	<.001	1.28 (1.09-1.51)	.003
3 Biomarkers combined						
Level, multivariable adjusted†	2.06 (1.53-2.79)	<.001	1.41 (0.93-2.13)	.104	1.38 (1.01-1.89)	.047
Level, medication adjusted‡	2.08 (1.54-2.80)	<.001	1.46 (0.95-2.23)	.083	1.38 (1.01-1.90)	.044
Slope, multivariable adjusted†	2.04 (1.51-2.78)	<.001	1.47 (0.94-2.16)	.093	1.41 (1.02-1.94)	.036
Slope, medication adjusted‡	2.06 (1.51-2.80)	<.001	1.44 (0.94-2.21)	.089	1.42 (1.02-1.99)	.040
Area, multivariable adjusted†	1.99 (1.49-2.66)	<.001	1.42 (0.94-2.13)	.092	1.32 (0.96-1.80)	.084
Area, medication adjusted‡	2.01 (1.49-2.73)	<.001	1.42 (0.94-2.15)	.098	1.26 (0.91-1.75)	.16

* Hazard ratios are given per doubling of level, slope, or area under the curve at any point in time.
† Adjusted for age, gender, BMI, renal function, NYHA class, and diabetes mellitus type 2.
‡ Adjusted for age; gender; and temporally changing total daily doses of equivalents of carvedilol, enalapril, furosemide, and spironolactone.

patient). The associations between the temporal biomarker patterns and the primary end point are shown in Table IV.

The temporal NT-proBNP pattern derived from the repeated measurements was a significant predictor of the primary end point after adjustment for age, gender, BMI, renal function, NYHA class, and DM (HR per doubling of NT-proBNP: 2.28, CI 1.82-2.86, P < .001). Figure 2, A displays the curves depicting the temporal NT-proBNP pattern of patients who reached the primary end point versus those who did not.

Figure 2, B depicts temporal HsTNT patterns of patients who reached the primary end point and those who did not. We found an association between the temporal HsTNT pattern and the primary end point, which remained present after multivariable adjustment (HR per doubling of biomarker: 2.05, CI 1.63-2.58, P < .001).

As shown in Figure 2, C, the temporal CRP pattern was also a significant predictor of the primary end point (HR per doubling of CRP after multivariable adjustment: 1.65, CI 1.30-2.08, P < .001).

NT-proBNP, HsTNT, and CRP patterns and the primary end point using a combined 3-biomarker model. When we combined temporal patterns of all 3 biomarkers in 1 model, we found independent associations of NT-proBNP (HR per doubling of NT-proBNP level at any given time point: 2.06, CI 1.53-2.79, P < .001) and CRP (HR per doubling of CRP level: 1.38, CI 1.01-1.89, P = .047) with the primary end point. These associations were also independent of temporally changing total daily doses of equivalents of carvedilol, enalapril, furosemide,

and spironolactone (Table IV). However, HsTNT was no longer associated with the primary end point in this model (HR per doubling of HsTNT: 1.41, CI 0.93-2.13, P = .10), illustrating that its predictive value was not independent of NT-proBNP and CRP.
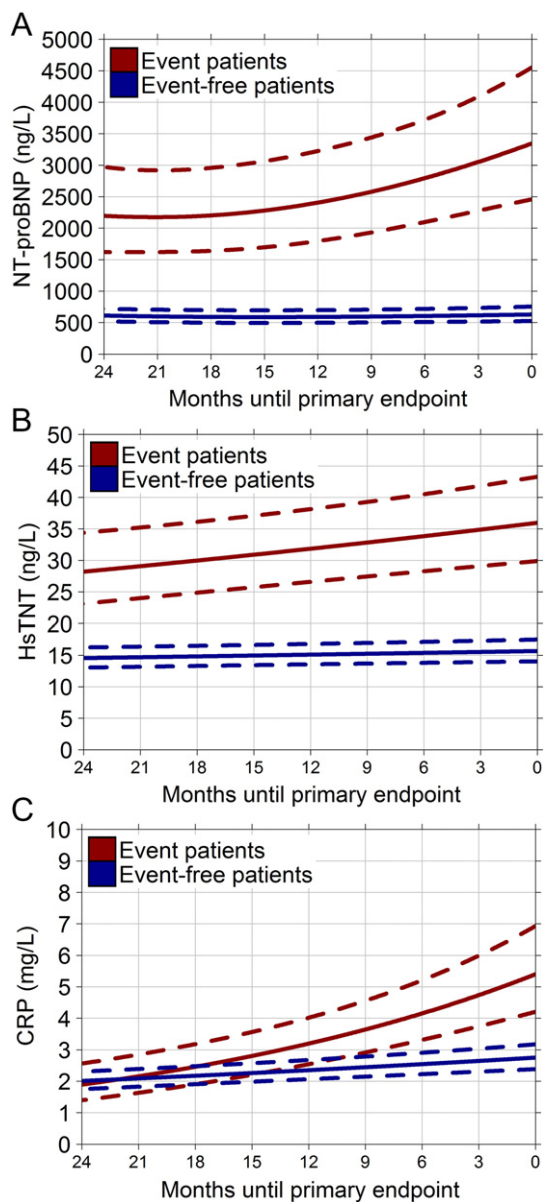
Slopes and areas under the curve of temporal patterns. Table IV displays HRs for the doubling of the instantaneous slopes and areas under the curve of the temporal biomarker patterns. The instantaneous slopes of the temporal patterns as well as the areas under the curve of NT-proBNP, HsTNT, and CRP were all associated with the primary end point after multivariable adjustment, including adjustment for temporally changing total daily doses of equivalents of carvedilol, enalapril, furosemide, and spironolactone.

When we entered the instantaneous slopes of the temporal biomarker patterns of the 3 biomarkers into 1 model, they remained independent predictors for NT-proBNP and CRP, but not for HsTNT. Simultaneously entering the areas under the curve of the 3 temporal biomarker patterns into 1 model showed that only NT-proBNP was independently associated with the primary end point.

Personalized prediction: individual, dynamic risk estimation

Figure 3 shows the temporal patterns of the biomarkers in several individual patients from our data set together with their corresponding individual probabilities of survival as estimated by the joint model. The figure shows that each time an additional measurement is

Figure 2

Temporal patterns. The temporal patterns, displayed as time until event, of (**A**) NT-proBNP, (**B**) HsTNT, and (**C**) CRP of patients who reached the primary end point versus those who did not.

by clinicians in an easy, user-friendly manner. Joint models, like those we have constructed, can be uploaded into an app (http://shiny.rstudio.com/) that creates an interface into which a clinician can add the characteristics, and consecutive biomarker measurements, of an individual patient. Subsequently, the app returns the curve depicting individual prognosis (Supplemental Figure 1).

Model performance. Discriminative ability of models containing the temporal patterns of the biomarker levels and baseline measurements only is shown in Table V. For all 3 biomarkers, models containing temporal biomarker patterns showed higher C-indices than those containing baseline measurements only. The highest C-index resulted from the multivariable model containing all 3 temporal biomarker patterns as well as age, gender, BMI, renal function, NYHA class, and DM (C-index 0.84).
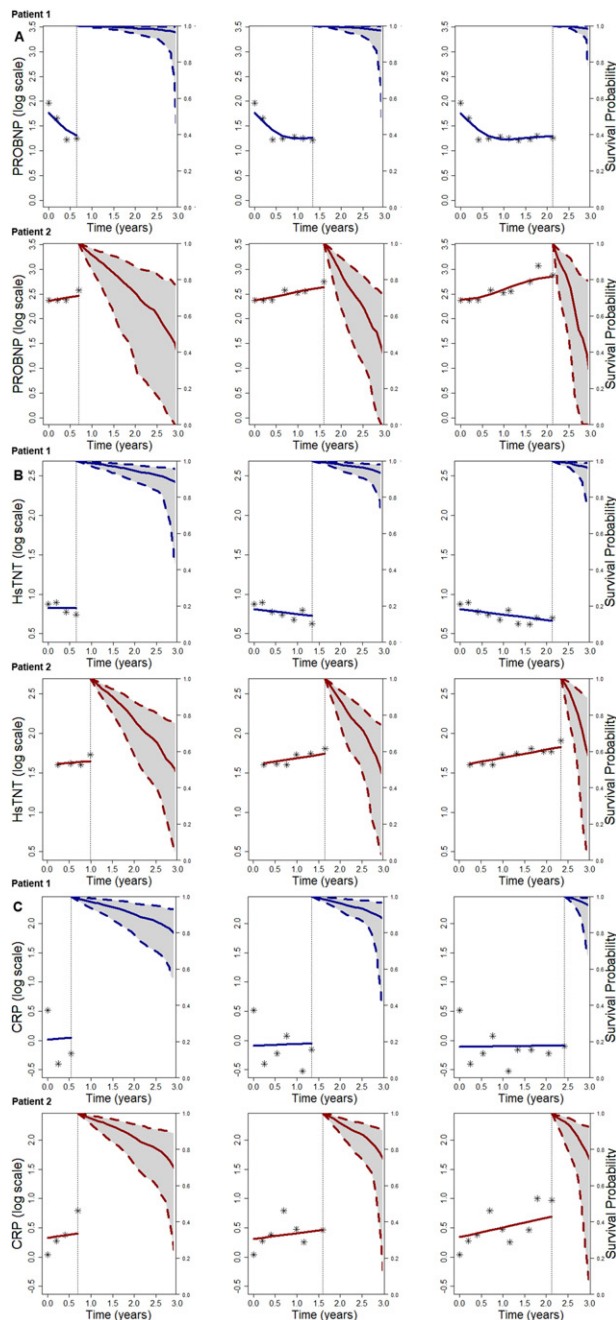
Temporal patterns of NT-proBNP, HsTNT, and CRP in relation to hospitalization for acute or worsening HF (secondary end point)

All 3 biomarker patterns were strong individual predictors of HF hospitalizations (age- and gender-adjusted HRs per doubling of biomarker: NT-proBNP 2.17, CI 1.57-2.66, $P < .001$; HsTNT 2.18, CI 1.72-2.76, $P < .001$; and CRP 1.99, CI 1.53-2.59, $P < .001$). These associations remained statistically significant after multivariable adjustment (HRs per doubling of biomarker: NT-proBNP 2.31, CI 1.77-3.01, $P < .001$; HsTNT 1.95, CI 1.49-2.55, $P < .001$; and CRP 1.80, CI 1.37-2.35, $P < .001$). After creating a time-dependent Cox model using all 3 temporal biomarkers patterns, derived from the individual joint models, we found that each of the 3 biomarkers remained independent predictors of HF hospitalizations (HR per doubling of biomarker: NT-proBNP 1.51, CI 1.26-1.80, $P < .001$; HsTNT 1.57, CI 1.24-2.00, $P = .001$; and CRP 1.41, CI 1.15-1.74, $P < .001$). These associations persisted after adjusting for temporally changing total daily doses of equivalents of carvedilol, enalapril, furosemide, and spironolactone (HR per doubling of biomarker: NT-proBNP 1.49, CI 1.23-1.80, $P < .001$; HsTNT 1.50, CI 1.18-1.91, $P = .001$; and CRP 1.39, CI 1.11-1.74, $P = .004$).

Discussion

We performed a prospective, observational study that comprised CHF patients with mostly systolic dysfunction and predominantly favorable NYHA class (I-II). Here, in the first inclusion round, we demonstrate that the dynamic, temporal patterns of serially measured NT-proBNP and CRP levels are strong and independent predictors of adverse clinical events. Moreover, instantaneous slope of these biomarkers' temporal trajectories, as well as the area under the curve of their temporal trajectories, is associated with adverse events. The

performed in the patient, the individual probability of survival is updated. Specifically, rising marker levels and worsening prognosis can be seen in the example patients who ultimately reached the composite end point versus stable or decreasing marker levels and more favorable prognosis in the example patients who stayed event-free. These individual estimates of prognosis can be obtained

Figure 3

Dynamic profiling of an individual patient's risk using patient-specific temporal trajectories. The solid red lines depict patients who experienced the study end point, and the solid blue lines depict patients who did not. The x-axis depicts follow-up time starting from baseline. Biomarker levels (on the log scale) are displayed on the left y-axis and survival probability (%) on the right y-axis. Patient-specific temporal biomarker trajectories are displayed left of the vertical dotted black line. To the right of this line, the corresponding conditional survival probability curve is displayed with 95% CIs (gray area). To show how this conditional survival probability curve is dynamically updated every time an extra measurement is recorded, we provide the curves for 3 time points at which risk was updated.

temporal patterns of HsTNT also significantly predict adverse events but lose their predictive capability when combined with temporal NT-proBNP and CRP patterns.

We also demonstrate, based on these dynamic models, how individual, temporal biomarker trajectories can be used for calculating patient-specific risk estimates, which

Table V. Discriminative ability of models containing the temporal patterns of NT-proBNP, HsTNT, and CRP level, as well as models containing baseline measurements only

	Baseline measurements	Temporal patterns
	C-index; multivariable model*	C-index; multivariable model*
NT-proBNP	0.78	0.83
HsTNT	0.73	0.75
CRP	0.67	0.69
Combined model†	0.79	0.84

* The multivariable models were corrected for age, gender, BMI, renal function, NYHA class, and diabetes mellitus type 2.
† NT-proBNP, HsTNT, and CRP are all included in the combined model.

are dynamically updated every time a patient has a new measurement performed.

Studies on the prognostic value of repeated natriuretic peptide measurements have mostly been performed in trial participants,[6,13] and studies on the prognostic value of repeated biomarker measurements other than natriuretic peptides are scarce.[3,4,12] Altogether, these existing studies describing temporal changes in biomarkers in relation to patient prognosis have 3 major limitations. Firstly, changes are often presented as a difference between just 2 measurements that are separated in time. Such an approach fails to fully capture the true biomarker pattern of the dynamic disease. Moreover, it fails to expose changes in biomarker level prior to clinically relevant end points because, on average, a long time period lies between the last (ie, second) biomarker measurement and the incident end point. To properly investigate whether an increase in biomarker level is present at the time an end point is approaching and whether this increase truly contributes to an individual's risk, the time period between the last measurement and the end point should be kept as brief as possible. This implies that a high frequency of blood sampling during prolonged follow-up is needed. Secondly, biomarkers are often studied in isolation, thus actually ignoring the different underlying etiologies that converge to adverse cardiac remodeling and HF progression. The third limitation of existing studies is related to the applied methods of data analysis. Often, absolute or relative differences between 2 measurements are calculated, or categorical changes across a threshold value are assessed. These various approaches to temporal change all render different estimates for associations between changes in biomarker level and outcome,[1] which are an illustration of their shortcomings. At best, Cox models with so-called time-dependent covariates are used to analyze the effects of temporal biomarker patterns. Although time-dependent Cox models assume that biomarker levels do not change between measurements, it is known that biomarker patterns are dynamic and continuously change

over time, parallel to the condition of the patient. All these limitations are overcome in Bio-SHiFT: we have performed a large number of frequent, repeated measurements (up to 11 trimonthly samples per patient); we have studied multiple biomarkers; and we have applied modern statistical methods ("joint modeling"), which, as stated above, take into account the continuous, dynamic changes in biomarker patterns and thus result in less bias.[21]

Several randomized trials have been performed to investigate whether using serial natriuretic peptide measurements to titrate medical therapy can improve clinical outcome of HF patients. However, because the results of these trials were not fully consistent, natriuretic peptide–guided therapy remains controversial.[8-10,15] It should be noted that most of these trials were based on protocols that used uniform natriuretic peptide targets in the intervention groups.[22-26] Existing trials that used individualized treatment targets are in the minority and often based their targets on natriuretic peptide levels that were measured briefly after the index episode of decompensation when titration of therapy was still ongoing.[27-29] Conversely, our study describes in detail the temporal biomarker patterns in stable CHF patients and reveals significant associations between temporal patterns of biomarker levels and adverse events. Patients with CHF who did not experience adverse cardiac events during prolonged follow-up were shown to have lower levels of NT-proBNP, HsTNT, and CRP at any moment in time compared with patients who did experience adverse cardiac events during follow-up. Additionally, the instantaneous rate of change in biomarker levels (represented by the slope of the temporal biomarker patterns), as well as the cumulative values the marker has taken in the past (represented by the area under the temporal biomarker patterns), was associated with adverse outcome. These findings support the concept of an individualized biomarker target level instead of a generally applicable uniform cutoff value for all patients. On top of this, they suggest that rate of change in biomarker level and the duration of biomarker level elevation merit attention to provide appropriate individual treatment targets as well as correct estimates of prognosis. Our study also demonstrates that temporal patterns of CRP predict adverse clinical outcome independently of NT-proBNP.

Future trials on biomarker-guided therapy may benefit from incorporating these findings. Firstly, future trials should use personalized biomarker cutoff values, that is, interpret a patient's biomarkers level in the context of his or her previous series of levels. This means that they should not only take into account the absolute biomarker level but also incorporate the instantaneous slope of the marker's trajectory. Secondly, upcoming trials should use a combination of multiple biomarkers, representing different pathophysiological pathways, to guide HF therapy. Finally, additional research should be performed

on the frequency of biomarker measurement and tailoring thereof to individual patients; subsequently, these findings should be incorporated into biomarker-guided trials as well.

Miller et al[12] published a study that might be considered comparable to Bio-SHiFT to a certain extent, as they evaluated serial measurements of cardiac troponin T and BNP in 190 ambulant CHF patients. Again, an important limitation of this study is the use of time-dependent Cox models. Still, Miller et al found that cardiac troponin T and BNP were both independent predictors of cardiac mortality or cardiac transplantation and that combined elevation of these biomarkers substantially adds to risk. We could only partly confirm these results. In Bio-SHiFT, although predictive as a separate marker, the HsTNT pattern appeared to be no longer significantly associated with the primary study end point after adjustment for the NT-proBNP and CRP patterns (and also after adjustment for NT-proBNP alone; data not shown). This may (at least in part) be due to the above-described differences in data analysis.

Some aspects of this study warrant consideration. With 263 patients, sample size is limited, and the majority of the patient population was in NYHA class I or II and had systolic dysfunction. Also, a large proportion had concomitant valvular heart disease. The results and conclusions should be judged accordingly because such a study population may not be fully representative of "real-life" CHF patients in general. Nevertheless, given the repeated-measures design, >2,000 blood samples were available, and all 3 investigated biomarkers, each having different pathophysiological properties, showed the hypothesized rising temporal pattern. This strengthens our findings and makes them less likely attributable to bias or chance. Further to this, the current investigation was an interim analysis of the patients enrolled in the first inclusion round. The full Bio-SHiFT cohort was designed to enroll 400 patients and to have sufficient statistical power to perform large-scale, hypothesis-free research on novel, lesser known biomarkers. In such cases, correction for multiple testing is warranted. The current investigation, however, examines 3 well-established biomarkers, which have been extensively implicated in HF in previous studies and which were chosen based on pathophysiological considerations, rendering correction for multiple testing redundant. Furthermore, additional investigations are needed to estimate the most efficient frequency of biomarker measurement so that optimal prognostic information can be gained without superfluous blood sampling. In our study, patients were monitored every 3 months to construct a data framework for our joint models. An extension to joint modeling is currently being developed to define optimal time frames for individual patients to return for consecutive measurements. In this context, the optimal frequency for biomarker measurement is expected to vary from patient to patient; it is likely that once a stable biomarker value is found in a patient, this patient could be reexamined after a longer time period, whereas if, for example, a biomarker value is found to have risen and thus prognosis is worsening, the patients may need to return more quickly. Moreover, in our study, repeatedly measured NT-proBNP and CRP were both independently associated with the primary end point. This implies that a multi-marker model would benefit monitoring of CHF patients (although a model combining both of these biomarkers seemed to have little incremental discriminative value over serial NT-proBNP assessment only as suggested by the C-index, the C-index is known to be rather insensitive to improvements in prediction performance, and it has been demonstrated previously that testing for improvement in prediction performance is actually redundant if a variable has already been shown to be an independent risk factor[30]). Future studies should investigate a broader spectrum of biomarkers to further improve risk assessment. Finally, although we have illustrated the application of biomarker-guided, personalized risk assessment in practice by means of an interface that uses joint modeling, we realize that many challenges remain to be resolved before truly implementing such a strategy.

In conclusion, detailed temporal patterns of NT-proBNP and CRP are strong, independent predictors of adverse clinical events in our study population of patients with stable CHF. Not only evolution of biomarker level but also instantaneous rate of change in level of NT-proBNP and CRP as well as the area under the curve of the trajectory of NT-proBNP was associated with adverse outcome. These findings suggest that individual patterns of change of biomarkers, as well as combinations of multiple biomarkers, should be taken into consideration for prognostication in patients with stable CHF. Overall, our study illustrates that several aspects of biomarker-guided risk stratification have been incompletely addressed so far and that there still seems to be room for improvement with regard to personalized risk assessment. Future steps could potentially include determining optimum timing of blood sampling, determining optimum combinations of biomarkers, and eventually a biomarker-guided trial that is based on personalized temporal patterns of multiple biomarkers.

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Disclosure

Contributors

Nick van Boven and Linda C. Battes are the main writers of the article and contributed to the analysis and

interpretation of data and revision of the article, and gave final approval of the article to be published.

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