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

9

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

Nick van Boven, Linda C. Battes, K. Martijn Akkerhuis, Dimitris Rizopoulos, Kadir Caliskan, Sharda S. Anroedh, Wisam Yassi, Olivier C. Manintveld, Jan-Hein Cornel, Alina A. Constantinescu, Eric Boersma, Victor A. Umans, Isabella Kardys

Am Heart J 2018;196:36-48

ABSTRACT

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

Methods: From 2011-2013, 263 CHF patients were included. NT-proBNP, HsTNT and CRP were measured at baseline and every 3 months. The primary endpoint (PE) comprised heart failure hospitalization, cardiovascular mortality, cardiac transplantation and LVAD-implantation. Associations between temporal biomarker patterns and the PE were investigated by joint modelling, which combines mixed models with Cox regression.

Results: Mean age was 67±12 years and 72% were men. Median follow-up was 2.2 (IQR 1.4–2.5) years. We used 2022 blood samples (median 9(IQR 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 HR per doubling of biomarker: NT-proBNP 2.28 (95%CI 1.82–2.86), HsTNT 2.05(1.63–2.58), 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 (HRs 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

are independently associated with adverse prognosis in CHF patients. Individual patterns of change and combining multiple biomarkers could carry value for prognostication and for therapy guidance.

INTRODUCTION

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 heart failure (HF) patients in several large studies.2-12

The majority of these studies have examined single, baseline measurements of these blood biomarkers. However, since 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 biomarker patterns over time have mostly focused on natriuretic peptides, generally used only few repeated biomarker measurements, and have utilized simplified representations of temporal biomarker evolution, such as change between two time-points.3-6,12-14 Results of these studies strongly depend on the statistical approach that was used.1 Subsequent trials on natriuretic peptide-guided therapy of HF have provided inconsistent results.8-10,15,16 Most of these trials did not use individualized target levels for natriuretic peptides, and did not take other biomarkers into consideration.

The above illustrates that in order to properly install personalized 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 endpoint of interest occurs, would provide further insight into the biomarkers' behaviour as this endpoint nearly approaches. This would enable an adequate investigation of whether, and to which degree, increasing (or decreasing) biomarker levels contribute to an individual'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 endpoint 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 two measurements over time and are thus not able to properly investigate the biomarker trajectory shortly before the endpoint 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 associations of the thus obtained temporal patterns with adverse clinical outcome. These 3 biomarkers were chosen because each of them represents different aspects of heart failure 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 multiple, 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 Ziekenhuisgroep, 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 (ESC).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 the current paper, we have performed an interim analysis on the 263 patients who were enrolled during the first inclusion period between October 2011 and June 2013.

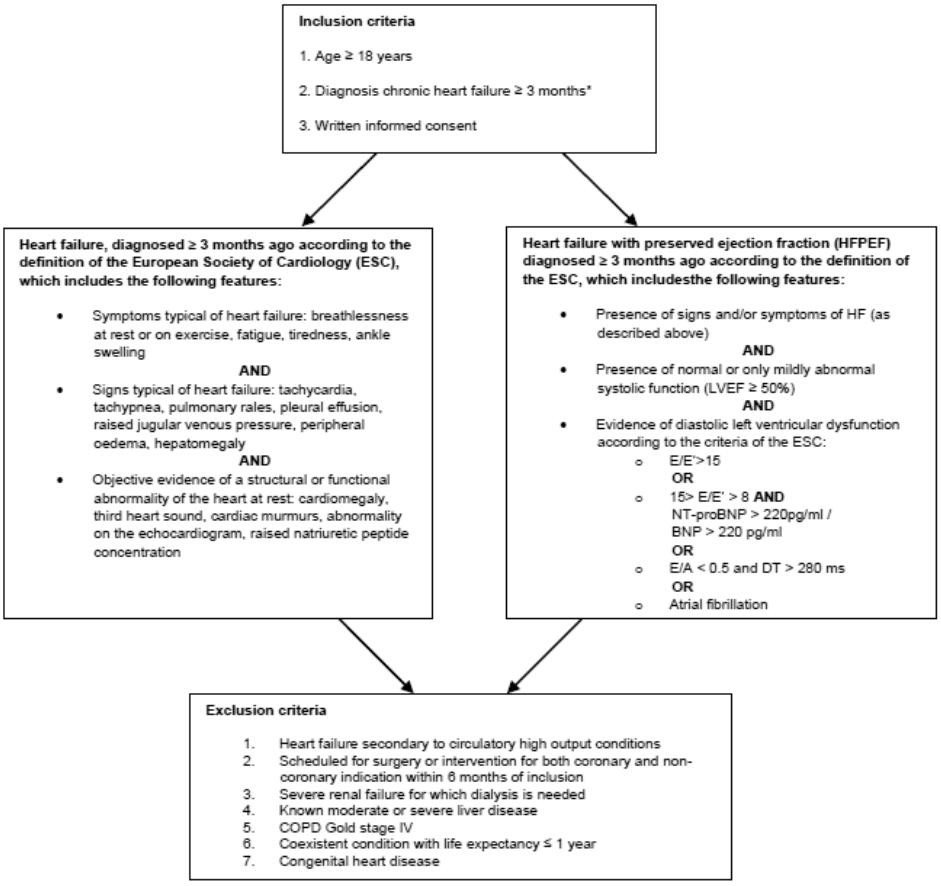

Figure 1 - Inclusion and exclusion criteria

Baseline assessment

At baseline, patients were evaluated by trained research physicians, who collected information on HF-related symptoms, including NYHA class,17,18 and performed physical examination, including blood pressure, heart rate and body mass index. Information on aetiology of heart failure, 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 (GFR) less than 60 mL/min/1.73 m2. Alcohol consumption was defined as drinking ≥1 alcoholic consumption per day. Data were entered into electronic case report forms. Electrocardiography and echocardiography were performed. Non-fasting 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 -80oC within 2 hours after blood collection. When applicable, samples were transported to the central laboratory (Erasmus MC, Rotterdam, The Netherlands) under controlled conditions (at a temperature of -80oC), 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, three biomarkers (NT-proBNP, HsTNT, and CRP) were measured in one batch in stored serum samples. Plasma NT–proBNP was analysed using an electrochemiluminesence immunoassay (Roche Diagnostics, Elecsys 2010, Indianapolis, Indiana, USA), which measures concentrations ranging from 5 to 35.000 pmol/L. Cardiac troponin T was also measured using an electrochemiluminesence immunoassay (Roche Diagnostics, Elecsys 2010 immunoassay analyser, Indianapolis, Indiana, USA), measuring concentrations ranging from 3-10000 ng/L. CRP was measured using an immunoturbidimetric assay (Roche Hitachi 912 chemistry analyser, Basel, Switzerland). This system measures concentrations ranging from 0.3 to 350 mg/L. All coefficients of variation were <5%.

Clinical study endpoints

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 implantation (LVAD) and mortality, were recorded in the electronic case report form by trained research physicians, and associated hospital records and discharge letters were collected.

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

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). Hospitalisation for acute or worsened HF was defined as a hospitalisation for an exacerbation of HF symptoms, in combination with 2 of the following: BNP or NT-proBNP >3x ULN, signs of worsening HF, such as pulmonary rales, raised jugular venous pressure or peripheral oedema, 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 ± standard deviation (SD). Non-normally 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 baseline patient characteristics, including baseline biomarker levels, and the primary endpoint, 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 <0.05) 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) sub model to assess the temporal evolution of the repeatedly measured marker with a Cox proportional-hazards

sub model to analyse 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 signifi- cantly 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 (i.e., 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 investigated 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 tests were two-tailed and p-values <0.05 were considered statistically significant.

Power calculation

The current investigation comprised 263 patients, of whom 70 reached the primary endpoint. For baseline measurements, these numbers are sufficient to detect odds ratios around 2 for the upper quintile of a biomarker associated with the endpoint (α-error 0.05, power of 80%) when comparing cases with non-cases. For repeated measurements, power is further enhanced. A median of 9 samples per patient were 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 (sigma, equal to 463) and the input parameter for the autoregressive correlation matrix (rho, equal to 0.49). Based on these input parameters, and using 1000 simulations, we calculated that a difference in change of NT-proBNP level over time of 6 ng/L per month can be demonstrated between cases and non-cases (α-error 0.05, power of 80%). This difference is small in clinical terms, demonstrating that the study has high statistical power.

Table 1 – Baseline characteristics

	Total (n=263)
	No. (%) / 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, bpm	67 (±12)
Systolic blood pressure, mmHg	122 (±20)
Diastolic blood pressure, mmHg	73 (±11)
Biomarker level	
NT-proBNP (pmol/L)	137.3 (51.9 – 272.6)
HsTNT (ng/L)	18.0 (9.6 – 33.2)
CRP (mg/L)	2.2 (0.9 – 4.8)
Features of heart failure	
Duration of heart failure, years	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 heart failure	
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)

Table 1 – Baseline characteristics (*continued*)

	Total (n=263)
	No. (%) / Mean (±SD) / Median (25th – 75th percentile)
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 unit/day)	108 (42)
Smoking	185 (71)
Ever	186 (72)
Current	26 (10)
Medication use	
ACE-i	173 (67)
ARB	75 (29)
Aldosteron antagonist	178 (68)
Diuretic	237 (90)
Beta-blocker	232(88)
Aspirin	45 (17)
Vitamin K antagonist	200 (77)
Nitrates	44 (17)
Digoxin	59 (23)
Antiarrhythmics	39 (15)

Normally distributed continuous variables are presented as mean (± standard deviation). Non-normally distributed continuous variables are expressed as median (25^{th} – 75^{th} percentile). Categorical variables are expressed as count (percentage). Valid percentages may vary for some counts, because of missing values. ACE-I = ace inhibitor; ARB = angiotensin II receptor blocker; CABG = coronary artery bypass grafting; COPD = chronic obstructive pulmonary disease; CRP = C-reactive protein; CRT = cardiac resynchronization therapy; CVA = cerebrovascular accident; HFPEF = heart failure with preserved ejection fraction; HsTNT = high sensitive cardiac troponin T; ICD = implantable cardioverter / defibrillator; LVEF = left ventricular ejection fraction; NT-proBNP = N-terminal pro-B-type natriuretic peptide NYHA = New York heart association; PCI = percutaneous coronary intervention; SD = standard deviation.

* Baseline echocardiograms were available in 72% of all patients because of logistic reasons.

RESULTS

Baseline findings

From October 2011 to August 2015, 263 patients were included. Baseline characteristics are shown in Table 1. Mean age of the study population was 67 years (SD ±12). The majority were men (72%) in New York heart association (NYHA) class I or II (73%). Median duration of HF was 4.6 years (IQR 1.7 – 9.9). Median baseline NT-proBNP was 137.3 pmol/L (IQR 51.9 – 272.6), HsTNT 18.0 ng/L (IQR 9.6 – 33.2) and CRP 2.2 mg/L (IQR 0.9 – 4.8). Positive associations were found between baseline NT-proBNP level and age (p=0.01), heart rate (p=0.01), NYHA class (p<0.001), and renal failure (p<0.001). Inverse associations were found between NT-proBNP and diastolic blood pressure (p<0.001) and BMI (p<0.001). Baseline HsTNT level was positively associated with age (p<0.001), NYHA class (p<0.001) and renal failure (p<0.001). Baseline CRP level showed positive associations with heart rate (p=0.01) and renal failure (p=0.045), and inverse associations with systolic (p=0.046) and diastolic blood pressure (p<0.001).

Clinical endpoints

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 re-hospitalized for worsened HF, 5 (1.9%) patients underwent heart transplantation and 3 (1.1%) patients received LVAD-implantation (Table 2). Since 21 patients were re-hospitalized for worsened HF before dying from cardiovascular causes eventually during further follow-up, 70 patients (27%) reached the composite primary endpoint. Overall all-cause mortality was 32 (12%).

Associations between baseline characteristics and the primary endpoint are shown in Table 3. 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 endpoint (Table 3).

Temporal biomarker patterns and the primary endpoint

During follow-up, we collected 2193 blood samples, of which 2022 were drawn before the occurrence of the primary endpoint (median of 9 (IQR 5-10) samples per patient). The associations between the temporal biomarker patterns and the primary endpoint are shown in Table 4.

Table 2 – Endpoints

Endpoint	N (%)
Primary	
Combined primary endpoint*	70 (27)
Secondary	
Hospitalization for acute or worsening heart failure	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 endpoint comprised heart failure hospitalization, cardiovascular mortality, cardiac transplantation and LVAD-implantation

Tabel 3 – Associations between baseline characteristics and the primary endpoint

Variabele	Crude HR (CI)	P	Adjusted HR (CI)‡	P
NT-proBNP (pmol/L)*	1.02 (1.02 – 1.03)	<0.001	1.02 (1.01 – 1.02)	<0.001
HsTNT (pg/mL)*	1.12 (1.08 – 1.16)	<0.001	1.08 (1.02 – 1.16)	0.020
CRP (mg/L)*	1.26 (1.06 – 1.50)	0.016	1.18 (0.96 – 1.45)	0.12
Age†	1.02 (1.01 – 1.05)	0.035	1.00 (0.98 – 1.02)	0.86
Male gender	1.27 (0.80 – 2.19)	0.40		
Systolic blood pressure†	0.99 (0.98 – 0.99)	0.040	0.99 (0.98 – 1.01)	0.26
Diastolic blood pressure†	0.98 (0.96 – 1.00)	0.055		
Heart rate†	1.01 (0.99 – 1.03)	0.24		
Body mass index kg/m^2†	1.00 (0.96 – 1.05)	0.88		
NYHA-class†	2.10 (1.56 – 2.54)	<0.001	1.61 (1.14 – 2.26)	0.006
Chronic renal failure	2.11 (1.28 – 3.50)	0.004	1.25 (0.72 – 2.18)	0.42
Diabetes mellitus	2.06 (1.29 – 3.29)	0.003	1.91 (1.17 – 3.11)	0.010
Hypercholesterolemia	1.37 (0.85 – 2.20)	0.20		
Hypertension	1.31 (0.82 – 2.10)	0.26		
Ever smoker	1.48 (0.84 – 2.62)	0.18		
History of CAD	1.56 (0.96 – 2.53)	0.074		
History of CVA	1.40 (0.78 – 2.51)	0.26		
ICD	1.20 (0.74 – 1.95)	0.47		
CRT	0.80 (0.47 – 1.36)	0.42		

CRP = C-reactive protein; CRT = cardiac resynchronization therapy; CVA = cerebrovascular accident; HsTNT = high sensitive cardiac troponin T; ICD = implantable cardioverter / defibrillator; NT-proBNP = N-terminal pro-B-type natriuretic peptide NYHA = New York heart association.

* HR per 10 units increase

† HR per unit increase

‡ All characteristics univariably associated with the primary endpoint ($p<0.05$) were entered into the multivariable Cox regression model.

Table 4 - Association between temporal patterns of logarithmically transformed NT-proBNP, hsTNT and CRP and the primary endpoint

	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)	<0.001	2.21 (1.79–2.72)	<0.001	1.80 (1.43–2.26)	<0.001
Multivariable adjusted†	2.28 (1.82–2.86)	<0.001	2.05 (1.63–2.58)	<0.001	1.65 (1.30–2.08)	<0.001
Instantaneous slope of temporal pattern						
Adjusted for age and gender	2.16 (1.79–2.62)	<0.001	2.14 (1.71–2.66)	<0.001	1.85 (1.43–2.41)	<0.001
Multivariable adjusted†	2.15 (1.71–2.68)	<0.001	2.02 (1.58–2.58)	<0.001	1.69 (1.32–2.18)	<0.001
Area under the curve of temporal pattern						
Adjusted for age and gender	1.68 (1.45–1.96)	<0.001	1.74 (1.46–2.07)	<0.001	1.32 (1.13–1.55)	<0.001
Multivariable adjusted†	1.54 (1.30–1.83)	<0.001	1.55 (1.29–1.86)	<0.001	1.28 (1.09–1.51)	0.003
Three biomarkers combined						
Level, multivariable adjusted†	2.06 (1.53–2.79)	<0.001	1.41 (0.93–2.13)	0.104	1.38 (1.01–1.89)	0.047
Slope, multivariable adjusted†	2.04 (1.51–2.78)	<0.001	1.47 (0.94–2.16)	0.093	1.41 (1.02–1.94)	0.036
Area, multivariable adjusted†	1.99 (1.49–2.66)	<0.001	1.42 (0.94–2.13)	0.092	1.32 (0.96–1.80)	0.084

* HR = hazard ratio. 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.

The temporal NT-proBNP pattern derived from the repeated measurements was a significant predictor of the primary endpoint 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<0.001). Figure 2a displays the curves depicting the temporal NT-proBNP pattern of patients who reached the primary endpoint versus those who did not.

Figure 2b depicts temporal HsTNT patterns of patients who reached the primary endpoint and those who did not. We found an association between the temporal HsTNT pattern and the primary endpoint, which remained present after multivariable adjustment (HR per doubling of biomarker: 2.05; CI 1.63 – 2.58; p<0.001).

As shown in figure 2c, the temporal CRP pattern was also a significant predictor of the primary endpoint (HR per doubling of CRP after multivariable adjustment: 1.65; CI 1.30 – 2.08; p<0.001).

NT-proBNP, HsTNT and CRP patterns and the primary endpoint 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 b .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 4 displays hazard ratios 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 endpoint after multivariable adjustment.

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 endpoint.

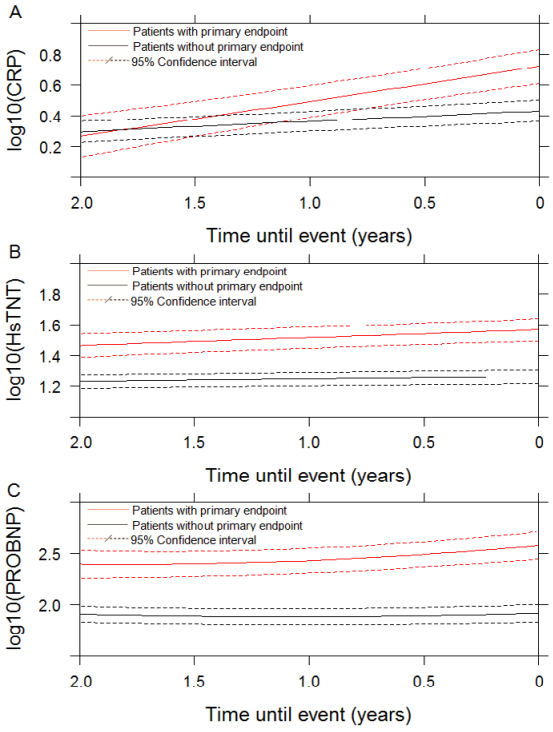

Figure 2 - Temporal patterns
The temporal patterns, displayed as time until event, of logarithmically transformed A) N-terminal pro B-type natriuretic peptide (NT-proBNP), B) high-sensitive troponin T (HsTNT) and C) C-reactive protein (CRP) of patients who reached the primary endpoint versus those who did not.

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 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 favourable prognosis in the example patients who stayed event-free. These individual estimates of prognosis can be obtained 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).

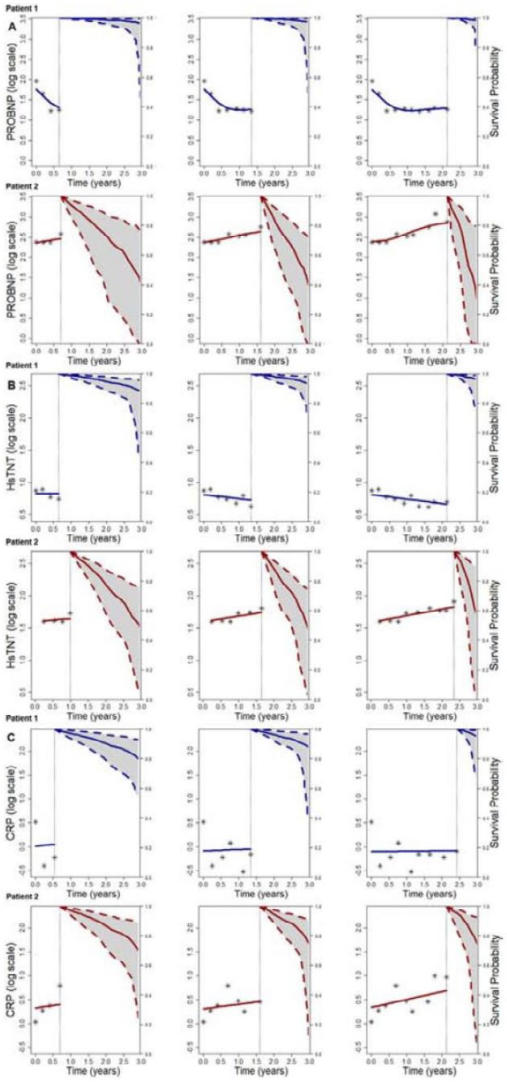

Figure 3. Dynamic profiling of an individual patient's risk using patient-specific temporal trajectories.

The solid red lines depict patients who experienced the study endpoints, 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% confidence intervals (grey area). To show this conditional survival probability curve is dynamically updated every time an extra measurement is recorded, we provide the curves for three time-points at which risk was updated.

Model performance

Discriminative ability of models containing the temporal patterns of the biomarker levels and baseline measurements only is shown in table 5. 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).

Table 5 – 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.

Temporal patterns of NT-proBNP, HsTNT and CRP in relation to hospitalisation for acute or worsening HF (secondary endpoint)

All three biomarker patterns were strong individual predictors of HF hospitalisations (age- and gender adjusted HRs per doubling of biomarker: NT-proBNP, 2.17; CI 1.57 – 2.66; p<0.001; HsTNT, 2.18; CI 1.72 – 2.76; p<0.001 and CRP, 1.99; CI 1.53 – 2.59; p<0.001). These associations remained statistically significant after multivariable adjustment (HRs per doubling of biomarker: NT-proBNP, 2.31; CI 1.77 – 3.01; p<0.001; HsTNT, 1.95; CI 1.49 – 2.55; p<0.001 and CRP, 1.80; CI 1.37 – 2.35; p<0.001). 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<0.001; HsTNT, 1.57; CI 1.24 – 2.00; p=0.001 and CRP, 1.41; CI 1.15 – 1.74; p<0.001). 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 b .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 favourable 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 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 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 (i.e., 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 changeover 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 cut off 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 cut off 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, N2,000 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 re-examined 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 ad- dressed 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 eventu-

ally a biomarker-guided trial that is based on personalized temporal patterns of multiple biomarkers.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ahj.2017.10.008.

REFERENCES

1. Masson S, Latini R, Anand IS, et al. Prognostic value of changes in N-terminal pro-brain natriuretic peptide in Val-HeFT (Valsartan Heart Failure Trial). J Am Coll Cardiol 2008;52:997-1003.
2. Anand IS, Latini R, Florea VG, et al. C-reactive protein in heart failure: prognostic value and the effect of valsartan. Circulation 2005;112:1428-1434.
3. Sato Y, Yamada T, Taniguchi R, et al. Persistently increased serum concentrations of cardiac troponin t in patients with idiopathic dilated cardiomyopathy are predictive of adverse outcomes. Circulation 2001;103:369-374.
4. Perna ER, Macin SM, Canella JP, et al. Ongoing myocardial injury in stable severe heart failure: value of cardiac troponin T monitoring for high-risk patient identification. Circulation 2004;110:2376-2382.
5. Bettencourt P, Azevedo A, Pimenta J, et al. N-terminal-pro-brain natriuretic peptide predicts outcome after hospital discharge in heart failure patients. Circulation 2004;110:2168-2174.
6. Anand IS, Fisher LD, Chiang YT, et al. Changes in brain natriuretic peptide and norepinephrine over time and mortality and morbidity in the Valsartan Heart Failure Trial (Val-HeFT). Circulation 2003;107:1278-1283.
7. O'Brien RJ, Squire IB, Demme B, et al. Pre-discharge, but not admission, levels of NT-proBNP predict adverse prognosis following acute LVF. Eur J Heart Fail. 2003;5:499-506.
8. Felker GM, Hasselblad V, Hernandez AF, et al. Biomarker-guided therapy in chronic heart failure: a meta-analysis of randomized controlled trials. Am Heart J 2009;158:422-430.
9. Savarese G, Trimarco B, Dellegrottaglie S, et al. Natriuretic peptide-guided therapy in chronic heart failure: a meta-analysis of 2,686 patients in 12 randomized trials. PLoS One. 2013;8:e58287.
10. Porapakkham P, Porapakkham P, Zimmet H, et al. B-type natriuretic peptide-guided heart failure therapy: A meta-analysis. Arch Intern Med 2010;170:507-514.
11. Latini R, Masson S, Anand IS, et al. Prognostic value of very low plasma concentrations of troponin T in patients with stable chronic heart failure. Circulation 2007;116:1242-1249.
12. Miller WL, Hartman KA, Burritt MF, et al. Serial biomarker measurements in ambulatory patients with chronic heart failure: the importance of change over time. Circulation 2007;116:249-257.
13. Yan RT, White M, Yan AT, et al. Usefulness of temporal changes in neurohormones as markers of ventricular remodeling and prognosis in patients with left ventricular systolic dysfunction and heart failure receiving either candesartan or enalapril or both. Am J Cardiol 2005;96:698-704.
14. Maisel A, Barnard D, Jaski B, et al. Primary results of the HABIT Trial (heart failure assessment with BNP in the home). J Am Coll Cardiol 2013;61:1726-1735.
15. Troughton RW, Frampton CM, Brunner-La Rocca HP, et al. Effect of B-type natriuretic peptide-guided treatment of chronic heart failure on total mortality and hospitalization: an individual patient meta-analysis. Eur Heart J 2014;35:1559-1567.

16. Adams KF, Jr., Felker GM, Fraij G, et al. Biomarker guided therapy for heart failure: focus on natriuretic peptides. Heart Fail.Rev 2010;15:351-370.
17. Dickstein K, Cohen-Solal A, Filippatos G, et al. ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2008: the Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2008 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association of the ESC (HFA) and endorsed by the European Society of Intensive Care Medicine (ESICM). Eur Heart J 2008;29:2388-2442.
18. McMurray JJ, Adamopoulos S, Anker SD, et al. ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2012: The Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2012 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association (HFA) of the ESC. Eur Heart J 2012;33:1787-1847.
19. Paulus WJ, Tschope C, Sanderson JE, et al. How to diagnose diastolic heart failure: a consensus statement on the diagnosis of heart failure with normal left ventricular ejection fraction by the Heart Failure and Echocardiography Associations of the European Society of Cardiology. Eur Heart J 2007;28:2539-2550.
20. Rizopoulos D. JM: An R Package for the Joint Modelling of Longitudinal and Time-to-Event Data. Journal of Statistical Software 2010;35:1-33.
21. Rizopoulos D, Takkenberg JJ. Tools & techniques--statistics: Dealing with time-varying covariates in survival analysis--joint models versus Cox models. Eurointervention 2014;10:285-288.
22. Troughton RW, Frampton CM, Yandle TG, et al. Treatment of heart failure guided by plasma aminoterminal brain natriuretic peptide (N-BNP) concentrations. Lancet 2000;355:1126-1130.
23. Jourdain P, Jondeau G, Funck F, et al. Plasma brain natriuretic peptide-guided therapy to improve outcome in heart failure: the STARS-BNP Multicenter Study. J Am Coll Cardiol 2007;49:1733-1739.
24. Pfisterer M, Buser P, Rickli H, et al. BNP-guided vs symptom-guided heart failure therapy: the Trial of Intensified vs Standard Medical Therapy in Elderly Patients With Congestive Heart Failure (TIME-CHF) randomized trial. JAMA 2009;301:383-392.
25. Januzzi JL, Jr., Rehman SU, Mohammed AA, et al. Use of amino-terminal pro-B-type natriuretic peptide to guide outpatient therapy of patients with chronic left ventricular systolic dysfunction. J Am Coll Cardiol 2011;58:1881-1889.
26. Berger R, Moertl D, Peter S, et al. N-terminal pro-B-type natriuretic peptide-guided, intensive patient management in addition to multidisciplinary care in chronic heart failure a 3-arm, prospective, randomized pilot study. J Am Coll Cardiol 2010;55:645-653.
27. Shah MR, Califf RM, Nohria A, et al. The STARBRITE trial: a randomized, pilot study of B-type natriuretic peptide-guided therapy in patients with advanced heart failure. J Card Fail. 2011;17:613-621.
28. Eurlings LW, van Pol PE, Kok WE, et al. Management of chronic heart failure guided by individual N-terminal pro-B-type natriuretic peptide targets: results of the PRIMA (Can PRo-brain-natriuretic peptide guided therapy of chronic heart failure IMprove heart fAilure morbidity and mortality?) study. J Am Coll Cardiol 2010;56:2090-2100.

29. Persson H, Erntell H, Eriksson B, et al. Improved pharmacological therapy of chronic heart failure in primary care: a randomized Study of NT-proBNP Guided Management of Heart Failure--SIGNAL-HF (Swedish Intervention study--Guidelines and NT-proBNP AnaLysis in Heart Failure). Eur J Heart Fail. 2010;12:1300-1308.