Incremental Prognostic Power of Novel Biomarkers (Growth-Differentiation Factor-15, High-Sensitivity C-Reactive Protein, Galectin-3, and High-Sensitivity Troponin-T) in Patients With Advanced Chronic Heart Failure

Dirk J. Lok, MD[a,*], IJsbrand T. Klip, MD[b], Sjoukje I. Lok, MD[c], Pieta W. Bruggink-André de la Porte, MD[a], Erik Badings, MD[a], Jan van Wijngaarden, MD[a], Adriaan A. Voors, MD[b], Rudolf A. de Boer, MD[b], Dirk J. van Veldhuisen, MD[b], and Peter van der Meer, MD[a,b]

Elevated natriuretic peptides provide strong prognostic information in patients with heart failure (HF). The role of novel biomarkers in HF needs to be established. Our objective was to evaluate the prognostic power of novel biomarkers, incremental to the N-terminal portion of the natriuretic peptide (NT-proBNP) in chronic HF. Concentrations of circulating NT-proBNP, growth differentiation factor 15 (GDF-15), high-sensitivity C-reactive protein (hs-CRP), galectin-3 (Gal-3), and high-sensitivity troponin T (hs-TnT) were measured and related to all-cause long-term mortality. Of 209 patients (age 71 ± 10 years, 73% male patients, 97% New York Heart Association class III), 151 (72%) died during a median follow-up of 8.7 ± 1 year. The calculated area under the curve for NT-proBNP was 0.63, GDF-15 0.78, hs-CRP 0.66, Gal-3 0.68, and hs-TnT 0.68 (all p <0.01). Each marker was predictive for mortality in univariate analysis. In multivariate analysis, elevated concentrations of GDF-15 (hazard ratio [HR] 1.41, confidence interval [CI] 1.1 to 178, p = 0.005), hs-CRP (HR 1.38, CI 1.15 to 1.67, p = 0.001), and hs-TnT (HR 1.27, CI 1.06 to 1.53, p = 0.008) were independently related to mortality. All novel markers had an incremental value to NT-proBNP, using the integrated discrimination improvement. In conclusion, in chronic HF, GDF-15, hs-CRP, and hs-TnT are independent prognostic markers, incremental to NT-proBNP, in predicting long-term mortality. In this study, GDF-15 is the most predictive marker, even stronger than NT-proBNP. **(Am J Cardiol 2013;112:831−837)**

Concentrations of natriuretic peptides (NP) are useful in the diagnosis and management of heart failure (HF),[1] and provide powerful prognostic information in these patients, independent of left ventricular ejection fraction.[2] NPs are produced by the myocardium as a reaction to an increase in myocardial wall stress.[3] However, concentrations of circulating NP do not necessarily provide reliable information about the mechanism, etiology, and intensity of myocardial distress. Furthermore, a high intraindividual variation of NP has been described in patients with stable chronic HF.[4] Hence, there is a need for additive biomarkers with respect to pathophysiology, treatment effect, and prognosis. Several novel markers, such as growth differentiation factor-15 (GDF-15),[5,6] high-sensitivity C-reactive protein (hs-CRP),[7,8] galectin-3 (Gal-3),[9,10] and high-sensitivity troponin T (hs-TnT)[11,12] are being tested and introduced for their clinical use in chronic HF. However, the added value of these markers is still under debate, and long-term data are lacking. Therefore, we analyzed the power of these markers head to head, compared with and added to N-terminal pro-brain-type natriuretic peptide (NT-proBNP), with respect to all-cause mortality during long-term follow-up in a population with advanced chronic HF.

Methods

The present study was conducted as a substudy from the Deventer-Alkmaar Heart Failure study (DEAL-HF), which has been described elsewhere.[13,14] In brief, 240 patients with typical signs and symptoms of chronic HF combined with findings of a reduced left ventricular ejection fraction (45%) or diastolic dysfunction, according to the 2001 guidelines for the diagnosis of HF of the European Society of Cardiology, were included.[15] Main exclusion criteria were an expected survival of <1 year, kidney function replacement therapy, and planned hospitalization.

In the present multimarker study, a complete set of data was available of 209 patients at baseline, due to missing blood samples (n = 28) and loss to follow-up (n = 3). The study was approved by the local medical ethics committees

[a]Deventer Hospital, Deventer, the Netherlands; [b]University of Groningen, University Medical Center Groningen, Groningen, the Netherlands; and [c]University Medical Center Utrecht, Utrecht, the Netherlands. Manuscript received January 21, 2013; revised manuscript received and accepted May 2, 2013.

A.A. Voors is funded through Grant 242209 from the European Commission FP7-Health-2009-BIOSTAT-CHF.

See page 836 for disclosure information.

*Corresponding author: Tel: 31-570-535001; fax: 31-570-627044.

E-mail address: lokd@dz.nl (D.J. Lok).

0002-9149/13/$ - see front matter
http://dx.doi.org/10.1016/j.amjcard.2013.05.013

Table 1
Baseline characteristics in relation to the occurrence of the end point.

Variable	Total (n = 209)	Survivors (n = 58)	Nonsurvivors (n = 151)	p Value
Age (yrs)	71 ± 10	67 ± 11	72 ± 9	<0.001
Men	73%	62%	77%	0.02
HF etiology, ischemic	66%	50%	72%	0.003
Body mass index (kg/m^2)	26 ± 5	27 ± 5	26 ± 5	0.105
NYHA class, III/IV	97%/3%	98%/2%	96%/4%	0.418
Diabetes mellitus	27%	17%	32%	0.028
Chronic obstructive pulmonary disease	27%	29%	26%	0.405
Hypercholesterolemia	48%	50%	48%	0.119
Sodium (mmol/L)	138 ± 3	139 ± 3	138 ± 3	0.026
Hemoglobin (mmol/L)	8.4 ± 1.0	8.5 ± 0.8	8.3 ± 1.0	0.242
Estimated glomerular filtration rate (ml/min/1.73m^2)	52 ± 14	55 ± 13	50 ± 15	0.038
NT-proBNP (pg/ml)*	1,771 (1,008−4,483)	1,729 (720−3,144)	2,373 (1,093−5,813)	0.004
GDF-15 (ng/L)	1,606 (1,087−2,412)	1,082 (802−1,502)	1,900 (1,357−2,671)	<0.001
hs-CRP (mg/dl)	4.7 (1.9−11.2)	3.1 (1.4−5.4)	5.8 (2.0−14.9)	0.003
Gal-3 (ng/ml)	17.6 (13.3−21.4)	15.0 (11.8−18.0)	18.5 (13.9−22.3)	<0.001
hs-TnT (ng/ml)	25.6 (17.2−39.3)	18.8 (10.4−30.8)	28.2 (18.9−1,900)	<0.001
Use of angiotensin converting enzyme inhibitors	86%	90%	84%	0.306
Use of angiotensin receptor blockers	11%	8%	11%	0.578
Use of beta blockers	64%	67%	62%	0.502
Use of diuretics	97%	96%	97%	0.757
Use of statins	41%	52%	36%	0.044
Use of spironolactone	31%	24%	34%	0.187

Values are presented as means ± SD, medians ± interquartile ranges, or as frequencies and percentages.
* To convert to pmol/L, devide by 0.118.

and complied with the Declaration of Helsinki. All patients gave written informed consent.

Routine laboratory measurements and blood samples for biomarker analysis were obtained at baseline. Baseline was defined as the moment of signing the informed consent, with patients preferably in a stable condition. Blood samples were taken on the same day, just after signing the informed consent. Patients could be included before discharge after hospitalization for HF (31%) or at the out patient clinic (69%). EDTA plasma was separated and stored at −70°C. Renal function, assessed using the estimated glomerular filtration rate (ml/min/1.73m^2), was calculated using the Modification of Diet in Renal Disease equation.[16]

Circulating concentrations of NT-proBNP, GDF-15, hs-CRP, Gal-3, and hs-TnT were analyzed according to the description of the manufacturer (NT-proBNP, Roche Diagnostics, Rotkreutz, Switzerland; GDF-15, Roche Diagnostics; hs-CRP, Roche Diagnostics; Gal-3, BG Medicine, Waltham, Massachusetts; hs-TnT, Roche Diagnostics).

GDF-15 was analyzed by electrochemiluminescence immunoassay (precommercial assay). The interassay coefficient of variation was 2.3% at 1,100 ng/l and 1.8% at 17,200 ng/L with a lower limit of detection level of <10 ng/l. The reference value for GDF-15 was 1,109 ng/l (97.5th percentile). Quality control data were acquired with spiked plasma.

Gal-3 concentrations were measured by enzyme-linked immunosorbent assay; the lower limit of detection was 1.13 ng/ml. The 90th, 95th, and 97.5th percentile of the normal reference interval were 17.6, 20.3, and 22.1 ng/ml, respectively. Imprecision studies demonstrated that the total coefficient of variation was <10% at a low concentration of 6 ng/ml, 7% near the midlevel concentration of 21 ng/ml, and 15% at the high level of 70 ng/ml.

High-sensitivity CRP and NT-proBNP concentrations were measured by a electrochemiluminescence immunoassay using an Elecsys (Roche Diagnostics). The lower limit of detection level for hs-TnT was ≤3 pg/ml, for hs-CRP ≤0.15 mg/L, and for NT-proBNP 20 pg/ml. The coefficient of variation for hs-TnT was ≤5% in the range 25 to ≤100 pg/ml; for hs-CRP, ≤5% for values >1.0 mg/L; and for NT-proBNP, 5% for values >100 pg/ml.

The end point of the current study was all-cause mortality. Patients were followed up to 10 years after randomization at the outdoor patient clinic. In case of no show, information regarding survival was obtained from the hospital system, relatives, or general practitioner. Baseline medication was up-titrated according to the 2001 guidelines.[15]

Data are expressed as mean ± SD when normally distributed, as median with interquartile range when distribution was skewed and as frequencies and percentages. The intergroup differences were tested using Student *t* test, Mann-Whitney U test, or Pearson chi-square test when appropriate. For further analyses, logarithmic transformation was performed to achieve a normal distribution for skewed variables. To assess the ability of NT-proBNP, GDF-15, hs-CRP, Gal-3, and hs-TnT in predicting all-cause mortality, areas under the curve (AUCs) of receiver operating characteristics (ROC) curves were constructed. The statistical significance of differences in AUCs was estimated using the approach by DeLong et al.[17] Optimal cut-off points were calculated using ROC curves. Log-rank tests were used to assess the significance between the concentrations of GDF-15, hs-CRP, Gal-3, and hs-TnT, above and below the optimal cut-off point.

Unadjusted hazard ratios (HR) of log-transformed biomarkers were calculated for univariate Cox regression

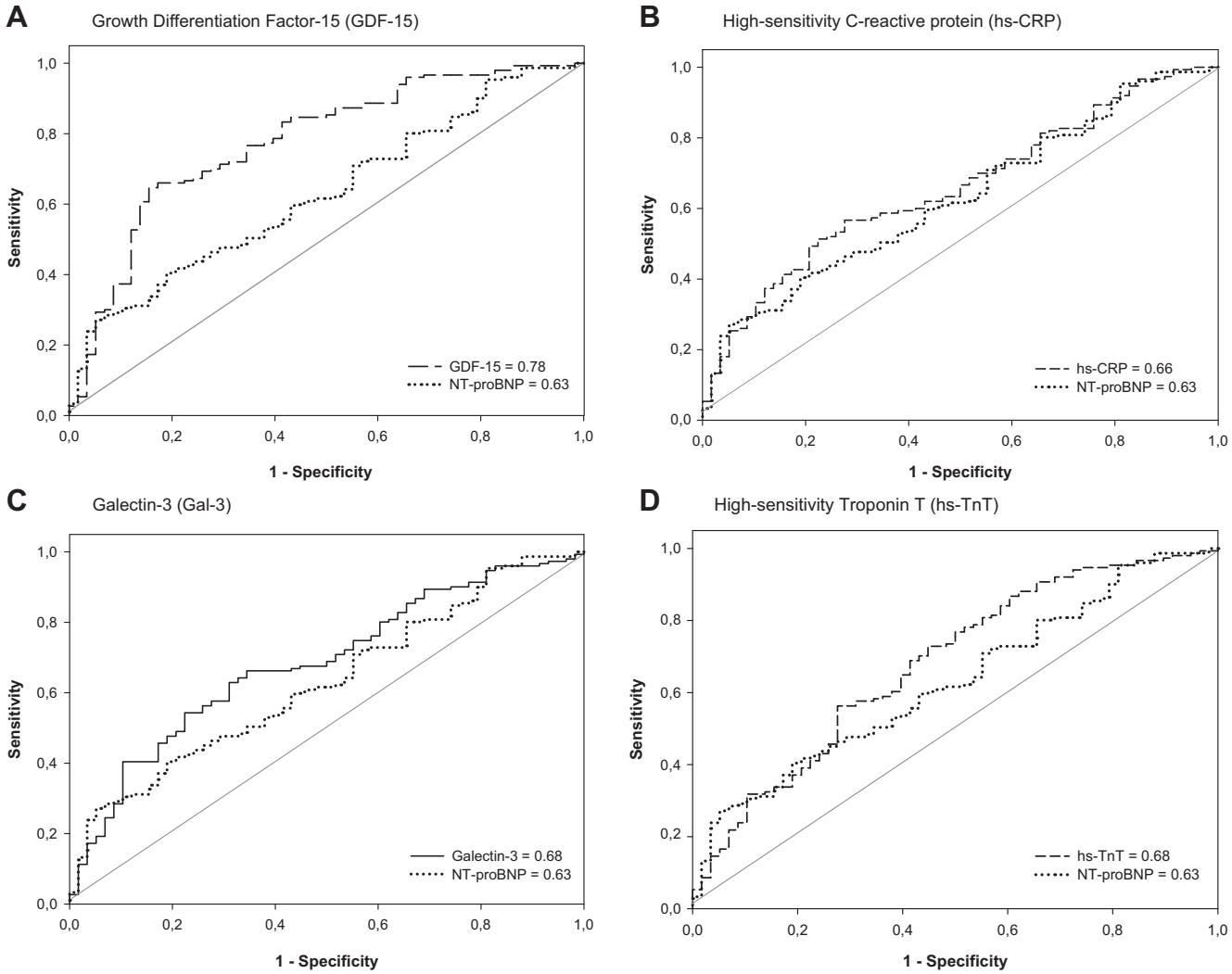

Figure 1. ROC curves for NT-proBNP, GDF-15, hs-CRP, Gal-3, and hs-TnT to predict all-cause mortality.

Table 2
Areas under the curve of novel markers versus N-terminal pro-brain-type natriuretic peptide

Novel Marker vs NT-proBNP	p Value	AUC for Combination of Markers	Incremental Power of Novel Marker	p Value
GDF-15 vs NT-proBNP	<0.001	GDF-15 + NT-proBNP = 0.78	NT-proBNP vs GDF-15 + NT proBNP	<0.001
hs-CRP vs NT-proBNP	0.488	hs-CRP + NT-proBNP = 0.68	NT-proBNP vs hs-CRP + NT-proBNP	0.068
Gal-3 vs NT-proBNP	0.279	Gal-3 + NT-proBNP = 0.69	NT-proBNP vs Gal-3 + NT-proBNP	0.039
hs-TnT vs NT-proBNP	0.235	hs-TnT + NT-proBNP = 0.68	NT-proBNP vs hs-TnT + NT-proBNP	0.067

analyses (depicted as per SD increase). In consecutive multivariate models, GDF-15, hs-CRP, Gal-3, and hs-TnT were adjusted for age and gender, renal function, HF etiology, NT-proBNP concentrations, and all other univariate significant biomarkers. Finally, the net reclassification improvement (NRI) and the integrated discrimination improvement (IDI), described by Pencina et al, were also calculated.[18] The aim of the NRI was to examine the prognostic discrimination of integrated discrimination (ID) on top of clinical risk factors on the primary end point. Clinical risk factors included age, gender, renal function, HF etiology, and NT-proBNP concentrations. We used risk categories of <25%, 25% to 50%, and >50%.

All tests were 2-sided, and a p value <0.05 was considered statistically significant. All statistical analyses were performed using STATA version 11.0 (StataCorp LP, College Station, Texas) and SPSS version 18.0 (SPSS, Chicago, Illinois).

Results

Characteristics of the study population are described in Table 1. Medical care was provided according to the guidelines of the European Society of Cardiology prevailing at the time of inclusion and execution of the study with optimal application of therapy (baseline medication,

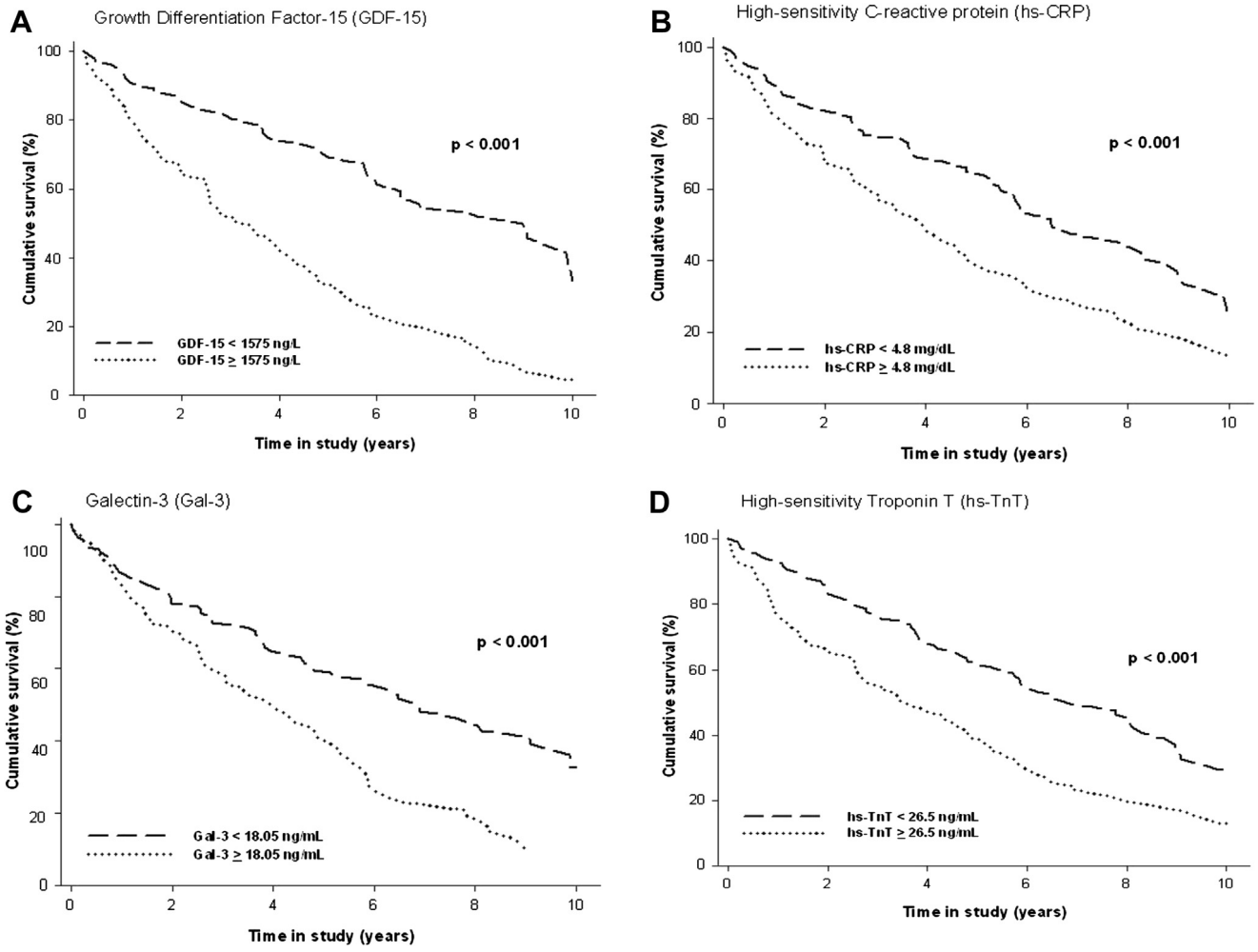

Figure 2. Kaplan-Meier curves reflecting the difference in event-free survival rates for GDf-15, hs-CRP, Gal-3, and hs-TnT above or below their optimal cut-off points.

Table 1). At baseline, beta blockers were prescribed in 60% of the patients. This figure went up to 69% after 1 year of follow-up. Almost all patients used a blocker of the renin-angiotensin system (96%) and diuretic therapy (97%) at baseline. Nonsurvivors were older, more often male, more frequently had ischemic etiology and diabetes mellitus, and had lower sodium and estimated glomerular filtration rate.

The median follow-up for survivors was 8.4 (interquartile range 7.8 to 9.8) years. In total, 151 (72%) patients died. Nonsurvivors had significantly higher baseline concentrations of all biomarkers. For each individual marker, the AUC was plotted against the AUC of NT-proBNP (Figure 1). Only GDF-15 was significantly better than NT-proBNP in predicting mortality (Table 2; $p < 0.001$). Figure 2 depicting Kaplan-Meier survival curves show that all biomarkers significantly predicted outcome (all $p < 0.001$).

The power of each novel marker incremental to NT-proBNP was calculated (Table 2). GDF-15 and Gal-3 showed to be of significant additive value when combined with NT-proBNP (Table 2; $p < 0.001$ and $p = 0.039$, respectively) whereas the other markers only showed marginal changes.

Univariate and multivariate (Table 3) Cox-proportional hazard regression models were conducted for each variable. GDF-15 ($p = 0.005$), hs-CRP ($p = 0.001$), and hs-TnT ($p = 0.008$) were significant independent predictors for all-cause mortality, whereas Gal-3 was not ($p = 0.638$).

Data of survivors and nonsurvivors were adjusted for age, gender, renal function, HF etiology, and NT-proBNP, and patients were classified separately into risk categories (<25%, 25% to 50%, and >50%). The NRI and IDI are presented in Table 4. For the NRI, all markers except Galectin-3 were able to improve the index significantly. For the IDI, all novel markers had an incremental value to NT-proBNP.

Discussion

Our study examined the predictive power of GDF-15, hs -CRP, Gal-3, and hs-TnT incremental to NT-proBNP, with respect to long-term outcome in patients with advanced chronic HF. The main findings of the present study are that GDF-15, hs-CRP, and hs-TnT have independent predictive power for long-term mortality, incremental to established clinical and biochemical risk factors. GDF-15 showed to be the strongest prognosticator. Second, the presence of each individual marker significantly enhanced integrated discrimination improvement.

Table 3
Univariate and multivariate Cox-proportional hazard analysis

Variable	GDF-15 (per SD)	Z	p Value	hs-CRP (per SD)	Z	p Value	Galectin-3 (per SD)	Z	p Value	hs-TnT (per SD)	Z	p Value
	HR (95% CI)			HR (95% CI)			HR (95% CI)			HR (95% CI)		
Univariate	1.69 (1.43−1.98)	6.31	<0.001	1.55 (1.31−1.83)	5.15	<0.001	1.41 (1.20−1.64)	4.30	<0.001	1.53 (1.32−1.78)	5.65	<0.001
Model 1	1.56 (1.31−1.86)	4.94	<0.001	1.47 (1.26−1.73)	4.70	<0.001	1.30 (1.10−1.53)	3.11	0.002	1.45 (1.24−1.71)	4.54	<0.001
Model 2	1.45 (1.18−1.79)	3.45	0.001	1.42 (1.19−1.70)	3.93	<0.001	1.27 (1.04−1.57)	2.35	0.019	1.36 (1.14−1.62)	3.39	0.001
Model 3	1.41 (1.11−1.78)	2.81	0.005	1.38 (1.15−1.67)	3.41	0.001	1.06 (0.84−1.32)	0.47	0.638	1.27 (1.06−1.53)	2.63	0.008

Model 1: adjusted for age and gender. Model 2: adjusted for model 1 + renal function (estimated glomerular filtration rate), HF etiology and NT-proBNP. Model 3: adjusted for model 2 + novel biomarkers (combination of GDF-15, hs-TnT, Gal-3 and/or hs-CRP).

Table 4
Net reclassification improvement and integrated discrimination improvement of multiple markers related to N-terminal pro-brain-type

	c Statistics	NRI*	p Value	IDI	p Value
NT-proBNP	0.62	referent		referent	
GDF-15	0.67	18.9	0.006	0.073	<0.001
hs-CRP	0.63	13.5	0.022	0.035	0.015
Gal-3	0.60	6.4	0.184	0.024	0.024
hs-TnT	0.64	12.9	0.027	0.023	0.023

Adjusted for age, gender, renal function, HF etiology, and NT-proBNP.
* Risk categories <25%, 25% to 50%, and >50%.

NPs have emerged as important biomarkers with a well-known role in the diagnosis and prognosis of HF.[1] However, concentrations of NP can be elevated in several nonprimary cardiac diseases, such as pulmonary embolism and/or hypertension.[19,20] After treatment, NP concentrations may lower, sometimes even normalize, falsely suggesting no disease at all. Besides myocyte stress, other processes such as inflammation, neurohormonal modulation, myocyte injury, oxidative stress, and extracellular matrix remodeling are recognized as important mechanisms associated with HF (Figure 3).[21]

Inflammation is a key process in the development and progression of chronic HF. GDF-15 has alleged anti-inflammatory activity and is weakly expressed under normal conditions.[22] GDF-15 belongs to the transforming growth factor-beta family and has been associated with myocardial fibrosis.[23] Myocardial expression of GDF-15 rapidly increases during cardiac distress.[24] GDF-15 is strongly associated with adverse outcome in patients with an acute coronary syndrome and those with stable coronary heart disease, providing prognostic information beyond hs-CRP and hs-TnT.[6,24,25] However, the role of GDF-15 in chronic HF patients has been less studied. In a study by Kempf et al, GDF-15 was an independent predictor of mortality in CHF, beyond New York Heart Association (NYHA) class, left ventricular ejection fraction, and NT-proBNP. Mortality rates at 48 months increased with increasing quartiles of GDF-15.[5] Interestingly, and in contrast to our findings, in the study by Kempf et al, addition of GDF-15 did not improve the c statistic of NT-proBNP.[5] Our study population consisted of older patients with more advanced HF with a longer follow-up, presumably leading to subsequent enhanced predictive power of GDF-15. These differences likely resulted in subsequent enhanced predictive power for GDF-15 in our trial.

The prognostic role of GDF-15 in the current study is in agreement with a previous trial from Anand et al in which GDF-15 and changes over time were associated with mortality and first morbid event in HF patients, independently of hs-CRP, hs-TnT, and NT-proBNP.[6] Yet this study focused on the value of GDF-15 and compromised a median follow-up of only 2 years. Patients in this trial had less advanced heart failure (NYHA class III and IV in 43%) compared with our study population (NYHA class III and IV in 100%). Moreover, in the study from Anand, only 33% of the patients used a beta-blocking agent and only 2% spironolactone. In our long-term follow-up study, with a median follow-up of 8.4 years, patients were treated with

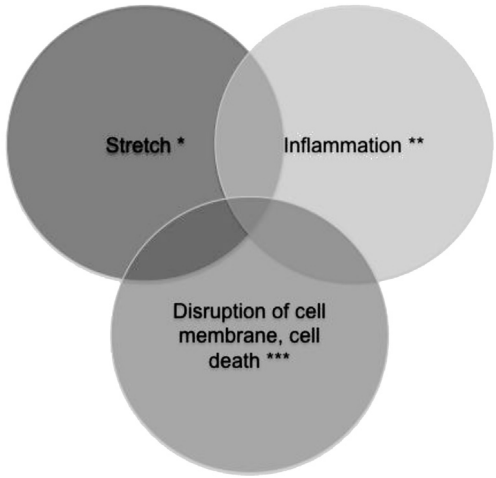

Figure 3. Schematic representation of biomarkers in chronic HF. Increased wall stress, inflammation, and disruption of cell membrane cause a cascade of biomarker release that can be measured to monitor disease severity and ongoing myocardial insult. *NPs; **GDF15; hs-CRP; Gal-3; ***hs-TnT.

beta-blocking agents at entry in 64% of the cases (after 1 year 69%), and 36% of the patients used spironolactone at baseline (after 1 year 30%). In the ambulatory community-based Framingham Heart Study, GDF-15 showed to be the strongest prognosticator with respect to the end points of HF and death compared with other biomarkers (soluble ST2, high-sensitivity troponin I, hs-CRP, and BNP).[26] These data, combined with our results, indicate that elevated concentrations of GDF-15 and changes related to this are independently associated with prognosis of chronic HF patients and overall prognosis.

Another more commonly used inflammatory marker hs-CRP, is a hepatocyte-derived cytokine and currently under attention for diagnostic and prognostic purposes in HF. Tang et al concluded that high plasma hs-CRP concentrations portend poor long-term outcomes in a heart failure with a reduced ejection fraction (HFrEF) patient cohort independently of NP.[27] In our multivariate analyses, the additive value on long-term term outcome of hs-CRP was confirmed.

Adverse and excessive extracellular matrix remodeling leads to fibrosis and impaired cardiac function. Gal-3 plays an important regulatory role in cardiac fibrosis and remodeling[28] and has been suggested as a potential therapeutic target. In chronic HF, increased plasma concentrations of Gal-3 were detected. Moreover, Gal-3 concentrations have additive prognostic value to NT-proBNP in chronic HF with respect to mortality.[9,10] Interestingly, in the present study, Gal-3 did not show to have independent prognostic value.

Presumably, the adjustments for baseline concentrations of additional markers (including NT-proBNP) and established risk factors, which were not performed in previously trials, may have attributed to this discrepancy.

Ultimately, because of excessive strain, ischemia, stress, or inflammation, myocardial cell disruption occurs. This will lead to release of cytokines, activation of macrophages, and release of troponins, originating from cardiomyocytes. Elevated concentrations of high-sensitivity troponin I and hs-TnT were studied in large cohorts of chronic HF patients and appeared to be independent predictors for outcome.[11,12,29] The independent prognostic value from hs-TnT is consistent with our data from multivariate analysis. In addition, we demonstrated that hs-TnT enhanced risk classification and integrated discrimination improvement.

The most important limitation of this study is the relatively small sample size. For the present study, we evaluated the superiority and incremental power of 4 novel markers predominantly in patients with chronic advanced HFrEF. Our results cannot be extrapolated to less advanced forms of HFrEF, acute HF, and/or HF with a preserved left ventricular systolic function (HFpEF). The end point of the present study was all-cause mortality. Because all patients were in NYHA class III or IV, it is to be expected that the vast majority died of a cardiac cause, resulting in an extremely small group of patients in the study dying of a non-cardiovascular cause. Although interesting, whether the selected biomarkers would also predict noncardiac mortality, we feel that our cohort is too small for a meaningful analysis of noncardiac death. Our findings on the additive value of novel markers in chronic HF need to be confirmed in a larger cohort of patients.

Disclosures

BG Medicine, Inc., has rights related to Galectin-3 measurements. BG Medicine, Inc., provided an unrestricted research grant to the Department of Cardiology of the University Medical Center Groningen, which employs P.v.d.M., R.A.d.B., and D.J.v.V. D.J.v.V and R.A.d.B. have received consultancy and speakers fees from BG Medicine, Inc. The Deventer Cardiology Research department received an unrestricted research grant from BG Medicine, Inc. Roche Diagnostics performed laboratory assessments for GDF-15, hs-CRP, hs-TNT, and NT-proBNP in a blinded manner.

1. McMurray JJV, Adamopoulos S, Anker SD, Auricchio A, Böhm M, Dickstein K, Falk V, Filippatos G, Fonseca C, Gomez-Sanchez MA, Jaarsma T, Køber L, Lip GYH, Maggioni AP, Parkhomenko A, Pieske BM, Popescu BA, Rønnevik PK, Rutten FH, Schwitter J, Seferovic P, Stepinska J, Trindade PT, Voors AA, Zannad F, Zeiher A. 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 J Heart Fail* 2012;14:803−869.
2. van Veldhuisen DJ, Linssen GCM, Jaarsma T, van Gilst WH, Hoes AW, Tijssen JPG, Paulus WJ, Voors AA, Hillege HL. Brain natriuretic peptide and prognosis in heart failure patients with preserved and reduced ejection fraction. *J Am Coll Cardiol* 2013;61:1498−1506.
3. Daniels LB, Maisel AS. Natriuretic peptides. *J Am Coll Cardiol* 2007;50:2357−2368.
4. Bruins S, Fokkema MR, Römer JWP, Dejongste MJL, van der Dijs FPL, van den Ouweland JMW, Muskiet FAJ. High intraindividual variation of B-type natriuretic peptide (BNP) and amino-terminal proBNP in patients with stable chronic heart failure. *Clin Chem* 2004;50:2052−2058.
5. Kempf T, Haehling von S, Peter T, Allhoff T, Cicoira M, Doehner W, Ponikowski P, Filippatos GS, Rozentryt P, Drexler H, Anker SD, Wollert KC. Prognostic utility of growth differentiation factor-15 in patients with chronic heart failure. *J Am Coll Cardiol* 2007;50:1054−1060.

6. Anand IS, Kempf T, Rector TS, Tapken H, Allhoff T, Jantzen F, Kuskowski M, Cohn JN, Drexler H, Wollert KC. Serial measurement of growth-differentiation factor-15 in heart failure: relation to disease severity and prognosis in the Valsartan Heart Failure Trial. *Circulation* 2010;122:1387−1395.
7. Niizeki T, Takeishi Y, Kitahara T, Suzuki S, Sasaki T, Ishino M, Kubota I. Combination of conventional biomarkers for risk stratification in chronic heart failure. *J Cardiol* 2009;53:179−187.
8. Ky B, French B, Levy WC, Sweitzer NK, Fang JC, Wu AHB, Goldberg LR, Jessup M, Cappola TP. Multiple biomarkers for risk prediction in chronic heart failure. *Circ Heart Fail* 2012;5:183−190.
9. Lok DJA, Van Der Meer P, la Porte de PWB-A, Lipsic E, Van Wijngaarden J, Hillege HL, van Veldhuisen DJ. Prognostic value of galectin-3, a novel marker of fibrosis, in patients with chronic heart failure: data from the DEAL-HF study. *Clin Res Cardiol* 2010;99: 323−328.
10. de Boer RA, Lok DJA, Jaarsma T, Van Der Meer P, Voors AA, Hillege HL, van Veldhuisen DJ. Predictive value of plasma galectin-3 levels in heart failure with reduced and preserved ejection fraction. *Ann Med* 2011;43:60−68.
11. Masson S, Anand I, Favero C, Barlera S, Vago T, Bertocchi F, Maggioni AP, Tavazzi L, Tognoni G, Cohn JN, Latini R. Serial measurement of cardiac troponin T using a highly sensitive assay in patients with chronic heart failure: data from two large randomized clinical trials. *Circulation* 2012;125:280−288.
12. Egstrup M, Schou M, Tuxen CD, Kistorp CN, Hildebrandt PR, Gustafsson F, Faber J, Goetze J-P, Gustafsson I. Prediction of outcome by highly sensitive troponin T in outpatients with chronic systolic left ventricular heart failure. *Am J Cardiol* 2012;110:552−557.
13. Bruggink-André de la Porte PWF, Lok DJA, Van Wijngaarden J, Cornel JH, Pruijsers-Lamers D, van Veldhuisen DJ, Hoes AW. Heart failure programmes in countries with a primary care-based health care system. Are additional trials necessary? Design of the DEAL-HF study. *Eur J Heart Fail* 2005;7:910−920.
14. la Porte de PWFB-A, Lok DJA, van Veldhuisen DJ, Van Wijngaarden J, Cornel JH, Zuithoff NPA, Badings E, Hoes AW. Added value of a physician-and-nurse-directed heart failure clinic: results from the Deventer-Alkmaar heart failure study. *Heart* 2007;93:819−825.
15. Remme W. Guidelines for the diagnosis and treatment of chronic heart failure. *Eur Heart J* 2001;22:1527−1560.
16. Smilde TDJ, van Veldhuisen DJ, Navis G, Voors AA, Hillege HL. Drawbacks and prognostic value of formulas estimating renal function in patients with chronic heart failure and systolic dysfunction. *Circulation* 2006;114:1572−1580.
17. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics* 1988;44:837−845.
18. Pencina MJ, D'Agostino RB, D'Agostino RB, Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. *Stat Med* 2008;27:157−172; discussion 207−12.
19. Tulevski II, Hirsch A, Sanson BJ, Romkes H, van der Wall EE, van Veldhuisen DJ, Büller HR, Mulder BJ. Increased brain natriuretic peptide as a marker for right ventricular dysfunction in acute pulmonary embolism. *Thromb Haemost* 2001;86:1193−1196.
20. Andreadis EA, Georgiopoulos DX, Tzavara CK, Katsanou PM, Fragouli EG, Mavrokefalou EM, Chatzis NA, Ifanti GK, Diamantopoulos EJ. Plasma brain natriuretic peptide: a biochemical marker of effective blood pressure management? *J Hypertens* 2009;27:425−432.
21. Giannessi D. Multimarker approach for heart failure management: perspectives and limitations. *Pharmacol Res* 2011;64:11−24.
22. Kempf T, Zarbock A, Widera C, Butz S, Stadtmann A, Rossaint J, Bolomini-Vittori M, Korf-Klingebiel M, Napp LC, Hansen B, Kanwischer A, Bavendiek U, Beutel G, Hapke M, Sauer MG, Laudanna C, Hogg N, Vestweber D, Wollert KC. GDF-15 is an inhibitor of leukocyte integrin activation required for survival after myocardial infarction in mice. *Nat Med* 2011;17:581−588.
23. Lok SI, Winkens B, Goldschmeding R, van Geffen AJP, Nous FMA, van Kuik J, van der Weide P, Klöpping C, Kirkels JH, Lahpor JR, Doevendans PA, de Jonge N, de Weger RA. Circulating growth differentiation factor-15 correlates with myocardial fibrosis in patients with non-ischaemic dilated cardiomyopathy and decreases rapidly after left ventricular assist device support. *Eur J Heart Fail* 2012 Nov;14: 1249−1256.
24. Wollert KC, Kempf T, Peter T, Olofsson S, James S, Johnston N, Lindahl B, Horn-Wichmann R, Brabant G, Simoons ML, Armstrong PW, Califf RM, Drexler H, Wallentin L. Prognostic value of growth-differentiation factor-15 in patients with non-ST-elevation acute coronary syndrome. *Circulation* 2007;115:962−971.
25. Kempf T, Björklund E, Olofsson S, Lindahl B, Allhoff T, Peter T, Tongers J, Wollert KC, Wallentin L. Growth-differentiation factor-15 improves risk stratification in ST-segment elevation myocardial infarction. *Eur Heart J* 2007;28:2858−2865.
26. Wang TJ, Wollert KC, Larson MG, Coglianese E, McCabe EL, Cheng S, Ho JE, Fradley MG, Ghorbani A, Xanthakis V, Kempf T, Benjamin EJ, Levy D, Vasan RS, Januzzi JL. Prognostic utility of novel biomarkers of cardiovascular stress: the Framingham Heart Study. *Circulation* 2012;126:1596−1604.
27. Tang WHW, Shrestha K, Van Lente F, Troughton RW, Martin MG, Borowski AG, Jasper S, Klein AL. Usefulness of C-reactive protein and left ventricular diastolic performance for prognosis in patients with left ventricular systolic heart failure. *Am J Cardiol* 2008;101:370−373.
28. de Boer RA, Voors AA, Muntendam P, van Gilst WH, van Veldhuisen DJ. Galectin-3: a novel mediator of heart failure development and progression. *Eur J Heart Fail* 2009;11:811−817.
29. Kawahara C, Tsutamoto T, Sakai H, Nishiyama K, Yamaji M, Fujii M, Yamamoto T, Horie M. Prognostic value of serial measurements of highly sensitive cardiac troponin I in stable outpatients with non-ischemic chronic heart failure. *Am Heart J* 2011;162:639−645.