ORIGINAL RESEARCH

Prognostic Value of Serial Galectin-3 Measurements in Patients With Acute Heart Failure

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***Background*—**Several clinical studies have evaluated the association between galectin-3 levels and outcome in patients with heart failure (HF). However, little is known about the predictive value of repeated galectin-3 measurements. This study evaluates the prognostic value of repeated time-dependent galectin-3 measurements in acute HF patients.

***Methods and Results*—**In the TRIUMPH (Translational Initiative on Unique and Novel Strategies for Management of Patients with Heart Failure) clinical cohort study, 496 acute HF patients were enrolled in 14 hospitals in The Netherlands, between 2009 and 2014. Repeated blood samples (7) were drawn during 1-year follow-up. Associations between repeated biomarker measurements and the primary end point were assessed using a joint model. Median age was 74 years and 37% were women. The primary end point, composite of all-cause mortality and HF rehospitalization, was reached in 188 patients (40%), during a median follow-up of 325 days (interquartile range 85–401). The median baseline galectin-3 level was 24 ng/mL (interquartile range 18–34). The mean number of galectin-3 measurements available per patient was 4.3. After adjustment for clinical factors and N-terminal pro-brain natriuretic peptide, there was a weak association between baseline galectin-3 and risk of the primary end point. When repeated measurements were taken into account, the adjusted hazard ratio per 1 SD increase of the galectin-3 level (on the log2 scale) at any time point increased to 1.67 (95% confidence interval, 1.24–2.23, $P<0.001$). After additional adjustment for repeated N-terminal pro-brain natriuretic peptide measurements, the association remained statistically significant.

***Conclusions*—**Repeated galectin-3 measurements appeared to be a strong predictor of outcome in acute HF patients, independent of N-terminal pro-brain natriuretic peptide. Hence, galectin-3 may be helpful in clinical practice for prognostication and treatment monitoring. **(*J Am Heart Assoc*. 2017;6:e003700. DOI: 10.1161/JAHA.116.003700.)**

Key Words: biomarker • galectin-3 • heart failure • repeated measurements

Most studies on serum biomarkers in heart failure (HF) populations conducted so far have related adverse outcome during follow-up with a single measurement at baseline.[1–3] Although this approach has demonstrated the prognostic value of a variety of biomarkers, among which are the well-known natriuretic peptides,[4] it does not explore the biological variation within patients with evolving disease. In fact, HF is a highly variable, heterogeneous, and progressive condition.[5] Thus, repeated biomarker measurements may be required to more accurately reflect this dynamic and progressive nature of the underlying pathophysiologic processes, such as mechanical overload, atherosclerosis,

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Clinical Perspective

What Is New?

- This study has a unique study design, including 7 measurements scheduled during 1-year follow-up, along with a state-of-the-art statistical approach using joint modeling to evaluate the prognostic significance of repeated galectin-3 measurements in acute heart failure patients.

What Are the Clinical Implications?

- This study demonstrates that repeated galectin-3 measurements are a strong and independent predictor of adverse outcome and offer incremental prognostic value to that conferred by other known risk factors and, importantly, repeated measurements of N-terminal pro-brain natriuretic peptide.
- These results suggest that repeated galectin-3 measurements in addition to repeated N-terminal pro-brain natriuretic peptide measurements may be used in clinical practice to identify patients with heart failure who are at increased risk of adverse outcome.

inflammation, and cardiac fibrosis. Therefore, we expect that risk models that account for repeated measurements may more adequately reflect the current status of the patient compared with models that only use single measurements.

The TRIUMPH (Translational Initiative on Unique and Novel Strategies for Management of Patients with Heart Failure) study was designed to identify and validate novel biomarkers to improve prognostication in HF.[6] TRIUMPH was designed as a translational study program, combining biological discovery of novel biomarkers, technologic advances, and clinical validation in patients presenting with acute HF. In the clinical validation study, both the novel and established HF biomarkers were evaluated for their prognostic properties using a unique design of 7 planned repeated measurements during 1-year follow-up. Based on previous clinical and epidemiological studies, galectin-3 was earmarked as a biomarker with high potential for improving prognostication.

Galectin-3 is a member of a large family of β-galactoside-binding animal lectins.[7] Galectin-3 expression has been detected in macrophages, neutrophils, eosinophils, and mast cells. In response to a variety of mechanical and neurohormonal stimuli, macrophages secrete galectin-3.[8] Galectin-3 stimulates additional macrophages, pericytes, myofibroblasts, and fibroblasts, which are all involved in the initiation and progression of tissue scarring. Consequently, galectin-3 appears to be involved in cardiac fibrosis. In addition, galectin-3 plays an important role in the inflammatory response, which is an important step in the process of cardiac remodeling.[9–11] Galectin-3 is expressed in numerous tissues such as heart, kidney, lung, uterus, and colon.[12] The level of galectin-3 expression is relatively low in heart tissue under normal conditions, but may increase substantially under pathophysiological circumstances.[13]

Several clinical studies have evaluated the prognostic value of galectin-3. Higher levels of galectin-3 have been associated with an increased risk of incident HF and all-cause mortality in the general population.[14,15] Furthermore, single galectin-3 levels have shown to be an independent risk factor of mortality in both stable and acute HF patients, although it still remains uncertain whether galectin-3 confers independent prognostic information when added to N-terminal pro-brain natriuretic peptide (NT-proBNP).[2,3,16–18] A few studies have been performed to assess the prognostic value of galectin-3 when measured multiple times. The change in galectin-3 level over time was predictive of outcome.[19–21] However, given the dynamic and progressive nature of HF, the number of galectin-3 measurements needed for adequate estimation of the true galectin-3 level is expected to be high. Therefore, in the present study, we assessed the independent association between the estimated instantaneous galectin-3 level, using frequently measured galectin-3 levels, and the incidence of all-cause mortality and HF readmission during 1-year follow-up in the 496 patients with acute HF who compose the TRIUMPH clinical cohort.

Methods

Objective and Study Design

TRIUMPH was designed as a translational bench-to-bedside study program encompassing the entire spectrum of biomarker discovery to clinical validation.[6] The clinical validation study was an observational prospective study enrolling patients admitted with acute HF in 14 hospitals in The Netherlands, between September 2009 and December 2013. This cohort study was designed to validate the clinical value of biomarkers successfully passing the bio-informatics and early-validation stages of TRIUMPH, and to further evaluate more established biomarkers of HF. There was a particular interest in the change in biomarker levels over time. The study was approved by the medical ethics committee at all participating centers.

Patient Selection

Patients ≥18 years of age were eligible for enrollment if they were hospitalized with decompensation of known chronic HF or newly diagnosed HF. Furthermore, 3 other criteria had to be met: (1) natriuretic peptide levels had to be elevated to ≥3 times the upper limit of normal, (2) there had to be evidence of sustained systolic or diastolic left ventricular dysfunction, and (3) patients had to be treated with intravenous diuretics. Patients with HF precipitated by a noncardiac condition, by severe valvular dysfunction without sustained left ventricular

dysfunction, or by an acute ST-segment elevation myocardial infarction were excluded. Furthermore, patients scheduled for a coronary revascularization procedure, on a waiting list for a heart transplantation, with severe renal failure for which dialysis was needed, or with a coexistent condition with a life expectancy <1 year could not participate. All study participants provided written informed consent.

Patient Management

Patient management was at the discretion of the treating physician, and in accordance with the guidelines of the European Society of Cardiology.[22] Importantly, the biomarker data that were generated in the context of this observational study were not used for treatment decisions.

Study Procedures

During hospitalization, blood samples were obtained at admission (day 1), once during days 2 to 4 and, subsequently, on the day of discharge. Afterwards, repeated blood samples were also obtained at outpatient follow-up visits, which were planned at 2 to 4 weeks, 3 months, 6 months, and 9 to 12 months after discharge. The baseline blood sample was defined as the first sample obtained after inclusion, up to a maximum of 2 days after inclusion. At each visit, HF symptoms were assessed using the New York Heart Association classification. Medication use was determined at discharge using 3 categories: (1) use of an angiotensin-converting enzyme inhibitor or an angiotensin II receptor antagonist or both, (2) use of a β-blocker, and (3) use of diuretics. Patients underwent physical examination and systematic measurements of weight, blood pressure, and heart rate.

Blood Collection

Nonfasting blood samples were obtained by venipuncture and transported to the clinical chemistry laboratory of each participating hospital for further processing according to a standardized protocol. The collected material was centrifuged at 1700*g*/relative centrifugal force, after which citrate-, EDTA-, heparin-, and trasylol-plasma was separated, as well as blood serum. Buffy coats were collected from EDTA tubes to enable analysis of genetic factors. Dimethylsulfoxide was added to an additional EDTA tube for cryopreservation of blood cells. All blood aliquots were subsequently stored at a temperature of −80°C within 2 hours after venipuncture.

Galectin-3 Measurements

Serum and heparin-plasma were transported under controlled conditions to a central laboratory (Future Diagnostics Solutions B.V.) for batch analysis of galectin-3 and NT-proBNP levels. Galectin-3 concentrations were determined in serum, using the BGM Galectin-3 Test as instructed by the manufacturer (BG Medicine, Inc, Waltham, MA). NT-proBNP concentrations were determined in heparin plasma using the Elecsys NT-proBNP assay on a Cobas 8000 analyzer (Roche Diagnostics Limited, Rotkreuz, Switzerland). Analysts were blinded for patient characteristics and end points.

End Points

Information on vital status and hospital readmissions was obtained until at least 9 months with a maximum of 400 days after the index hospitalization. We approached the civil registry, screened all medical records, and asked patients for information during their follow-up visits.

The primary end point is the composite of all-cause mortality and readmission for HF. Readmission for HF was defined as an unplanned rehospitalization because of decompensation of HF, with at least 2 of the following 3 criteria being present: elevated natriuretic peptide levels ≥3 times the upper limit of normal, symptoms of cardiac decompensation (rales, edema, or elevated central venous pressure), and treatment with intravenous diuretics. Secondary end points included the individual components of the primary end point and cardiovascular mortality. An event adjudication committee, blinded for biomarker information, was established for reviewing and adjudication of end points.

Statistical Analysis

The distributions of continuous variables, including biomarker levels, were evaluated for normality by visual examination of the histogram and Kolmogorov–Smirnov tests. Variables with a normal distribution are presented as mean± SD, whereas the median and interquartile range (IQR) are presented in case of non-normality. Categorical variables are presented as counts and percentages. Galectin-3 and NT-proBNP levels had a non-normal distribution and were therefore log-transformed for further analysis.

Patients were classified according to the quartiles of the galectin-3 distribution, and differences in baseline characteristics between these quartiles were evaluated by χ^2 tests (categorical variables), analysis of variance, or Kruskal–Wallis tests, as appropriate.

We applied Cox proportional hazards models to evaluate the association of baseline galectin-3 levels with the study end points. Subjects were censored at the time of occurrence of the end point under investigation, death, and at the scheduled end of follow-up. No deviations of the proportional hazards assumption were found by inspecting log minus log plots of the

survival functions. We performed univariate analyses to obtain the crude estimates of the effect of baseline galectin-3 level (model 1), analyses that were adjusted for age and sex only (model 2), and analyses that were additionally adjusted for systolic blood pressure, diabetes mellitus, left ventricular ejection fraction, previous hospitalization for HF during the past 6 months, ischemic HF, body mass index, estimated glomerular filtration rate, and baseline NT-proBNP level (model 3). The results are presented as adjusted hazard ratios (HR) per 1 SD increase of the biomarker level (on the log2 scale) with 95% confidence intervals (CI). We calculated the estimated glomerular filtration rate using the Modification of Diet in Renal Disease equation.[23]

Joint models were fitted to assess the association between estimated instantaneous biomarker levels, calculated using the repeated biomarker levels, and the specified study end points. A joint model combines a mixed-effects linear regression model for the serial measurements with a Cox proportional hazards model for the risk of the specified study end points.[24] We used cubic splines, with knots set at 1 week and 1 month after initial hospitalization. For the analyses with the repeated galectin-3 measurements, we used similar univariate and multivariate models as mentioned above (models 1, 2, and 3), except for model 3 in which we added medication use at discharge to the mixed-effects linear regression model. We also tested whether the instantaneous slope of the galectin-3 trajectories itself, when added to model 3, was an independent predictor. Finally, we combined the repeated measurements of galectin-3 and NT-proBNP to assess their respective independent prognostic value. Taking into account the limitations of the R packages for Joint Modeling, we were able to combine the estimated galectin-3 trajectory (using a mixed-effects linear regression model) and the estimated NT-proBNP trajectory (using a time-dependent Cox proportional hazards model) in 1 joint model. Since the model did not converge when we adjusted for all the covariates in model 3, baseline systolic blood pressure had to be left out in this final model (model 4). Diagnostics and sensitivity analyses were performed to evaluate the joint models. To account for the correlation structure between serial biomarker measurements collected from the same patient, we obtained the SD from the total variance of a random intercepts linear mixed model fitted on the postdischarge data. The final results are presented as adjusted HR per 1 SD increase of the biomarker level (on the log2 scale) at any point in time with 95% CI.

The TRIUMPH sample size was chosen to achieve a power of 80% ($1-\beta=0.8$) to detect an odds ratio of at least 2.0 ($\alpha=0.05$, 2-sided test) for a biomarker value above the 75% percentile of its distribution comparing end point cases with noncases. The incidence of the primary end point was initially estimated at 25% to 30%, based on observations in historical HF populations. Then, 780 patients are required. During the course of the study, based on evolving evidence, the estimated incidence was adjusted to 30% to 35%, and the sample size was eventually determined at 490 patients. TRIUMPH enrolled 496 patients, and 40% reached the primary end point.

Data on covariates were complete in 93% of patients, except for left ventricular ejection fraction, which

Table 1. Baseline Parameters According to Overall Sample in Study Population (N=475)

Variables	Overall Sample
Demographic characteristics, median (IQR) or %	
Age, y	74 (65–80)
Female	37
White	95
Measurements at baseline, median (IQR) or %	
Body mass index, kg/m^2	28 (25–31)
Systolic blood pressure, mm Hg	125 (110–147)
Diastolic blood pressure, mm Hg	74 (65–85)
Heart rate, bpm	85 (72–100)
eGFR, mL/min per 1.73 m^2	46 (34–62)
Left ventricular ejection fraction, %	30 (21–41)
NYHA classification	
II	17
III	55
IV	27
Medical history, %	
Newly diagnosed heart failure	36
Heart failure with reduced ejection fraction	83
Previous heart failure admission within 6 mo	20
Ischemic heart failure	49
Myocardial infarction	40
Hypertension	51
Atrial fibrillation	42
Diabetes mellitus	36
Stroke	17
Medication use at discharge, %	
ACE-I and/or ARB	78
β-Blocker	78
Diuretics	93
Biomarkers, median (IQR)	
Galectin-3, ng/mL	24 (18–34)
NT-proBNP, pg/mL	4152 (2089–9387)

ACE-I indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor antagonist; bpm, beats per minute; eGFR, estimated glomerular filtration rate; IQR, interquartile range; NT-proBNP, N-terminal pro-brain natriuretic peptide; NYHA, New York Heart Association.

Table 2. Baseline Characteristics According to Quartiles of Galectin-3 Level

Variables	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P* Value*
Demographic characteristics, median or %					
Age, y	70	73	76	75	0.010
Female	45	31	33	38	0.13
White	92	93	97	96	0.27
Measurements at baseline, median (IQR) or %					
Body mass index, kg/m^2	27	27	29	29	0.035
Systolic blood pressure, mm Hg	130	125	125	122	0.29
Diastolic blood pressure, mm Hg	80	73	74	70	<0.001
Heart rate, bpm	94	85	84	80	0.002
eGFR, mL/min per 1.73 m^2	63	55	42	32	<0.001
Left ventricular ejection fraction, %	30	30	34	31	0.020
NYHA classification					
II	23	18	11	14	0.12
III	50	51	63	60	
IV	27	28	25	26	
Medical history, %					
Newly diagnosed heart failure	57	40	26	21	<0.001
Heart failure with reduced ejection fraction	88	88	76	81	0.080
Previous heart failure admission within 6 mo	8	17	24	29	<0.001
Ischemic heart failure	40	45	55	56	0.036
Myocardial infarction	28	32	54	48	<0.001
Hypertension	40	50	56	60	0.016
Atrial fibrillation	32	44	45	46	0.089
Diabetes mellitus	20	32	41	50	<0.001
Stroke	14	14	17	22	0.29
Biomarkers, median					
NT-proBNP, pg/mL	3180	3970	4372	7544	<0.001
Galectin-3, ng/mL	16	21	28	40	<0.001

bpm indicates beats per minute; eGFR, estimated glomerular filtration rate; IQR, interquartile range; NT-proBNP, N-terminal pro-brain natriuretic peptide; NYHA, New York Heart Association.
**P* value for differences between groups.

was complete in 78%. Single imputation was applied to account for missing values of covariates. Data are imputed using predictive mean matching for continuous variables, logistic regression for binary variables, and polytomous regression for unordered categorical data. Baseline covariates used in the full model and survival information were used in the imputation. The software used was R package MICE (https://cran.r-project.org/web/packages/mice/mice.pdf). A sensitivity analyses was performed on the full model for the primary end point on the complete cases.

The Statistical Package for Social Sciences, version 21.0 (SPSS, IBM Corp., Armonk, NY) was used for descriptive data analysis. R statistical software (version 2.15.0, available at: www.r-project.org) was used for advanced statistical analyses of the longitudinal biomarker data and study end points (packages JMBayes and JM). All statistical tests were 2-tailed and $P<0.05$ were considered statistically significant.

Results

Patients

A total of 496 patients were enrolled in the TRIUMPH clinical cohort. Three patients withdrew their informed consent.

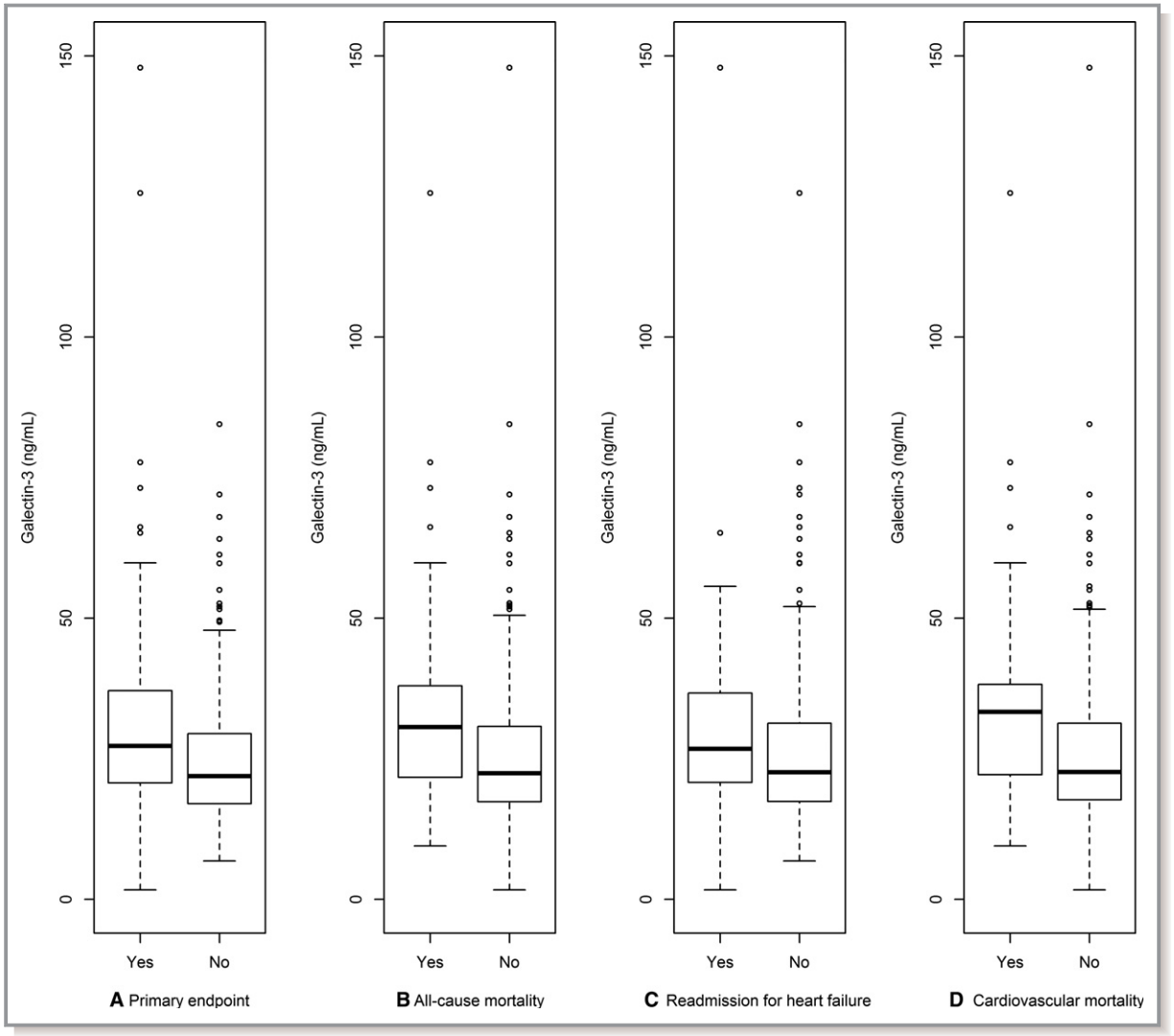

Figure 1. Distributions of baseline galectin-3 levels within the subpopulations of patients who had an event and those who did not experience an event for: (A) the primary end point; (B) the single end point of all-cause mortality; (C) the single end point of readmission for heart failure; and (D) the single end point of cardiovascular mortality.

Eighteen patients were withdrawn from statistical analyses because of inclusion violation. These patients had no evidence of sustained systolic or diastolic left ventricular dysfunction on echocardiography. Accordingly, 475 patients compose the analysis set. Their median age was 74 years (IQR 65–80) and 37% were women (Table 1). Median systolic blood pressure was 125 mm Hg (IQR 110–147) and median left ventricular ejection fraction was 30% (IQR 21–41). At discharge 78% used an angiotensin-converting enzyme inhibitor or an angiotensin II receptor antagonist or both, 78% used a β-blocker, and 93% used diuretics. Median baseline galectin-3 level was 24 ng/mL (IQR 18–34) and NT-proBNP was 4152 pg/mL (IQR 2089–9387). Table 2 shows the baseline characteristics of patients in different quartiles of galectin-3 level. Patients in quartiles with a higher galectin-3 level were older and had a worse kidney function. In the higher galectin-3 quartiles, more patients had a history of myocardial infarction and diabetes mellitus, had ischemic HF, and had been admitted to the hospital for HF during the past 6 months. In the lower galectin-3 quartiles, more patients had newly diagnosed HF during the initial hospitalization.

Baseline Galectin-3 Levels and the Incidence of Study End Points

During the median follow-up of 325 days (IQR 85–401), 188 patients (40%) reached the primary composite end point of all-cause death (n=113) or readmission for HF (n=123). This corresponds with an incidence rate of 55.9 per 100 patient-years for the primary end point. In the highest quartile of baseline galectin-3, 65 patients (59%) reached the primary

Table 3. Hazard Ratios for Different End Points Per 1 SD Increase of the Baseline Galectin-3 Level (on the log2 Scale)

	Mean Value*			Baseline Level†	
	M−SD	M	M+SD		
	15.9	24.7	38.2	HR (95% CI)	*P* Value
Primary end point					
Model 1				1.50 (1.30–1.75)	<0.001
Model 2				1.49 (1.28–1.73)	<0.001
Model 3				1.12 (0.93–1.36)	0.241
Number of events/patients		188/475			
All-cause mortality					
Model 1				1.54 (1.29–1.85)	<0.001
Model 2				1.52 (1.26–1.83)	<0.001
Model 3				1.26 (1.01–1.59)	0.044
Number of events/patients		113/475			
HF hospitalization					
Model 1				1.47 (1.22–1.76)	<0.001
Model 2				1.47 (1.23–1.76)	<0.001
Model 3				1.05 (0.82–1.33)	0.720
Number of events/patients		123/475			
Cardiovascular mortality					
Model 1				1.60 (1.28–1.99)	<0.001
Model 2				1.57 (1.26–1.97)	<0.001
Model 3				1.24 (0.93–1.67)	0.147
Number of events/patients		77/475			

Model 1 unadjusted; model 2 adjusted for age and sex; model 3 adjusted for age, sex, systolic blood pressure, diabetes mellitus, LVEF, previous hospitalization for heart failure during the past 6 mo, ischemic heart failure, body mass index, eGFR, and baseline NT-proBNP. CI indicates confidence interval; eGFR, estimated glomerular filtration rate; HF, heart failure; HR, hazard ratio; LVEF, left ventricular ejection fraction; M, mean; NT-proBNP, N-terminal pro-brain natriuretic peptide.

*Mean±1 SD of the patient-specific geometric mean galectin-3 value at baseline (presented on the linear scale).

†Hazard ratios are related to a 1-SD increase of galectin-3 (on the log scale) at baseline.

end point compared with 27 patients (24%) in the lowest quartile. The number of events in the highest quartile compared with the lowest quartile of galectin-3 was also higher for all-cause mortality (n=44 [40%] and n=14 [13%], respectively) and readmission for HF (n=44 [40%] and n=19 [17%], respectively).

Baseline galectin-3 levels were higher in patients who reached a study end point when compared with those who remained event-free (Figure 1). The baseline galectin-3 level was associated with an increased risk of reaching the primary end point, as well as with all-cause mortality, cardiovascular mortality, and HF readmission (Table 3). After adjustment for all selected potential confounders including baseline NT-proBNP level (model 3), the association between baseline galectin-3 and the different end points became weaker, but remained present.

Repeatedly Measured Galectin-3 Levels and the Incidence of Study End Points

On average, galectin-3 was available 4.3 times during follow-up. The mean galectin-3 level during follow-up was 23.8 ng/mL; an increase of 1 SD galectin-3 level on the log2 scale from the mean was 13 ng/mL. A decrease of 1 SD galectin-3 level on the log2 scale was 8 ng/mL. After adjustment for age and sex (model 2), the HR per SD increase of the galectin-3 level (on the log2 scale) at any point in time was 2.09 (95% CI, 1.71–2.56) for the primary end point. After adjustment for the broader range of potential confounders including medication use at discharge and baseline NT-proBNP level (model 3), the association remained highly statistically significant with a HR of 1.67 (95% CI, 1.24–2.23) (Table 4). Results were similar for the secondary end points. The instantaneous slope of the galectin-3 level trajectories itself was not an independent predictor of the primary end point.

After adjustment for repeated NT-proBNP measurements (model 4), the association between repeated galectin-3 levels and adverse outcome remained statistically significant with a HR of 1.54 (95% CI, 1.16–2.05) for the primary end point corresponding with 1 SD increase of galectin-3 level (on the log2 scale) at any point in time (Table 5). The HR corresponding with a 1-SD increase of NT-proBNP level (on the log2 scale) at any point in time was 2.10 (95% CI, 1.63–2.74) after adjustment for repeated galectin-3 levels.

Figure 2A shows the average estimated galectin-3 level in patients with and without the primary end point according to model 3 and the individual galectin-3 measurements. During hospitalization the average galectin-3 level remains steady for patients who remained free of the primary end point. For patients who reached the primary end point during follow-up, the average estimated galectin-3 level decreased slightly after the initial hospitalization. Apparently, throughout follow-up, patients who reached the primary end point had, on average, higher levels than their counterparts who remained free of the primary end point. Furthermore, the average estimated galectin-3 levels appeared to elevate several weeks before the time of the primary end point (Figure 2B).

Discussion

This study clearly demonstrates that, in patients admitted with acute HF, repeated galectin-3 measurements are a strong and independent predictor of the composite end point of all-cause mortality or readmission for HF during 1-year follow-up. Our results illustrate that repeated measurements of galectin-3 offer incremental prognostic value to (repeatedly

Table 4. Hazard Ratios for Different End Points Per 1 SD Increase of the Galectin-3 Level (on the log2 Scale) at Any Point in Time, Using a Joint Model

	Mean Value*			Instantaneous Level†	
	M−SD	M	M+SD	HR (95% CI)	*P* Value
Primary end point	15.4	23.8	36.6		
Model 1				2.07 (1.71–2.53)	<0.001
Model 2				2.09 (1.71–2.56)	<0.001
Model 3				1.67 (1.24–2.23)	<0.001
All-cause mortality	15.4	23.8	36.9		
Model 1				2.41 (1.83–3.15)	<0.001
Model 2				2.36 (1.78–3.08)	<0.001
Model 3				2.14 (1.47–3.16)	<0.001
HF hospitalization	15.4	23.8	36.6		
Model 1				1.87 (1.47–2.39)	<0.001
Model 2				1.92 (1.48–2.46)	<0.001
Model 3				1.41 (1.02–1.93)	0.035
Cardiovascular mortality	15.4	23.8	36.9		
Model 1				2.46 (1.79–3.34)	<0.001
Model 2				2.43 (1.76–3.35)	<0.001
Model 3				2.22 (1.48–3.36)	<0.001

Model 1 unadjusted; model 2 adjusted for age and sex; model 3 adjusted for age, sex, systolic blood pressure, diabetes mellitus, LVEF, previous hospitalization for HF during the past 6 mo, ischemic HF, body mass index, eGFR, medication use at hospital discharge (ACE-I and/or ARB, β-blocker, and diuretics) and baseline NT-proBNP level. ACE-I indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor antagonist; CI, confidence interval; eGFR, estimated glomerular filtration rate; HF, heart failure; HR, hazard ratio; LVEF, left ventricular ejection fraction; M, mean; NT-proBNP, N-terminal pro-brain natriuretic peptide.

*Mean±1 SD of the patient-specific geometric mean galectin-3 value during follow-up (presented on the linear scale).

†HRs are related to a 1-SD increase of galectin-3 (on the log scale) at any point in time.

measured) NT-proBNP, which is considered the criterion standard biomarker in HF patients.

Our observation that baseline galectin-3 level was associated with mortality confirms earlier findings both in acute and stable HF patients.[2,3,25,26] Similar to previous studies, the association between baseline galectin-3 level and mortality attenuated after adjustment for established risk factors, including kidney function and NT-proBNP level.[16,17,27] The association between baseline galectin-3 level and readmission for HF was less apparent. However, the decision to hospitalize a patient for decompensation of HF may be influenced by several subjective patient- and physician-related factors that are unlikely to have an association with the galectin-3 level. Furthermore, several risk factors such as kidney function, diabetes mellitus, and NT-proBNP level influence this decision and are related to galectin-3. Therefore, the association between baseline galectin-3 level and HF readmission attenuated after adjustment for these risk factors. Since the primary end point is a composite of all-cause mortality and readmission for HF, the relationship between the galectin-3 level and the mortality end points per se are stronger compared with the primary end point.

Repeated galectin-3 measurements were strongly and independently related to the primary end point, as well as its separate components. Repeated measurements take into account the dynamic and continuous change in galectin-3 level over time, which better reflects the true nature of the underlying pathophysiology in HF. In this study, the number of galectin-3 measurements per patient was high and therefore the repeated galectin-3 measurements could be used to estimate instantaneous galectin-3 levels (ie, the estimated galectin-3 level at any point in time during the follow-up period). When compared with baseline galectin-3 levels, the estimated instantaneous galectin-3 levels identified patients at an even higher risk for reaching an end point. The estimated instantaneous galectin-3 level more accurately approximates the true galectin-3 level and therefore reflects the actual condition of the patient at that point in time during follow-up. This is expected to be important since HF is a dynamic and often progressive disease in which inflammation, cardiac fibrosis, and remodeling are ongoing processes that cannot be captured in a single biomarker assessment at 1 point in time.[5] Furthermore, baseline galectin-3 measurements were all taken during hospitalization for

Table 5. Hazard Ratios for Different End Points Per 1-SD Increase of Galectin-3 Level or NT-proBNP Level (on the log2 Scale) at Any Point in Time Using Repeated Galectin-3 and NT-proBNP Measurements in a Joint Model

	Mean Value*			Instantaneous Level†	
	M−SD	M	M+SD	HR (95% CI)	*P* Value
Primary end point					
Galectin-3	15.4	23.8	36.6	1.54 (1.16–2.05)	0.003
NT-proBNP	742	2445	8062	2.10 (1.63–2.74)	<0.001
All-cause mortality					
Galectin-3	15.4	23.8	36.9	1.77 (1.22–2.52)	<0.001
NT-proBNP	739	2480	8321	2.68 (1.90–3.86)	<0.001
HF hospitalization					
Galectin-3	15.4	23.8	36.6	1.29 (0.92–1.81)	0.160
NT-proBNP	742	2445	8062	1.71 (1.27–2.25)	<0.001
Cardiovascular mortality					
Galectin-3	15.4	23.8	36.9	1.89 (1.25–2.85)	0.002
NT-proBNP	739	2480	8321	2.62 (1.70–4.27)	<0.001

Model 4 adjusted for age, sex, diabetes mellitus, LVEF, previous hospitalization for heart failure during the past 6 mo, ischemic heart failure, body mass index, eGFR, medication use at hospital discharge (ACE-I and/or ARB, β-blocker, and diuretics) and baseline NT-proBNP level. ACE-I indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor antagonist; CI, confidence interval; eGFR, estimated glomerular filtration rate; HF, heart failure; HR, hazard ratio; LVEF, left ventricular ejection fraction; M, mean; NT-proBNP, N-terminal pro-brain natriuretic peptide.

*Mean±1 SD of the patient-specific geometric mean biomarker level during follow-up (presented on the linear scale).

†Hazard ratios are related to a 1-SD increase of biomarker level (on the log scale) at any point in time.

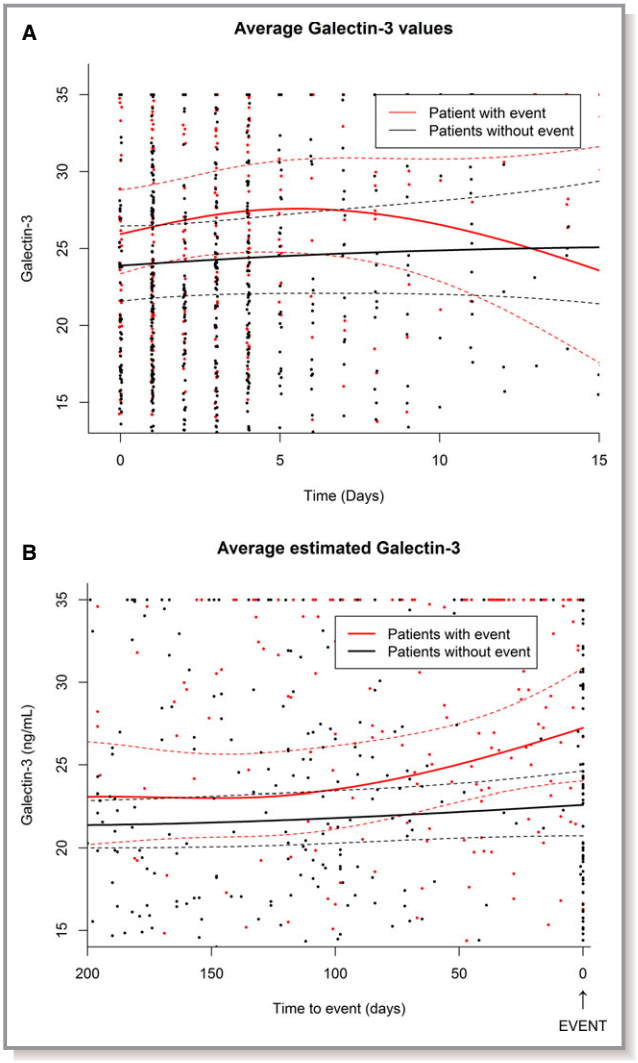

Figure 2. A, Average estimated galectin-3 pattern during initial hospitalization for decompensated heart failure for patients with and without the primary end point. The figure includes the individual galectin-3 measurements for patients with and without the primary end point. B, Average estimated galectin-3 pattern before the primary end point or end of follow-up for patients with and without the primary end point. The figure includes the individual galectin-3 measurements for patients with and without the primary end point. The average estimated galectin-3 levels are adjusted for age, sex, systolic blood pressure, diabetes mellitus, LVEF, previous hospitalization for heart failure during the past 6 months, ischemic heart failure, body mass index, eGFR, medication use at hospital discharge (ACE-I and/or ARB, β-blocker, and diuretics), and baseline NT-proBNP (model 3). ACE-I indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor antagonist; eGFR, estimated glomerular filtration rate; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-brain natriuretic peptide.

decompensated chronic HF or new-onset HF. It is known that galectin-3, in contrast to natriuretic peptides, does not respond to volume overload and unloading directly, which occurs during hospitalization.[28] As galectin-3 is involved in the process of myocardial fibrosis, it is more likely that galectin-3 is of more prognostic value when patients enter a more chronic phase of HF.[11]

Interestingly, the slope of the galectin-3 trajectory did not add prognostic information to the estimated instantaneous galectin-3 level. An explanation could be that galectin-3 is helpful in identifying high-risk patients when their galectin-3 level rises above a certain threshold. The change in galectin-3 level before reaching this threshold is not essential for risk stratification. However, to be able to estimate whether a patient's galectin-3 level rises above the threshold, repeated measurements are required. A few studies have been conducted on the prognostic value of multiple galectin-3 measurements in acute and stable HF patients.[19,20] These studies showed that change in galectin-3 level is associated with mortality. A possible explanation as to why in the present study slope of the galectin-3 trajectory did not add further prognostic information might be that the number of galectin-3 measurements during follow-up was substantially higher in our study, which allowed us to estimate an instantaneous slope of the galectin-3 trajectory, rather than the slope of the

difference ("delta") between the level at baseline and that at a fixed point in time.

The statistical method (Joint Model) used to estimate the trajectory of the galectin-3 level takes into account the continuous changes in biomarker levels and adequately analyzes the relation between these biomarker trajectories and different end points considering the changing population because of censoring at the time of occurrence of an end point. Previous studies presented changes in biomarker level as a "delta" between just 2 measurements that are separated in time. If >2 samples are taken into account, patients have often been categorized according to the number of high or low biomarker levels. Obviously, both approaches do not fully capture the true biomarker pattern of the dynamic disease. Additionally, the power to predict adverse outcome is reduced.

An important finding of the present study is that repeated galectin-3 measurements conferred additional and independent prognostic information to that offered by baseline as well as repeated NT-proBNP measurements. The fact that NT-proBNP and galectin-3 reflect different underlying pathophysiological processes in HF may be the most important reason for this observation. Galectin-3 is a marker of cardiac fibrosis, inflammation, and remodeling, whereas NT-proBNP is a marker of volume overload.[13,29] As such, galectin-3 might be a marker that more directly reflects the pathophysiological processes that lead to adverse cardiac remodeling and deterioration of cardiac function, whereas NT-proBNP reflects the volume overload resulting from the actual (left) ventricular dysfunction. In this way, the galectin-3 and NT-proBNP level provide complementary information on the pathophysiological state, as well as with respect to the assessment of prognosis. With respect to prognostication in HF, the results of the present study, therefore, not only provide evidence for the use of repeated galectin-3 measurements, but also for the combined use with (repeatedly measured) NT-proBNP.

Although this study is a large multicenter prospective observational study, it seems that the studied population is not completely representative for the average HF population. The mean age in our study population is 74 years and the women are underrepresented. Moreover, only 18% of the included HF patients have a preserved ejection fraction. de Boer et al[30] showed that galectin-3 levels did not differ between HF patients with a reduced and preserved ejection fraction and the predictive value of galectin-3 was stronger in patients with a preserved ejection fraction. By underrepresenting the HF patients with a preserved ejection fraction in our study, we possibly underestimated the prognostic value of galectin-3.

Future studies should evaluate the value of repeated galectin-3 measurements when used to guide treatment decisions. It may be hypothesized that treatment is to be intensified in patients with high galectin-3 levels or unfavorable galectin-3 patterns. On the other hand, repeated galectin-3 measurements might be helpful to identify patients who are more likely to respond to certain treatments.[31] Furthermore, it remains to be addressed whether galectin-3 may be targeted by specific antigalectin-3 therapies. Additional studies should also determine the number of galectin-3 measurements needed for optimal prognostication and therapy monitoring. The frequency by which galectin-3 levels should be measured may not be identical for each patient, but depends on the clinical condition of the patient, the treatment given, the galectin-3 level, and the progression of galectin-3 levels during follow-up.

Conclusions

The TRIUMPH study clearly demonstrates that repeated measurements of galectin-3 are a strong and independent predictor of adverse outcome in patients following admission for acute HF. The estimated instantaneous galectin-3 level identified patients at a higher risk of reaching adverse events than baseline galectin-3 levels alone. In addition, repeated galectin-3 measurements offer incremental prognostic value to that conferred by other known risk factors and, importantly, repeated measurements of NT-proBNP. These results suggest that repeated galectin-3 measurements in addition to NT-proBNP measurements may be helpful in clinical practice to identify HF patients who are at increased risk of adverse outcome.

Appendix

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Disclosures

None.

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