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ORIGINAL RESEARCH ARTICLE

The diagnostic and prognostic value of galectin-3 in patients at risk for heart failure with preserved ejection fraction: results from the DIAST-CHF study

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Abstract

Aims Galectin-3 (Gal-3) predicts long-term outcome among patients with heart failure (HF) with preserved ejection fraction (HFpEF). The ability of Gal-3 to diagnose and predict incident HFpEF in a cohort at risk for HFpEF is of particular interest. We aimed to determine the association between Gal-3 and clinical manifestations of HFpEF, the relationship between Gal-3 and all-cause mortality, or the composite of cardiovascular hospitalization and death.

Methods and results The observational Diast-CHF study included patients aged 50 to 85 years with ≥1 risk factor for HF (e.g. hypertension, diabetes mellitus, and atherosclerotic disease) or previously suspected HF. Patients were followed for 10 years. The association between Gal-3, evidence of diastolic dysfunction, and Framingham criteria for HF was examined. All deaths and hospitalizations were adjudicated as cardiovascular or non-cardiovascular. The analysis population was composed of 1386 subjects (67 years old, 50.9% female). The area under the receiver operating characteristic curve to diagnose HFpEF was 0.71. At a cut-off value of 13.57 ng/mL, sensitivity was 0.61 and specificity was 0.73 for Gal-3, and the diagnostic power to detect HFpEF was superior to N-terminal pro-brain natriuretic peptide (area under the receiver operating characteristic curve 0.59, $P > 0.001$). Baseline Gal-3 was associated with risk factors for HF ($P < 0.001$). Higher levels of Gal-3 predicted incident HFpEF ($P < 0.05$), adjusted all-cause mortality ($P < 0.001$), and the adjusted composite of cardiovascular hospitalization and death ($P < 0.001$), both independent from N-terminal pro-brain natriuretic peptide.

Conclusions Gal-3 differentiated patients with HFpEF from an overall cohort of well-characterized patients with risk factors for HFpEF. Independent of other factors, baseline Gal-3 levels were associated with a higher risk for incident HFpEF, mortality, or the composite of cardiovascular hospitalization and death over 10 year follow-up. In conjunction with clinical parameters, Gal-3 adds a statistically significant value for the diagnosis of HFpEF within this study, yet the clinical relevance remains debatable.

Keywords Galectin-3; Heart failure; HFpEF; Mortality; Risk prediction; Biomarkers

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Introduction

Galectin-3 (Gal-3), a beta-galactoside-binding lectin, is a biomarker expressed by macrophages and involved in inflammatory and fibrotic pathways.[1–3] Gal-3 levels significantly correlate with impaired left ventricular (LV) filling in patients with stable coronary artery disease and cardiac ventricular remodelling.[4] Although not cardio-specific, the utility

of Gal-3 as a prognostic marker in patients with heart failure (HF) has been evaluated in multiple studies, and it has been shown to independently predict outcome.[5–15] While guideline recommendations suggest that Gal-3 may be considered for additive risk stratification in patients with ambulatory or acute HF,[16] Gal-3 is rarely used in routine clinical patient work-up to date. Gal-3 has shown to predict mortality in a general population after adjustment for other factors[17] and predicts long-term outcome among patients with HF with preserved ejection fraction (HFpEF).[5,13] The ability and utility of Gal-3 to diagnose and predict incident HFpEF in a cohort of patients at risk for HFpEF is of particular interest.[18]

Brouwers *et al*. showed that Gal-3 predicted new-onset HF among high-risk but not low-risk patients, and no association between Gal-3 and incident HFpEF was observed.[19] Gal-3 predicted incident HF in the Framingham Offspring Cohort data, and baseline Gal-3 levels were not different among patients who developed HFpEF vs. HFrEF.[20] Data from other small studies suggest that Gal-3 is sensitive to detect HFpEF but less specific than B-type natriuretic peptide.[21] Thus, the data supporting a role for Gal-3 to diagnose and predict incident HF are inconclusive. This uncertainty may continuously unsettle clinicians and HF practitioners.

Heart failure with preserved ejection fraction remains difficult to define, and the field evolved towards the recognition that measuring LV ejection fraction (LVEF) alone is insufficient to characterize the HFpEF syndrome. Rather, multiple factors including symptoms and structural and functional LV abnormalities should be considered for the diagnosis.[22,23,24] Thus, previous Gal-3 studies may have been limited by the lack of variables needed to fully characterize HFpEF.

The multicentre, non-interventional, observational study on Prevalence and Clinical Course of Diastolic Dysfunction and Diastolic Heart Failure (Diast-CHF) is a unique database with detailed clinical and echocardiographic data at baseline and during follow-up to describe characteristics consistent with HFpEF. Hence, the Diast-CHF cohort allows to evaluate the relationship between Gal-3 and characteristics consistent with incident HFpEF in a population with cardiovascular risk factors and to determine the prognostic relationship between Gal-3 and clinical outcomes.

Methods

The observational Diast-CHF study was conducted within the German Competence Network Heart Failure project.[25] Patients were referred by primary care physicians for inclusion. Eligible patients were aged 50 to 85 years with $\geq$1 risk factor for HF [e.g. hypertension, diabetes mellitus, sleep apnoea, and atherosclerotic disease (defined as symptomatic peripheral arterial occlusive disease, angiographically documented coronary artery disease, carotid artery stenosis, history of myocardial infarction, or history of stroke)] or a history of HF (ICD-10 diagnosis in the medical record). Diast-CHF exclusion criteria were limited to an inability to consent or participate because of language barriers or geographical reasons.

Six centres participated between 2004 and 2006 for inclusion of patients, between 2004 and 2016 for follow-up, and the majority of patients were enrolled at either University of Göttingen or Charité—Berlin University of Medicine, Germany. The study was conducted according to the Declaration of Helsinki ethical standards for research. Ethics committees at each centre reviewed and approved the protocol, and all subjects provided written informed consent prior to any study-related procedures.

Demographics, medical history, medications, and HF signs and symptoms were obtained at baseline and during follow-up. On the day of baseline assessment, peripheral venous blood was drawn after 15 min of rest in the supine position and in accordance with a pre-specified standardized operating protocol, into ethylendiaminetetraacetic acid-containing tubes, centrifuged immediately, frozen to −80°C, and sent to the University of Göttingen for analysis. Gal-3 was analysed using an enzyme-linked immunosorbent assay developed by BG Medicine (BG Medicine, Inc., Waltham, MA, USA) with a lower limit of detection of 1.13 ng/mL and no cross-reactivity with collagens or other galectins.[26] Both, patients and physicians, were unaware of baseline Gal-3 levels during follow-up. Personnel at the core laboratory were blinded to the patients' clinical data. N-terminal pro-brain natriuretic peptide (NT-proBNP) was measured with a commercially available electrochemiluminescence immunoassay on an Elecsys® analyser (all Roche Diagnostics GmbH, Mannheim, Germany). Creatinine clearance was calculated using the Modification of Diet in Renal Disease Study equation [estimated glomerular filtration rate = 186.3 × serum creatinine$^{-1.154}$ × age$^{-0.203}$ × 0.742 (for women) × 1.21 (for African American)].[27]

Detailed echocardiography was performed to evaluate parameters of diastolic function according to published recommendations and guidelines[22,23,24,28] at the time of inclusion and repeated at regular follow-up visits. Patients were followed for up to 10 years to ascertain incident HF in patients at risk but without HF at baseline. Survival status and hospitalizations were also collected, and all deaths and hospitalizations were adjudicated as cardiovascular or non-cardiovascular by two independent, experienced cardiologists.

The primary objectives of this analysis within Diast-CHF were (i) to determine the performance of Gal-3 compared with NT-proBNP to diagnose HFpEF, (ii) to detect incident HFpEF over a 10 year follow-up, (iii) to evaluate the association between Gal-3 and characteristics consistent with HFpEF, and (iv) to evaluate the association between Gal-3 and all-cause death and the composite of adjudicated cardiovascular hospitalization and death.

Characteristics consistent with incident heart failure with preserved ejection fraction

For the purpose of this analysis, the diagnosis of HFpEF, both baseline and incident, was diagnosed analogous to the 2016 European Society of Cardiology guidelines for the diagnosis of HF.[23] HFpEF was diagnosed in the presence of signs and symptoms of HF, ≥2 Framingham criteria for HF, a preserved LVEF > 50%, and echocardiographic findings of LV diastolic dysfunction. The diagnosis was established when left atrial volume index >34 mL/m^2, or LV mass index (LVMI) ≥115 g/m^2 for men and ≥95 g/m^2 for women, or E/e' ≥ 13, or mean e' septal and lateral wall <9 cm/s.[22,23,24] Given that use of natriuretic peptides is recommended for ruling out, but not necessarily to establish the diagnosis of HF, natriuretic peptides were not used to diagnose HFpEF, to allow for a comparison of the performance of Gal-3 and NT-proBNP in this analysis on non-acute patients. For the purpose of this analysis, patients with unclassified LV function or LVEF ≤ 50% were excluded from the analysis set.

Statistical analysis

Baseline data are presented as frequencies and percentages for categorical variables, or means and standard deviations for quantitative measures, with the exception of neurohormones and echocardiographic data, which are presented as median and inter-quartile ranges. For comparison, the cohort was split into tertiles according to levels of Gal-3, being Gal-3 < 10.5 ng/mL for the lower tertile, Gal-3 ≥ 10.5 and <13.4 ng/mL for the intermediate tertile, and Gal-3 ≥ 13.4 ng/mL for the higher tertile. Patients with Gal-3 levels in the higher tertile were compared with the pooled lower and intermediate tertile by *t*-test for independent samples concerning continuous variables. Frequencies were compared by χ^2 test. In view of the skewed distribution, neurohormones were tested by the Wilcoxon–Mann–Whitney rank procedure. Cohen's *D* (for scale variables), odds ratio (for binary), and median difference (for skew distributed variables) were calculated as effect measures indicating differences between subgroups.

The first objective of this analysis was to determine the capability of Gal-3 to detect HFpEF as defined earlier. Receiver operating characteristic (ROC) curves were created, and the areas under the ROC curve (AUCs) were calculated and compared by DeLong's test. Sensitivity and specificity were determined at the upper tertile and the point of maximal Youden index. We complemented the research of diagnostic ability by means of a logistic model for NT-proBNP and Gal-3 adjusted for age, sex, kidney function, diabetes mellitus, hypertension, and body mass index. Comparing the diagnostic ability of NT-proBNP and Gal-3, we calculated the net reclassification index (NRI) at the thresholds: NT-proBNP: 220 pg/mL (660 pg/mL for patients with atrial fibrillation)[29] and Gal-3: 13.4 ng/mL.

Second, we were interested to see which characteristics are reflected by a high Gal-3 level. We logarithmized Gal-3 (to the base of 2), NT-proBNP, and high-sensitivity C-reactive protein (to the base of 10) for normalization for linear models. Starting with a full model with the variables: age, sex, number of Framingham criteria,[30,31] diagnosis of coronary heart disease, hypertension, smoking, atrial fibrillation, estimated glomerular filtration rate, NT-proBNP, high-sensitivity C-reactive protein, and the echo parameters E/A, mean E/e', left atrial volume index, LV mass index, LVEF, and diagnosis of HFpEF, variables were stepwise eliminated following the Akaike information criterion.

Third, we used two approaches to explore the additional prognostic ability of Gal-3 beyond clinical criteria and NT-proBNP to predict (i) incident HFpEF, (ii) all-cause mortality, and (iii) cardiovascular hospitalization and death. On one hand, we extended a clinical model by NT-proBNP and Gal-3. The additional information was tested by likelihood ratio tests.

On the other hand, we built an exploratory model by stepwise backward variable selection starting with possibly confounding variables. In general, *C*-statistics were calculated. The prognostic model for incident HFpEF during the 10 year follow-up bases on patients without a history of HF or HFpEF at baseline.

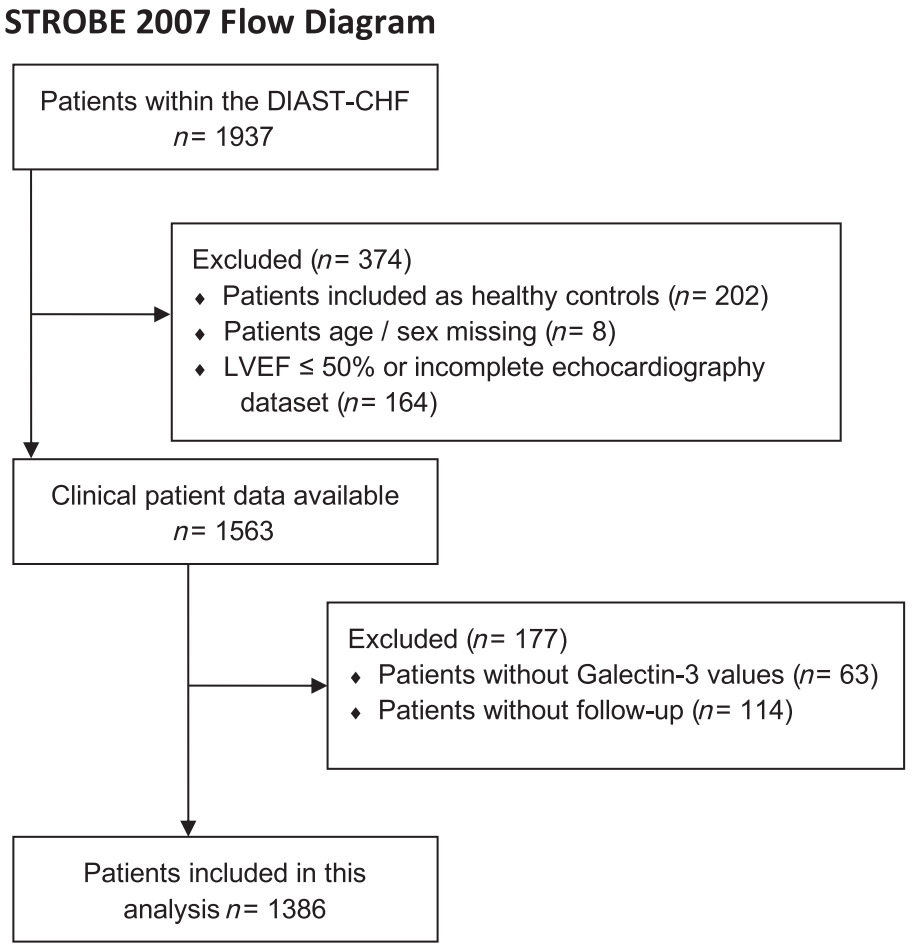
We depicted mortality by Kaplan–Meier curves for the Gal-3 subgroups and the combined endpoint by cumulative incidence curves. Data preparation and descriptive statistics were performed by SPSS, Version 26, IBM, Armonk, New York, United States. Multiple models were fitted, and ROC analysis and creation of charts were performed with R inclusive the packages *pROC*, *survival*, *survAUC*, *cutpointr*, and *lmtest*.[32,33] Tests are two sided at significance level of 0.05.

Results

A total of 1937 subjects were enrolled in the Diast-CHF study. For the purposes of this analysis, 202 controls and 349 patients were excluded because of LVEF ≤ 50%, large ventricular volumes, or missing key data variables (i.e. echocardiographic measures of diastolic function, left atrial volume index, age, or sex) (*Figure 1*). Thus, the analysis population was composed of 1386 subjects. Subjects were further classified according to a tertile split according to Gal-3 levels.

Baseline characteristics of the study population are shown in *Table 1*. Patients were older adults with a mean age of 67 years, and about half of the population was female. The majority had a history of hypertension, and other major co-morbidities were also common.

Figure 1 Derivation of the study cohort (STROBE diagram). LVEF, left ventricular ejection fraction.



A total of 170 patients had HFpEF at baseline. The AUC of Gal-3 was 0.71, and at a cut-off value of 13.57 ng/mL, sensitivity was 0.61 and specificity was 0.73 for diagnosis of HFpEF (*Figure 2*). This AUC was higher than the AUC = 0.59 of NT-proBNP. At a cut-off value of 143.7 ng/mL, the sensitivity of NT-proBNP was 0.52 and specificity was 0.65 for diagnosis of HFpEF ($P = 0.004$, *Figure 2*). Logistic models for the baseline diagnosis of HFpEF with a biomarker model of age, sex, NT-proBNP, and Gal-3 ($P < 0.001$), a clinical parameter model consisting of age, sex, previous diagnosis of HF, dyspnoea on exertion, and peripheral oedema (Model 1A, $C = 0.923$), the aforementioned clinical parameters plus NT-proBNP (Model 1B, $C = 0.922$), clinical parameters plus Gal-3 (Model 1C, $C = 0.922$), and clinical parameters plus NT-proBNP and Gal-3 (Model 1D, $C = 0.932$) (see Supporting Information, *Table S1A–S1D*) do show a significant contribution of Gal-3 within the respective models ($P < 0.05$), when added on top of NT-proBNP and clinical model parameters ($P < 0.05$) and superior diagnostic ability for model of clinical parameters and Gal-3 ($P = 0.017$, when compared with Model 1A) and a combined clinical and multi-biomarker model with NT-proBNP and Gal-3 ($P < 0.001$) in comparison with a clinical model alone (and a clinical model with NT-proBNP, $P = 0.0091$), displayed in the ROC curve (*Figure 3*).

In a head-to-head comparison for Gal-3 and NT-proBNP, Gal-3 $\geq$ 13.4 ng/mL outperformed NT-proBNP $\geq$ 220/660 pg/mL with an NRI of 0.22, $z = 3.96$, $P < 0.001$, for the diagnosis of HFpEF at baseline. For a comparison with different biomarker thresholds (Gal-3 $<$ 13.4 or $\geq$13.4 ng/mL, Gal-3 $\leq$ 17.8 or $>$17.8 ng/mL, and NT-proBNP 220/660 pg/mL), see Supporting Information, *Table S2A* and *S2B*. The association analysis between higher Gal-3, evidence of diastolic dysfunction, Framingham criteria for HF, and clinical characteristics examined is displayed in *Table 2*.

Over a median follow-up of 10 years, additional 107 patients experienced incident HFpEF. Patients with baseline

Table 1 Baseline characteristics of the DIAST-CHF galectin-3 analysis population

	Galectin-3 < 13.4 ng/mL *n* = 924 Mean (SD)	Galectin-3 ≥ 13.4 ng/mL *n* = 462 Mean (SD)	Total galectin-3 cohort *n* = 1386 Mean (SD)	Cohen's *D*	*P*-value[a] Raw	*P*-value[a] Adjusted
Age (years)	65.2 (7.6)	70 (7.7)	66.8 (8)	0.63	<0.001	
Body mass index (kg/m^2)	28.8 (4.7)	29.9 (5)	29.1 (4.8)	0.24	<0.001	
Blood pressure (systolic) (mmHg)	150.1 (21.2)	148.5 (21)	149.6 (21.1)	−0.08	0.186	
Blood pressure (diastolic) (mmHg)	85.1 (11.8)	81.4 (11.3)	83.9 (11.8)	−0.31	<0.001	
Heart rate (b.p.m.)	66.4 (11.6)	66.2 (11.6)	66.3 (11.6)	−0.02	0.787	

	Galectin-3 < 13.4 ng/mL *n* (%)	Galectin-3 ≥ 13.4 ng/mL *n* (%)	Total galectin-3 cohort *n* (%)	Odds ratio	Raw	Adjusted
Risk factors						
Female sex	414 (44.8%)	292 (63.2%)	706 (50.9%)	2.12	<0.001	<0.001
History of coronary heart disease	157 (17%)	115 (24.9%)	272 (19.6%)	1.62	<0.001	0.003
Hypertension	812 (87.9%)	430 (93.1%)	1242 (89.6%)	1.85	0.003	0.018
Hyperlipidaemia	400 (43.3%)	224 (48.5%)	624 (45%)	1.23	0.067	0.335
Type 2 diabetes mellitus	233 (25.2%)	135 (29.2%)	368 (26.6%)	1.22	0.112	0.336
Sleep apnoea	60 (6.5%)	28 (6.1%)	88 (6.3%)	0.93	0.755	0.755
Atrial fibrillation	53 (5.7%)	38 (8.2%)	91 (6.6%)	1.47	0.078	0.312
Current smoking	106 (11.5%)	41 (8.9%)	147 (10.6%)	0.75	0.139	0.278
Clinical characteristics						
Previous history of HF	56 (6.1%)	77 (16.7%)	133 (9.6%)	3.10	<0.001	<0.001
Dyspnoea at rest	19 (2.1%)	15 (3.2%)	34 (2.5%)	1.60	0.177	0.354
Exertional dyspnoea	204 (22.1%)	186 (40.3%)	390 (28.1%)	2.38	<0.001	<0.001
Orthopnoea	25 (2.7%)	30 (6.5%)	55 (4%)	2.51	0.001	0.002
Peripheral oedema	136 (14.7%)	139 (30.1%)	275 (19.8%)	2.49	<0.001	<0.001
Cough at night	33 (3.6%)	38 (8.2%)	71 (5.1%)	2.42	<0.001	0.001
Nycturia	517 (56.1%)	318 (68.8%)	835 (60.3%)	1.73	<0.001	<0.001
NYHA Class I	22 (38.6%)	16 (20.8%)	38 (28.4%)	—	0.008	0.024
NYHA Class II	31 (54.4%)	42 (54.5%)	73 (54.5%)			
NYHA Class III	4 (7%)	19 (24.7%)	23 (17.2%)			
Medication						
Antihypertensives	56 (6.1%)	77 (16.7%)	133 (9.6%)	2.10	<0.001	<0.001
Diuretics	19 (2.1%)	15 (3.2%)	34 (2.5%)	2.28	<0.001	<0.001
ACE inhibitors	204 (22.1%)	186 (40.3%)	390 (28.1%)	1.55	<0.001	<0.001
AT-1 antagonists	25 (2.7%)	30 (6.5%)	55 (4%)	1.40	0.020	0.081
Beta-blockers	136 (14.7%)	139 (30.1%)	275 (19.8%)	1.44	0.001	0.008
Calcium channel antagonists	33 (3.6%)	38 (8.2%)	71 (5.1%)	1.43	0.008	0.041
Anti-platelet therapy	517 (56.1%)	318 (68.8%)	835 (60.3%)	1.69	<0.001	<0.001
Anticoagulants	2 (0.2%)	0 (0%)	2 (0.1%)	2.09	0.001	0.009
Statins	22 (38.6%)	16 (20.8%)	38 (28.4%)	1.22	0.108	0.216
Insulin	31 (54.4%)	42 (54.5%)	73 (54.5%)	1.09	0.659	0.659
Oral antidiabetics	4 (7%)	19 (24.7%)	23 (17.2%)	1.38	0.035	0.105

	Galectin-3 < 13.4 ng/mL Median (quartiles)	Galectin-3 ≥ 13.4 ng/mL Median (quartiles)	Total galectin-3 cohort Median (quartiles)	Median difference	*P*-value[a] Raw	*P*-value[a] Adjusted
Laboratory values						
Galectin-3 (ng/mL)	10.5 [9.3, 11.9]	15.6 [14.3, 17.5]	11.9 [9.9, 14.3]	5.40	<0.001	<0.001
NT-proBNP (ng/L)	84.5 [45.4, 163]	143 [71.3, 299]	103 [52.7, 202]	46.90	<0.001	<0.001
hs-CRP (mg/dL)	1.53 [0.81, 3.27]	2.32 [1.17, 4.89]	1.8 [0.9, 3.8]	0.58	<0.001	<0.001

(*Continues*)

Table 1 (*Continued*)

	Galectin-3 < 13.4 ng/mL Median (quartiles)	Galectin-3 ≥ 13.4 ng/mL Median (quartiles)	Total galectin-3 cohort Median (quartiles)	Median difference	*P*-value[a]	
					Raw	Adjusted
Haemoglobin (g/dL)	14.2 [13.4, 15]	13.8 [12.9, 14.5]	14 [13.3, 14.9]	0.40	<0.001	<0.001
Estimated glomerular filtration rate (mL/min)	74.8 [63.4, 85.7]	61.7 [51.1, 73.2]	70.4 [58.5, 82.7]	12.90	<0.001	<0.001
Echocardiography						
Left ventricular (LV) ejection fraction (%)	61 [56, 65]	60 [56, 65]	61 [56, 65]	0.00	0.756	0.756
LV end-diastolic (ED) diameter (mm)	50 [46, 53]	49 [45, 52]	49 [45, 53]	−1.00	0.003	0.027
Interventricular septum (ED) (mm)	12 [11, 13]	12 [11, 14]	12 [11, 13]	0.00	0.066	0.264
LV posterior wall (ED) (mm)	11 [10, 12]	11 [10, 12]	11 [10, 12]	0.00	0.418	0.836
Left atrial volume index (mL/m^2)	23.9 [19.2, 29]	25 [20.2, 32]	24.1 [19.5, 29.6]	1.69	0.005	0.040
Left ventricular mass index (g/m^2)	76.5 [54.8, 111]	75.7 [53, 99.1]	76.2 [54, 107]	−2.17	0.310	0.930
Early wave (E) (cm/s)	71 [59, 83]	73 [60, 88]	72 [59, 84]	2.00	0.032	0.192
Atrial wave (A) (cm/s)	78 [66, 91]	83 [72, 97]	80 [67, 93]	5.50	<0.001	<0.001
e' mean (cm/s)	6.9 [5.8, 8.3]	6.3 [5.4, 7.6]	6.8 [5.6, 8]	−0.53	<0.001	<0.001
a' mean (cm/s)	10.7 [9.4, 11.9]	10.4 [9.1, 11.8]	10.6 [9.4, 11.9]	−0.20	0.058	0.290
E/e' average	10.1 [8.3, 12.4]	11.4 [9.4, 13.8]	10.6 [8.6, 12.8]	1.23	<0.001	<0.001
E/A	0.87 [0.72, 1.12]	0.82 [0.69, 1.06]	0.85 [0.71, 1.1]	−0.04	0.006	0.042

ACE, angiotensin-converting enzyme; AT-1, angiotensin-1; HF, heart failure; hs-CRP, high-sensitivity C-reactive protein; LV, left ventricular; NT-proBNP, N-terminal pro-brain natriuretic peptide; NYHA, New York Heart Association; SD, standard deviation.
Values are mean ± SD, median (quartiles), or *n* (%) unless otherwise noted.
[a]*P*-value is for the comparison of galectin-3 <13.4 vs. ≥13.4 ng/mL.

Gal-3 ≥ 13.4 ng/mL developed incident HFpEF significantly more often than patients with Gal-3 < 13.4 ($P < 0.001$). A clinical model consisting of age, sex, dyspnoea on exertion, and peripheral oedema (Model 3A, $C = 0.652$), a model of the aforementioned clinical parameters plus NT-proBNP (Model 3B, $C = 0.699$), the aforementioned clinical parameters plus Gal-3 (Model 3C, $C = 0.690$), and clinical parameters plus NT-proBNP plus Gal-3 (Model 3D, $C = 0.710$) (see Supporting Information, *Table S2*) demonstrate the independent, prognostic contribution ($P < 0.05$ in Models 3C and 3D) of Gal-3 for incident HFpEF and superior prognostic ability ($P < 0.001$) when compared with the clinical model, while the combination of clinical model with NT-proBNP and Gal-3 was superior to a clinical model and NT-proBNP alone ($P = 0.0026$).

During the 12 327 patient years at risk in this analysis, a total of 211 (15.2%) deaths and 415 (29.91%) combined endpoints (cardiovascular hospitalization or death) occurred. Gal-3 levels ≥13.4 ng/mL were associated with a higher risk of all-cause mortality (*Figure 4*), split by lower, intermediate, and higher tertiles (see Supporting Information, *Figure S1*); binary logarithm of Gal-3 was associated with a higher risk of all-cause mortality after adjustment for other important prognostic factors [hazard ratio (HR) 2.16, 95% confidence interval (CI) 1.43–3.27, $P < 0.001$] (*Table 3* and Supporting Information, *Figure S2*), indicating a more than two-fold increase in risk when Gal-3 doubles.

A clinical model of age, sex, previous diagnosis of HF, dyspnoea on exertion, and peripheral oedema alone (Model 4A, $C = 0.727$), a model of clinical parameters plus NT-proBNP (Model 4B, $C = 0.735$, $P < 0.001$, when compared with Model 4A), clinical parameters plus Gal-3 (Model 4C, $C = 0.742$, $P < 0.001$, when compared with Model 4A), and clinical parameters plus NT-proBNP plus Gal-3 (Model 4D, $C = 0.742$, $P < 0.001$, when compared with Model 4B) consistently demonstrate the superiority of clinical models with Gal-3 for prognosis of all-cause mortality (see Supporting Information, *Table S4A–S4D*). The *C*-statistics of 0.742 is further comparable with the explorative analysis of factors associated with all-cause mortality (*Table 3*). To display the cumulative incidence of the competing risks of incident HFpEF and death over 10 year follow-up stratified by Gal-3 < 13.4 or ≥13.4 ng/mL, see Supporting Information, *Figure S3*.

Gal-3 was similarly associated with an increased adjusted risk of the composite of cardiovascular hospitalization and death (HR 1.87, 95% CI 1.39–2.52, $P < 0.001$). The cumulative incidence of the composite endpoint components cardiovascular hospitalization ($P = 0.14$, non-significant) and death ($P < 0.001$) is displayed in *Figure 5*. In line with previous results, the respective models illustrate the prognostic ability of Gal-3 for the composite of cardiovascular hospitalization and death and can be accessed in Supporting Information, *Table S5A–S5D*.

Figure 2 Diagnostic ability for the detection of incidence heart failure with preserved ejection fraction (HFpEF): receiver operating characteristic curve for N-terminal pro-brain natriuretic peptide (NT-proBNP).

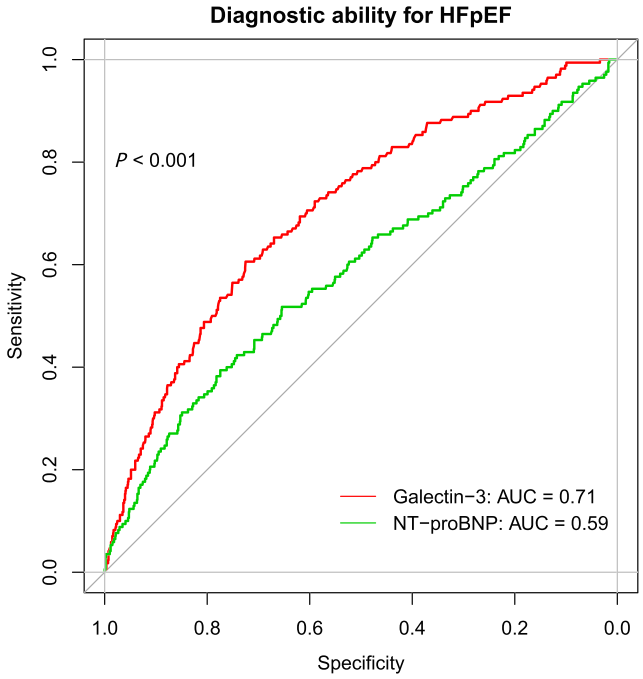
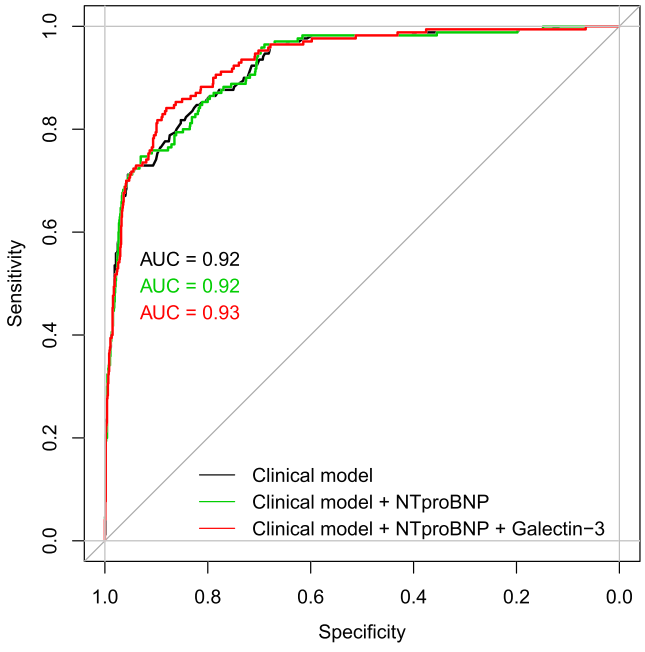


Figure 3 Diagnostic ability for the detection of incidence heart failure with preserved ejection fraction: adjusted receiver operating characteristic curve for a clinical model, clinical model plus N-terminal pro-brain natriuretic peptide (NT-proBNP), and clinical model plus NT-proBNP plus galectin-3. AUC, area under the receiver operating characteristic curve.



Discussion

In Diast-CHF, Gal-3 adds a statistically significant value to diagnose HFpEF. This was especially pronounced when evaluated outside of the context of clinical parameters. In conjunction with easy-to-obtain clinical parameters, its relevance in diagnosis and risk stratification does remain debatable. Gal-3 may be superior to NT-proBNP in regard to the diagnosis of baseline HFpEF and prognosis of all-cause mortality and a composite endpoint of cardiovascular hospitalization and death. While NT-proBNP was superior in prognosing incident HFpEF, Gal-3 yielded additional benefit to prognose incident HFpEF in combination with a clinical model and NT-proBNP. Moreover, increasing levels of Gal-3 were associated with co-morbidities, as well as typical clinical and echocardiographic characteristics consistent with HFpEF.

This analysis demonstrates that Gal-3 detected HFpEF, with moderate discriminatory power (AUC 0.71) in patients at risk, that is, presenting with signs and symptoms of HF, cardiovascular risk factors, and typical echocardiographic evidence of diastolic dysfunction. The Gal-3 value that was associated with these clinical characteristics is lower than the threshold used for additive risk stratification in patients with HFrEF (Gal-3 $>$ 17.8 ng/mL).

Table 2 Association analysis in multiplicative model for $\log_2$ galectin-3

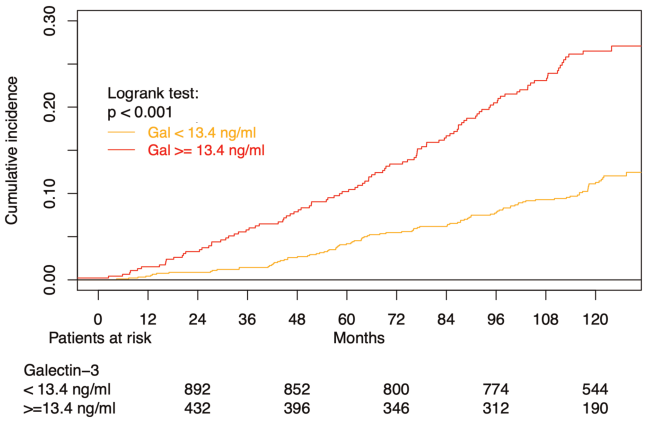
	Coefficient	95% confidence interval	*P*-value
(Constant)	12.9	11.7–14.3	<0.001
No. of Framingham criteria (ref. none)			
1	1.06	1.03–1.090	<0.001
2	1.18	1.12–1.23	<0.001
3 and more	1.17	1.07–1.27	<0.001
Smoking	1.04	0.99–1.08	0.011
eGFR (10 units)	0.95	0.95–0.96	<0.001
NT-proBNP (10-fold)	1.09	1.05–1.13	<0.001
E/A	0.93	0.89–0.98	0.002
E/e' (5 units)	1.01	1.00–1.01	<0.001
hs-CRP (10-fold)	1.08	1.04–1.11	<0.001

eGFR, estimated glomerular filtration rate; E/A, early-to-atrial wave ratio in mitral valve influx wave Doppler; E/e', ratio of early wave in mitral valve influx Doppler and tissue Doppler; hs-CRP, high-sensitivity C-reactive protein; NT-proBNP, N-terminal pro-brain natriuretic peptide.

In one study of a highly selected population of HFpEF, NT-proBNP identified diastolic dysfunction with an AUC of 0.83 on the ROC curve analysis, which was comparable with the AUCs for echocardiographic and invasive assessments of diastolic dysfunction used in the study.[34] In less select populations, the utility of NT-proBNP for the identification and risk stratification of HFpEF patients remains less certain.[35] In this population of patients at risk for HFpEF, Gal-3 was associated with a greater risk of all-cause mortality or a composite endpoint of cardiovascular hospitalization and death over a median follow-up of 10 years. Separation in the survival curves did not occur immediately, suggesting that Gal-3 may reflect a progressive, systemic process and relevant co-morbidities. It is plausible that Gal-3 might effectively identify patients most likely to develop HFpEF in this setting (and consequently be at risk for poor long-term outcomes) among an at-risk group of patients, especially with established risk factors.

These findings are consistent with the few studies that have examined Gal-3 as a predictor of new-onset HF. An analysis of the Framingham Offspring Cohort found that the incidence rate of HF increased with increasing Gal-3 quartiles. First HF events were reported in 166 patients over 11.2 years of follow-up. LV function was assessed in 140 of these patients, and 63 were classified as HFpEF and 77 as HFrEF. After adjustment for age and sex, the incident HF risk (including both HFpEF and HFrEF) increased 28% for every 1 standard deviation increase in log Gal-3 (95% CI 1.14–1.43, $P < 0.0001$).[20] Increasing Gal-3 quartiles were also associated with a higher adjusted risk of all-cause mortality.[20] A total of 2477 participants in the Framingham Heart Study Offspring cohort underwent measurement of plasma Gal-3 levels at 2 examinations. Change in Gal-3

Figure 4 All-cause mortality over 10 year follow-up stratified by galectin-3 $<$ 13.4 or $\geq$13.4 ng/mL.



was associated with future HF (HR 1.39 per 1 standard deviation increase; 95% CI 1.13–1.71) and all-cause mortality (HR 1.30; 95% CI 1.17–1.46). Change in Gal-3 was associated with both HFpEF and HF with reduced ejection fraction (HFrEF) ($P < 0.05$ for both).[36]

A small study ($n = 43$ controls; $n = 35$ HFpEF) reported an AUC of 0.891 (95% CI 0.808–0.974) for the diagnosis of HFpEF using Gal-3 levels >17.8 ng/mL.[21] HFpEF was defined by symptoms, LVEF > 45%, and normal LV size, and echocardiography confirmed LV diastolic dysfunction. The AUC in this study was higher than the finding from Diast-CHF, which in part could be due to the other selection criteria.

Our findings are also consistent with an analysis of the Prevention of Vascular and Renal Endstage Disease (PREVEND) study, which showed that Gal-3 was associated with new-onset HF among patients at high baseline cardiovascular risk (HR 1.30, 95% CI 1.12–1.50).[19] Of note, we observed an odds ratio of 1.77 for incident HFpEF (1.14–2.74, $P = 0.010$) in patients with Gal-3 above the median. In the PREVEND analysis, Gal-3 was associated with an increased risk of new-onset HFrEF (defined as signs, symptoms, and objective evidence of HF with LVEF ≤ 40%),[37] but not HFpEF (defined as signs, symptoms, and objective evidence of HF with LVEF ≥ 50%.[19,36] When evaluating the associations of 12 cardiovascular biomarkers with incident HFpEF vs. HFrEF across four longitudinal community-based cohorts: the Cardiovascular Health Study, the Framingham Heart Study, the Multi-Ethnic Study of Atherosclerosis, and the PREVEND study, de Boer *et al.* reported an HR of 1.02 (95% CI 0.93–1.12, n.s.) for Gal-3 and incident HFpEF.[2] Although the reason for this observation remains uncertain, it is possible that detailed echocardiographic criteria such as that collected in the Diast-CHF cohort or higher-risk entry criteria into the Diast-CHF cohort might have led to higher risk and enabled a more specific detection of HFpEF.

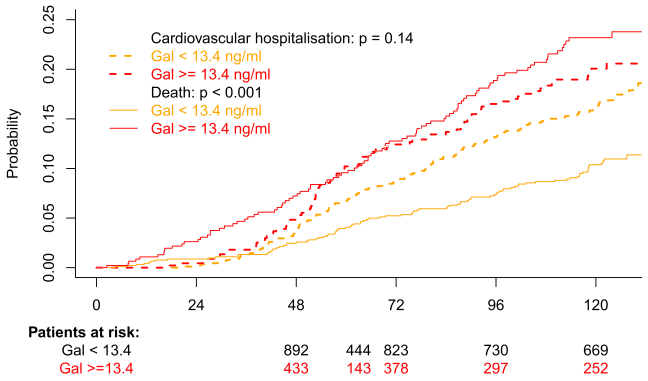
Several studies have evaluated the impact of combining Gal-3 with BNP or NT-proBNP to achieve better prognostic capability.[5,38,39] In the Pro-BNP Investigation of Dyspnea in the Emergency Department (PRIDE) study in 599 patients with acute HF, Gal-3 was a significant predictor of adjusted 60 day mortality (odds ratio 10.3, 95% CI 1.6–174.1, $P = 0.007$) and had an AUC of 0.74 ($P = 0.0001$).[38] The AUC for NT-proBNP was 0.67 ($P = 0.009$). The highest risk of 60 day mortality was observed in patients with elevations in both Gal-3 (>9.42 ng/mL) and NT-proBNP (>5562 pg/mL).[37] A substudy including 592 patients in the Coordinating Study Evaluating Outcomes of Advising and Counselling in Heart Failure (COACH) trial found that Gal-3 predicted adjusted all-cause death or HF hospitalization (HR 1.38, 95% CI 1.07–1.78, $P = 0.015$, adjusted for age, gender, BNP, estimated glomerular filtration rate, and diabetes). When LVEF was added to the model, the association was no longer significant, and a significant interaction with LVEF ($P = 0.047$) was observed. The AUC was 0.67 ($P = 0.004$) for the ROC analysis of Gal-3 as a predictor of death or HF hospitalization, and it was 0.65 ($P < 0.001$) for BNP. The AUC was greater for the combination of both Gal-3 and BNP than either parameter alone (AUC 0.69,

Table 3 Explorative analysis of factors associated with all-cause mortality

Term	Hazard ratio	95% confidence interval		*P*-value	C-statistics
Log_2(galectin-3)	2.16	1.43	3.27	<0.001	0.742
Age (decades)	2.06	1.62	2.62	<0.001	
Dyspnoea at exert.	1.65	1.23	2.22	0.001	
Smoking	1.95	1.23	3.11	0.004	
Log_{10}(NT-proBNP)	1.97	1.31	2.98	0.001	
E/A	0.36	0.20	0.65	<0.001	
E/e'	1.06	1.02	1.09	0.001	

E/A, early-to-atrial wave ratio in mitral valve influx wave Doppler; E/e', ratio of early wave in mitral valve influx Doppler and tissue Doppler; NT-proBNP, N-terminal pro-brain natriuretic peptide.

Figure 5 Cumulative incidence curves of the composite endpoint of cardiovascular hospitalization and death over 10 year follow-up stratified by galectin-3 < 13.4 or ≥13.4 ng/mL.



$P < 0.05$ vs. either alone).[39] The patient populations in PRIDE (acute HF) and COACH (chronic HFrEF) differ from the cohort in Diast-CHF. Yet evaluating single-marker and multi-marker strategies for diagnosis and prognosis may prove efficacious for HFpEF patients.

In addition to multi-marker strategies, repeated measurement of Gal-3 might be of additional use to obtain biomarker trajectories. Van der Velde *et al*. found persistently elevated Gal-3 to predict new-onset HF in PREVEND and serial measurements to provide more accurate prognostic information compared with single determination of Gal-3.[40] It is especially over longer follow-up periods, where this approach may add incremental value for our patients.

In regard to Gal-3 as a surrogate marker, that is, of inflammation or fibrogenesis on a systemic level, non-cardiac co-morbidities and risk factors, that is, renal insufficiency, have to be kept in mind. Hence, the clinical syndrome of HFpEF may require to be seen in the systemic context.

Of note, therapy guidance or Gal-3 as a potential target may be of particular interest. Yet, in a randomized, double-blind, two-arm, parallel-group, active-controlled clinical trial in 35 HFpEF patients with type 2 diabetes mellitus, a biomarker-driven approach, that is, to target collagen turnover in diabetic HFpEF patients, has not yielded positive results.[41] Edelmann *et al*. previously described Gal-3 levels to be modestly elevated in patients with stable HFpEF; Gal-3 related to functional performance and quality of life. Increasing Gal-3 was associated with worse outcome, independent of treatment or NT-proBNP.[13] Further, Ravassa *et al*. have described a biochemical phenotype of high collagen cross-linking identifies HFpEF patients resistant to the beneficial effects of spironolactone.[42] The Heart OMics in AGing Trial (HOMAGE) aims to investigate the effects of spironolactone on serum markers of collagen metabolism and on cardiovascular structure and function in people at risk of developing HF and potential interactions with Gal-3 as a marker of fibrogenic activity.[43] It remains of great interest if Gal-3 demonstrates additional benefit in stratification of people at risk of developing HF within HOMAGE.

From a clinical point of view, the currently used HFpEF prediction tools remain inaccurate in many settings. The majority of current diagnostic HFpEF biomarker studies have a high risk of bias, reducing the reproducibility and the potential for clinical care.[44] In this analysis of a patient cohort at risk with a decade of follow-up, we show that Gal-3 complements diagnosis with an NRI of 0.22 when compared with NT-proBNP and enhances risk stratification, helping to meet the pressing need for more accurate diagnosis and prediction tools. Although Gal-3 adds a statistically significant value for the diagnosis of HFpEF within this study, the clinical relevance, especially in co-function with clinical parameters, remains debatable.

Future and independent validations remain required to conclude on a suitable Gal-3 cut-off for diagnosis and prognosis in the setting of HFpEF. Nevertheless, including Gal-3 in the work-up of patients with suspected or manifest HFpEF may seem both feasible and advisable, especially when combined with NT-proBNP. This could lead to the introduction of dedicated surveillance programmes for patients at high risk to develop HFpEF and allow for additional risk stratification based on a multi-biomarker panel covering distinct pathophysiological axes in the future.

Strengths and limitations

These findings should be considered in the context of the following strengths and limitations. Diast-CHF was a population-based study that enrolled a large number of patients who subsequently underwent uniform standardized baseline evaluations at baseline. Importantly, all accrued clinical events were critically reviewed and confirmed by written reports. The proportion of patients lost to follow-up was low (4.5%) for a population-based study.

The diagnosis of incident HFpEF was established independently from natriuretic peptides and analogous to European Society of Cardiology 2016 guidelines.[24] We did not perform invasive haemodynamics to ascertain the diagnosis, not at baseline nor follow-up. A potential effect of diuretic treatment and other pharmaceutical therapy as differences in some baseline characteristics on Gal-3 levels was not accounted for. Also, changes in cardiovascular medications, Gal-3 levels, or neurohormonal activation were not assessed in the cohort; thus, their influence on the results cannot be estimated. Further, echocardiography was performed following the clinical standard at the time; hence, novel assessments, that is, strain analysis, are not available and the temporal dispersion of follow-up echocardiography in patients during later stages of the cohort may limit the accuracy of time to incident HFpEF. Only Caucasian patients were represented, which limits the generalizability of these findings to non-Caucasian populations.

Because of sample size and event rate, we did not calibrate our findings nor did we externally validate our findings in an independent cohort. Finally, this study took place within the German Health Care System, and event rates might be different in other geographical areas that apply different standards of care.

Conclusion

Galectin-3 differentiated patients with HFpEF from an overall cohort of well-characterized patients with cardiovascular risk factors. In conjunction with clinical parameters, Gal-3 has

statistically significant added value for the diagnosis of HFpEF within this study, yet the clinical relevance remains debatable. Gal-3 might be clinically useful to identify patients who are at high risk of developing HFpEF, especially when combined with NT-proBNP. Baseline Gal-3 level identified patients at high risk for death from any cause or the composite of cardiovascular hospitalization and death over 10 years of follow-up. Early identification could provide an opportunity to aggressively treat risk factors, which might delay or prevent the onset of HFpEF, a concept that should be tested in a prospective, randomized, clinical trial.

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Conflict of interest

T.D.T. reports personal fees from Novartis Pharma, Bayer Vital, and Berlin Chemie and non-financial support from Amgen and AstraZeneca, outside the submitted work. In addition, T.D.T. is co-inventor of patent WO/2015/028671 issued to Charité—Universitätsmedizin Berlin. M.M., D.H., J.P., K.N., and L.B. declared no conflicts of interest. H.-D. D. received grant support, paid to his institution, fees for presentations, fees for serving on an advisory board, and travel support, from Bayer and Amgen; grant support, paid to his institution, fees for presentations, and fees for serving on an advisory board, from Sanofi; grant support, paid to his institution, from Boehringer Ingelheim, Merck, CSL Behring, and Cytokinetics; and personal fees from Berlin Cures, Berlin Heals, LivaNova, and Stealth BioTherapeutics. C.H.-L. reports grants from the German Ministry of Education and Research, during the conduct of the study; personal fees from Hogrefe Huber Publishers, Servier, Novartis, and Heel; and grants from the German Ministry of Education and Research and Grun Foundation, outside the submitted work. G.H. reports personal fees from Berlin Chemie, Impulse Dynamics, Novartis, Servier, Springer, and Vifor Pharma and other fees from Corvia, outside the submitted work. B.P. reports personal fees from Bayer Healthcare, Novartis, Merck, Daiichi-Sankyo, MSD, Sanofi-Aventis, Stealth Peptides, and Vifor Pharma, outside the submitted work. R.W. reports grants from Boehringer Ingelheim, during the conduct of the study; personal and other fees from Bayer, Berlin Chemie, Boehringer Ingelheim, CVRx, Medtronic, Novartis, and Servier; other fees from Boston Scientific, Gilead, Johnson & Johnson, and Relypsa; and personal fees from Bristol Myers Squibb, Pfizer, and Sanofi, outside the submitted work. F.E. reports grants from the German Research Foundation (DFG) and the German Ministry of Education and Research, during the conduct of the study; personal fees and non-financial support from Novartis; grants and personal fees from Boehringer Ingelheim and Servier; personal fees from CVRx, Pfizer, Medtronic, MSD/Bayer, Bayer, ResMed, Berlin Chemie, Vifor Pharma, PharmaCosmos, and Merck; and grants from Thermo Fisher, outside the submitted work. ICMJE potential conflicts of interest disclosure statements can be accessed via the Editorial Office.

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Author contributions

T.D.T., R.W., and F.E. contributed to the conception of the work. All contributed to the acquisition, analysis, or interpretation of data for the work. T.D.T. and F.E. drafted the manuscript. M.M., H.-D.D., D.H., J.P., K.N., L.B., C.H.-L., G.H., and B.P. critically revised the manuscript. All authors gave final approval and agree to be accountable of the work ensuring integrity and accuracy. Detailed ICMJE author contribution forms in regard to this manuscript can be accessed via the Editorial Office.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. All-cause mortality by Galectin-3 tertiles
Figure S2. Error Bars for factors associated with all-cause mortality
Figure S3. Cumulative incidence for the competing risks of incident HFpEF and death over 10 yeary follow-up stratified by Galectin-3 $<$ or $\geq$ 13.4 ng/mL
Table S1. Models for diagnosis of HFpEF
Table S2. Net Reclassification Index (NRI) by Biomarker threshold
Table S3. Prognostic models for incident HFpEF
Table S4. Prognostic models for all-cause death
Table S5. Prognostic models for composite endpoint

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