

Growth-differentiation Factor-15: a Novel Biomarker in Patients with Diastolic Dysfunction?

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Abstract

Background: Growth differentiation factor-15 (GDF-15), a stress-responsive transforming growth factor- β -related cytokine, is elevated and independently related to an adverse prognosis in systolic heart failure.

Objective: This study aimed to investigate plasma levels of GDF-15 in patients with preclinical diastolic dysfunction or heart failure with normal ejection fraction (HF_nEF).

Methods: We evaluated 119 patients with normal ejection fraction referred for an elective coronary angiography, 75 (63%) of whom had coronary artery disease. Subjects were classified as having either mild left ventricular diastolic dysfunction (LVDD grade I, $n = 61$), HF_nEF (LVDD grade II or III, $n = 38$) or normal diastolic function (controls, $n = 20$). In a subgroup of 20 subjects, changes in cardiac output (CO) were measured by inert gas rebreathing (InnocorTM) in response to an orthostatic hemodynamic test.

Results: Growth differentiation factor-15 levels in HF_nEF [median 1.08, interquartile range (0.88-1.30) ng/ml] were significantly higher than in controls [0.60 (0.50-0.71) ng/ml, $p = 0.003$] and in patients with LVDD grade I [0.78 (0.62-1.04) ng/ml, $p < 0.001$]. In addition, GDF-15 was significantly elevated in patients with LVDD grade I compared to controls ($p = 0.003$). Furthermore, GDF-15 was correlated with echocardiographic markers of diastolic dysfunction and was correlated with the magnitude of CO response to the change in body position from standing to supine ($r = -0.67$, $p = 0.005$).

Conclusion: Growth differentiation factor-15 levels are elevated in subjects with HF_nEF and can differentiate normal diastolic function from asymptomatic LVDD. In addition, GDF-15 is associated with a reduced cardiac output response in the orthostatic hemodynamic test. (Arq Bras Cardiol. 2011; [online].ahead print, PP.0-0)

Keywords: Growth differentiation factor 15; heart failure; stroke volume; ventricular dysfunction.

Introduction

Nearly half of the patients presenting with symptoms and signs of heart failure have a normal ejection fraction (EF)^{1,2}, a condition termed "heart failure with normal ejection fraction (HF_nEF)". Recent data suggest that overall mortality due to HF_nEF is equivalent to that of systolic heart failure (SHF)^{1,2}. In the general population, asymptomatic left ventricular diastolic dysfunction (LVDD), which is considered as a precursor of HF_nEF³, is a powerful and independent predictor of death⁴.

Nevertheless, clinical trials in HF_nEF have been frequently disappointing, including those with drugs approved for SHF⁵⁻⁷. Notwithstanding the same overall mortality, this is

at least in part due to the lower proportion of heart failure-related death in the overall population of patients with HF_nEF⁸. The discrepancies in mortality rates from heart failure-related deaths may be attributed to the considerable heterogeneity among patients with HF_nEF, which can be explained by the use of different recruitment criteria⁹ or the complexity of echocardiographic diagnosis. Even though a specific guideline has been proposed recently by the American Society of Cardiology¹⁰, establishing this medical condition is difficult in clinical practice. Echocardiography can be misleading or inconclusive and will not always be sufficient to establish the diagnosis. Better characterization of patients with HF_nEF at greatest risk for heart failure-related complications and death could allow more effective use of specific interventions.

Biomarkers reflect distinct disease mechanisms, including neurohumoral activation, inflammation, myocyte injury and extracellular matrix turnover.

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Growth differentiation factor 15 (GDF-15) is a member of the transforming growth factor β (TGF- β) cytokine super-family and was first cloned as macrophage-inhibitory cytokine-1¹¹. Previous studies have shown that patients with SHF have increased circulating levels of GDF-15¹², and it has been demonstrated that GDF-15 provides prognostic information beyond that established by clinical and biochemical risk factors^{13,14}.

The aim of the present study was to test the diagnostic value of GDF-15 in identifying patients with preclinical, asymptomatic LVDD or HFnEF. Moreover, we analyzed the association of GDF-15 levels to different preload conditions.

Methods

One hundred-nineteen consecutive subjects referred to elective coronary angiography for stable or suspected coronary artery disease (CAD) or diagnostic workup of heart failure were enrolled in the study. Patients that needed coronary revascularization with either angioplasty or coronary bypass surgery were not enrolled in the study. The protocol was approved by the local Ethics Committee, and signed informed consent was obtained from all patients. Exclusion criteria were left ventricular EF < 50%, known CAD with progressive chest pain within the last month, coronary angioplasty or myocardial infarction < 6 weeks, hypertrophic cardiomyopathy, moderate-to-severe valvular heart disease, uncontrolled hypertension, atrial fibrillation or other severe arrhythmias, or serum-creatinine > 2,0 mg/dl. In patients without diabetes, a standardized oral glucose tolerance test (oGTT) was performed (75 g glucose) according to the World Health Organization protocol as previously described¹⁵. Body mass index (BMI) was calculated as weight (kg)/height (m²).

Echocardiography

Echocardiography was performed using a standard ultrasound system (Vivid 7, General Electric, Milwaukee, Wisconsin). Left ventricular EF was measured based on the modified biplane Simpson's method. The left atrium volume index¹⁶ was calculated using the biplane area-length method¹⁶. Dimensions were recorded by standard techniques according to current guidelines¹⁷. Left ventricular mass index (LVMI) was calculated by the Devereux formula indexed to the body surface area¹⁷. Conventional transmitral flow was measured with pw-Doppler. Early (E), late atrial (A) transmitral peak flow velocities and the ratio (E/A) were measured and three consecutive beats were averaged. Pulsed wave tissue Doppler imaging (TDI) was performed at the junction of the septal and lateral mitral annulus and three consecutive beats were averaged. Early diastolic velocities (E' medial, E' lateral) were recorded; the mean value (E' average) of E' at the medial and lateral mitral annulus was determined. Ratios of E/E' medial, E/E' lateral and average E/E' ratio were calculated. Diastolic dysfunction was classified according to the common consensus study by the American and European Society of Echocardiography (ASE, ESC)¹⁰.

Inert gas-rebreathing (Innocor™)

A spirometry (Innocor™ device INNO0500) was performed to exclude relevant pulmonary dysfunction. Cardiac output

(CO) was measured using an inert gas rebreathing (IGR) device (Innocor™, Innovision A/S, Denmark) in a subgroup with either normal diastolic function or asymptomatic mild LVDD (LVDD grade I, n = 10) and patients with HFnEF (LVDD grade II or III, n = 10). The method has been described in details elsewhere¹⁸ and has been validated in heart failure patients at rest and exertion^{19,20}. For the IGR method, O₂-enriched test gas with 0.5% N₂O (blood soluble gas) and 0.1% SF₆ (blood insoluble gas) was used. The gas volume was adjusted to meet the physiological demand during rest. Use of SF₆ enabled the measurement of the volume of lungs, valve and rebreathing bag. The test gas concentration was decreased during the rebreathing maneuver, with a rate proportional to pulmonary blood flow (PBF). The slope of the N₂ washout kinetics correlates with PBF and CO in the absence of pulmonary shunt.

Prior to the measurements, patients were instructed on the rebreathing technique. The interval for measurements was a minimum of 3 minutes to allow complete inert gas clearance. CO was measured in different body positions as a model of ventricular load change and indexed for body surface area (BSA, COi). After 5 minutes of rest in supine position, CO was measured 3 times, and then body position was changed from supine to upright. After a three-minute period prior to the minimum period necessary to achieve a hemodynamic equilibrium, three consecutive measurements were performed. The measurements were averaged in each position.

Laboratory analysis

Blood samples were drawn at rest for the analysis of routine laboratory parameters and were stored at -80°C for later analysis. After thawing, GDF-15 was measured in plasma with a pre-commercial electrochemiluminescence immunoassay and NT-proBNP with a commercially available electrochemiluminescence immunoassay on an automated Elecsys™ analyzer (all Roche Diagnostics GmbH, Mannheim, Germany) by an investigator blinded to patient characteristics.

Statistical analysis

All analyses were performed using SPSS statistical software (SPSS 17.0, Chicago, IL). The data are presented as medians (interquartile range) for continuous variables or absolute numbers (%) for categorical variables, unless otherwise specified. Log-transformed values were used for analysis as appropriate. A p value < 0.05 was considered statistically significant. Non-parametric tests for group differences between categories were performed. We used the Kruskal-Wallis test to test the equality of medians among more than two distinct groups. The Wilcoxon-Mann-Whitney U-test was used to analyze differences between the medians of two groups and the χ^2 test to evaluate differences in proportions in more than 2 sets of categorical variables. Fisher's Test was used for the comparison of two sets of binary variables.

Growth differentiation factor-15 levels were compared across different grades of LVDD and different categories of the E/e' average ratios and the LAi by the Jonckheere-Terpstra test. We used the Spearman rank correlation to identify variables associated with GDF15. Multivariate analysis of covariance

and logistic regression models, including most predictive variable for the dependent variables were built. Multiple linear regression analysis was applied to identify factors that were independently associated with GDF-15 levels.

Results

Patient characteristics

We included 119 patients with normal EF (66 years [59-73]) in the study (45% woman), 63% of whom had CAD without the need for revascularization. Subjects were classified as having either mild LVDD (grade I, $n = 61$), HFnEF (LVDD grade II or III, $n = 38$) or normal diastolic function (normal DF, $n = 20$). Patients with HFnEF were older, had a higher mean NYHA functional class, higher systolic and mean arterial blood pressure values, a lower resting heart rate and were more likely to have diabetes compared to the group with normal DF (all $p < 0.005$). An oGTT was performed in 80 individuals, of whom 35 (29%) had a normal glucose tolerance (NGT), 31 (26%) had impaired glucose tolerance (IGT) and 24 (20%) had newly detected diabetes. Twenty-nine patients had a history of type 2 diabetes mellitus before inclusion in the study; therefore, 53 (45%) individuals included in the study were identified as having type 2 diabetes mellitus (T2DM). Metabolic syndrome (MetS) was diagnosed in 51% of patients according to the amended National Cholesterol Education Program's Adult Treatment Panel III (ATP-III)²¹. The clinical characteristics in patients classified as to the presence or absence of LVDD or HFnEF are shown in Table 1, the laboratory data and parameters of cardiac assessment are highlighted in Table 2.

GDF-15 and diastolic function

In the whole study group, GDF-15 was significantly elevated in HFnEF patients (1.08 [0.88-1.30]) compared to those with normal diastolic function (0.60 [0.50-0.71], $p < 0.001$) or mild LVDD (0.78 [0.62-1.04], $p < 0.001$). In addition, GDF-15 plasma levels were higher in subjects with mild LVDD compared to patients with normal diastolic function ($p = 0.003$, Figure 1). When excluding subjects with CAD, GDF-15 levels remained significantly associated with the severity of diastolic dysfunction ($p < 0.001$). The GDF-15 levels were closely related to the New York Heart Association (NYHA) functional class. In a multivariate lineal regression model including variables known to be associated with the presence of LVDD, age ($\beta = 0.456$, $p < 0.001$) and the presence of mild LVDD or HFnEF ($\beta = 0.315$, $p = 0.001$) were identified as variables predictive of higher GDF-15 levels. Female gender, the presence or absence of CAD, BMI, a history of T2DM, a previous myocardial infarction, left ventricular muscle mass and hypertension did not add significantly to the model (adjusted $r^2 = 0.39$, $p < 0.001$ for overall model).

The relationship between GDF-15 quartiles and clinical echocardiographic characteristics and NT-pro-BNP levels is shown in Table 3. Increasing quartiles of GDF-15 levels were associated with a higher EF, a higher LAi, a higher E/E' septal and E/E' average ratio and a lower E' septal, lateral and average E' ratio. In addition, GDF-15 quartiles were associated with higher NT-pro-BNP levels. Particularly among the association

with echocardiographic parameters of diastolic dysfunction, GDF-15 levels were significantly increased across the LAi and the average E/E' ratio, with both being indicative of elevated left atrial filling pressures (Figure 2a and Figure 2b). There was a weak linear correlation between the LAi ($r = 0.354$, $p < 0.001$) and the average E/E' ratio with GDF-15 levels ($r = 0.613$, $p < 0.001$).

In contrast, the global longitudinal strain values, a very sensitive tool to detect subtle systolic dysfunction disregarding a normal EF, were not associated with GDF-15 levels. In addition, parameters of left ventricular geometry (left ventricular mass index, relative wall thickness and left ventricular end diastolic volume index) were not related to GDF-15 concentrations (Table 3).

GDF15 and hemodynamic orthostatic test

In a substudy, COi was measured with inert gas- rebreathing technique, in supine and upright body positions, as a model of ventricular load change. In subjects with HFnEF, COi was 1.96 [1.8-2.23] l/m² in the upright position vs 1.90 [1.6-1.93] l/min in subjects with normal DF or mild LVDD. After changes in body position from upright to supine, the HFnEF group increased COi by 7% [3-16%], whereas the non HFnEF group increased COi by 28% [23-16%], $p = 0.002$ (Figure 3a). The COi response plotted against GDF-15 quartiles is shown in Figure 3b. GDF-15 was negatively correlated with the magnitude of COi response ($r = -0.67$, $p = 0.005$).

Discussion

Growth differentiation factor-15 plasma levels are associated with the diagnosis of HFnEF and LVDD. Furthermore, GDF-15 levels are increased with more severe grades of LVDD and can differentiate subjects with normal DF and asymptomatic LVDD. Growth factor differentiation factor-15 levels correlates with the echocardiographic criteria implemented in the current guidelines for the diagnosis and classification of LVDD. This is the first study to show an association between GDF-15 levels and cardiac output response to different preload conditions serving as a functional parameter of hemodynamic reserve.

Experimental studies have shown that GDF-15 expression significantly increases in the heart after various forms of stress, including pressure overload^{22,23}. Animal studies have shown that GDF-15 promotes protective, antiapoptotic and anti-hypertrophic effects in the heart²³. Considering the fact that GDF-15 is produced by several other cell types in addition to cardiomyocytes (endothelial cells, adipocytes, macrophages, vascular smooth muscle cells), it is likely that this biomarker integrates information from different disease pathways, providing a pathophysiological insight in patients with HFnEF.

The human GDF-15 promoter contains two p53 and two Egr1 consensus binding sites²⁴. Increased expression of p53 has been detected in the failing human myocardium, and it has been suggested that it plays a role in the transition from cardiac hypertrophy to systolic heart failure²⁵. Egr1 is induced in human atherosclerotic plaques²⁶ and is a mediator of pressure overload hypertrophy in a mice model²⁷. Therefore, GDF-15 appears to have cardioprotective functions; those in SHF

Table 1 - Clinical characteristics

	Normal DF	mild LVDD	HFnEF	p-value ^a	all
Clinical variables					
Age (years)	51 (48-58)	67 (60-72)	73 (66-77)	<0.001*	66 (59-73)
Female gender, n (%)	7 (35)	27 (44)	20 (53)	0.426	54 (45)
BMI (kg/m ²)	26 (24-30)	28 (25-31)	27 (25-32)	0.2	27 (25-32)
Waist circumference (cm)	98 (88-110)	107 (96-117)	104 (100-111)	0.96	104 (97-115)
Hip circumference (cm)	98 (91-105)	109 (96-114)	105 (99-114)	0.176	104 (95-114)
Systolic BP (mmHg)	123(110-130)	130(124-140)	133(130-140)	0.002*	130(120-140)
Diastolic BP (mmHg)	77(70-80)	80(72-86)	80(70-88)	0.111	80(70-86)
MAP (mmHg)	94 (83-96)	96 (92-101)	99 (93-103)	0.015*	96 (90-102)
Pulse pressure	42 (40-50)	52 (48-60)	50 (46-70)	0.016*	50 (41-60)
Resting HR (beats/min)	73 (68-80)	74 (65-80)	69 (65-77)	0.047*	72 (65-78)
CAD, n (%)	10 (50)	40 (65)	25 (66)	0.417	75 (63)
History of MI, n (%)	5 (25)	11 (18)	12 (32)	0.299	28 (23)
History of stroke, n (%)	0	2 (3)	3 (8)	0.339	5 (4)
History of PTCA, n (%)	9 (45)	28 (46)	19 (50)	0.905	56 (47)
Mean NYHA-class	1 (0.5-2)	2 (1-2)	2.5 (2-3)	<0.001*	2 (1-2)
Cardiovascular risk factors					
Treated hypertension	14 (70)	55 (92)	37 (98)	0.004*	106 (90)
Smoking, n (%)	8 (44)	8 (13)	7 (19)	0.013*	23 (20)
Family history of CAD	7 (35)	40 (66)	16 (43)	0.019*	63 (53)
Hyperlipidemia, n (%)	8 (40)	44 (72)	26 (68)	0.029*	78 (66)
Glucose metabolism status					
NGT, n (%)	12 (60)	15 (25)	8 (21)		35 (29)
IGT, n (%)	5 (25)	17 (28)	9 (24)	0.013*	31 (26)
New detected T2DM, n (%)	2 (10)	10 (16)	12 (32)		24 (20)
Known T2DM, n (%)	1 (5)	19 (31)	9 (24)		29 (24)
Duration of T2DM (years)	2.5 (2-3)	5.5 (2-10)	14 (4-24.5)	0.076	5.5 (2.5-12.5)
Medications					
ACE inhibitor, n (%)	12 (60)	37 (61)	23 (61)	0.999	72 (61)
AT1 receptor blocker, n (%)	0	11 (18)	12 (32)	0.014*	23 (20)
Diuretics, n (%)	1 (%)	18 (30)	21 (55)	<0.001*	40 (34)
Ca ²⁺ blocker, n (%)	3 (15)	8 (13)	13 (34)	0.032*	24 (20)
β-Blocker, n (%)	11 (55%)	41 (67)	34 (89)	0.009*	86 (72)
Insulin therapy, n (%)	0	7 (14)	5 (16)	0.355	12 (13)
OAD, n (%)	1 (8)	12 (25)	3 (9)	0.109	16 (17)
Statin, n(%)	7 (35)	38 (62)	25 (65)	0.274	70 (59)
Acetyl salicylic acid, n (%)	10 (47)	51 (83)	29 (76%)	0.021*	90 (76)

Values are median (interquartile range) or n (%). ^aMann-Whitney-Test, Jonckheere-Terpstra test, Fisher or χ^2 - test were used as appropriate, * statistically significant ($p < 0.05$). BMI - Body mass index, BP - blood pressure, CAD - Coronary Artery Disease, CABG - coronary bypass graft, DF - diastolic function, HFnEF - heart failure with normal ejection fraction, IGT - impaired glucose tolerance, LVDD - left ventricular diastolic dysfunction, NGT - normal glucose tolerance, HR - heart rate, MAP - mean arterial pressure, OAD - oral anti-diabetic therapy, PTCA - percutaneous coronary angioplasty, T2DM - type 2 diabetes mellitus.

Table 2 - Laboratory data and Parameter of cardiac assessment according to diastolic function

	Normal DF	Mild LVDD	HFnEF	p-value ^a
Biomarker				
GDF-15	0.60 (0.50-0.71)	0.78 (0.62-1.04)	1.08 (0.88-1.30)	<0.001*
NT-pro-BNP	98.5 (56.5-129.0)	96.0 (58.5-178.5)	525.5 (349.0-1.147.0)	<0.001*
Routine parameter				
Total cholesterol (mg/dl)	182 (165-199)	195 (172-225)	198 (164-210)	0.483
LDL-cholesterol (mg/dl)	103 (86-125)	111 (92-135)	110 (93-137)	0.495
HDL-cholesterol (mg/dl)	51 (42-60)	53 (40-62)	46 (40-56)	0.201
Triglyceride (mg/dl)	133 (111-186)	146 (98-206)	161 (119-212)	0.120
Lp (a) (mg/dl)	7 (5-21)	15 (5-35)	14 (6-58)	0.180
TSH (mU/l)	1.3 (0.9-2.3)	1.4 (0.9-2.1)	1.3 (0.9-1.8)	0.784
Creatinine (mg/dl)	0.8 (0.7-0.9)	0.9 (0.7-0.9)	0.9 (0.8-1.1)	0.003*
hsCRP	0.2 (0.1-0.5)	0.3 (0.1-0.6)	0.3(0.2-0.7)	0.248
HOMA-IR	1.14 (0.7-1.3)	1.6 (1.1-2.7)	1.8 (1.1-3.4)	0.019*
2h-PG (mg/dl)	126 (106-162)	142 (112-191)	170 (130-215)	0.016*
Hba1c (%)	5.7 (5.5-6.0)	6.1 (5.7-6.8)	6.2 (5.8-6.6)	0.015*
Systolic function				
Ejection fraction (%)	64 (61-69)	65 (61-70)	70 (63-74)	0.028 [†]
Smax (cm/s)	6.25 (5.65-6.75)	5.90 (5.05-7.15)	6.00 (5.30-6.80)	0.640
GLS (-,%)	20.3 (21.6-18.2)	19.4 (21.8-16.6)	18.4 (21.2-16.4)	0.205
LV geometry				
LVEDD (mm)	45 (43-48)	42 (39-47)	45 (42-51)	0.418
LVMi (g/m ²)	79 (63-93)	81 (69-107)	104 (80-135)	0.003 [†]
RWT	0.45 (0.40-0.48)	0.51 (0.46-0.67)	0.55 (0.44-0.63)	0.016 [†]
Diastolic function				
LA- Index (ml/m ²)	25 (23-28)	29 (27-31)	42 (37-51)	<0.001 [†]
E (cm/s)	60 (60-75)	60 (50-70)	80 (70-100)	0.001 [†]
A (cm/s)	60 (50-70)	80 (70-90)	80 (70-90)	0.014 [†]
E/A ratio	1.2 (0.9-1.4)	0.8 (0.7-0.9)	1.1 (0.9-1.3)	0.148
E' septal (cm/s)	8.4 (7.6-9.3)	5.8 (5.1-6.7)	5.6 (4.4-6.2)	0.001 [†]
E' lateral (cm/s)	11.1 (10.4-13.0)	7.9 (7.0-9.0)	7.0 (5.4-8.2)	0.001 [†]
Average E'	9.7 (9.1-11.0)	7.1 (6.0-7.9)	6.3 (5.2-7.3)	0.001 [†]
E/E' septal ratio	7.9 (7.2-8.6)	10.4 (8.5-12.2)	15.0 (12.3-17.2)	0.001 [†]
E/E' average ratio	6.7 (5.9-7.4)	8.8 (7.4-10.3)	13.2 (10.9-15.4)	0.001 [†]

* Statistically significant ($p < 0.05$). Jonckheere-Terpstra test. A - late diastolic transmitral inflow velocity, EF - ejection fraction. Smax - systolic velocity, DF - diastolic function, GPS - global longitudinal strain, LA - left atrial, E - early diastolic transmitral inflow velocity, E' septal - early diastolic tissue Doppler velocity septal, E' lateral - early diastolic tissue Doppler velocity lateral, HFnEF - heart failure with normal ejection fraction, HOMA-IR - Homeostasis Model Assessment of Insulin Resistance, hsCRP - high sensitive c-reactive protein, LVEDD - left ventricular end-diastolic diameter, Lp (a) - Lipoprotein (a), LV - left ventricular, LVMi - left ventricular muscle mass index, NT-pro BNP - amino-terminal pro-B-type natriuretic peptide, PG - postprandial glucose, RWT - relative wall thickness, Smax - systolic velocity.

and higher levels are associated with adverse cardiovascular outcomes. Accordingly, higher levels of GDF-15 in patients with more advanced stages of LVDD (HFnEF, LVDD grad II or III) may reflect an adaptive response that is outweighed by the severity of the underlying disease. As GDF-15 predicts cardiac mortality in patients with CAD²⁸, acute myocardial infarction²⁹ or SHF¹³, GDF-15 might be a future biomarker in patients with HFnEF, probably indicating prognosis.

According to the current guidelines, patients can be classified as having HFnEF or LVDD without the need for objective evidence of hemodynamic limitation in exercise tolerance as a result of increasing preload or afterload conditions. Incoherent in the current diagnostic approach is that leading symptoms of patients with HFnEF are related to physical activity³⁰, whereas ventricular performance obtained at rest does not accurately reflect exercise tolerance and

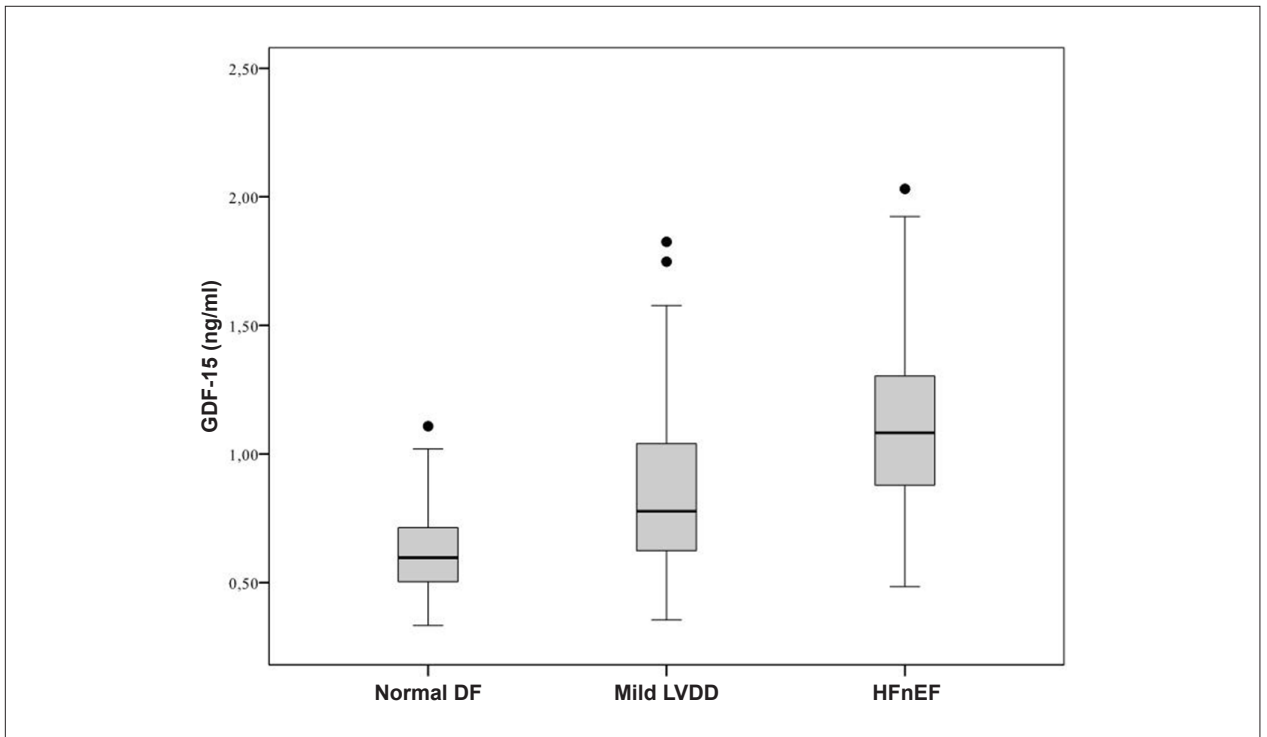


Figure 1 - Growth differentiation factor-15 levels in patients with normal diastolic function, mild diastolic dysfunction and heart failure with normal ejection fraction. GDF-15 levels are presented as box (25th percentile, median, 75th percentile), and whiskers plots and upper outliers are presented as black dots. DF - diastolic function, GDF-15 - growth differentiation factor-15, LVDD - left ventricular diastolic dysfunction.

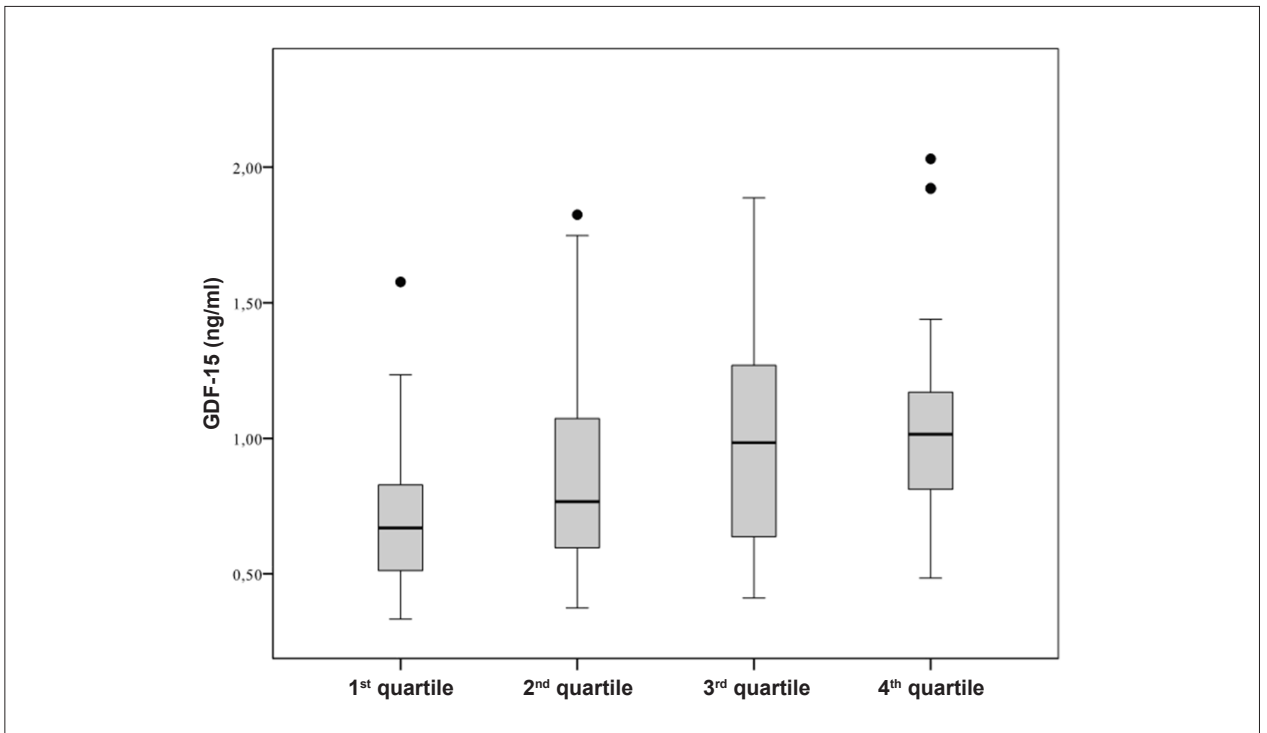


Figure 2a - Growth differentiation factor-15 levels stratified according to left atrial volume index quartiles. Growth differentiation factor 15 (GDF-15) levels are presented as box (25th percentile, median, 75th percentile), and whiskers plots and upper outliers are presented as black dots. GDF-15 - growth differentiation factor 15. The left atrial volume index in the first quartile ranged from 19.3 - 26.7 ml/m² body surface area (BSA), in the second quartile from 26.7 - 30.2 ml/m², in the third quartile from 30.2 - 37.5 ml/m² and in the fourth quartile from 37.2 - 82.0 ml/m² ($p < 0.001$ in the Jonckheere-Terpstra-Test).

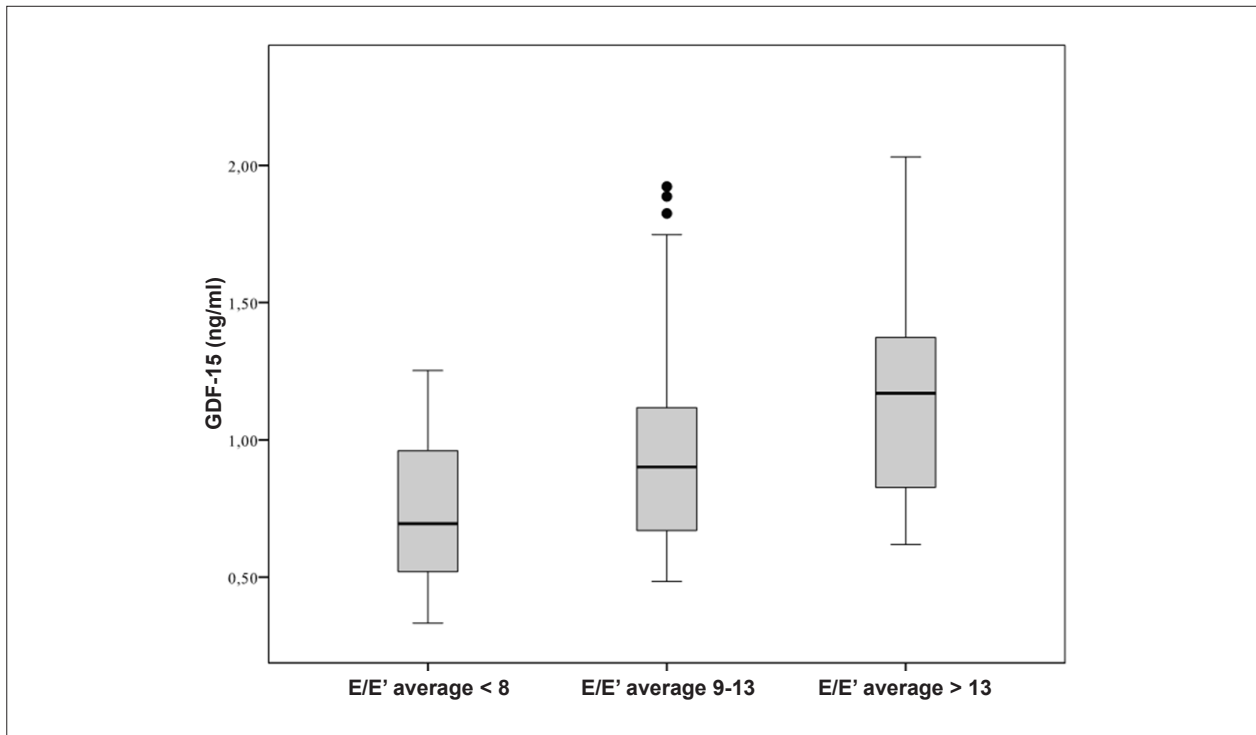


Figure 2b - Growth differentiation factor-15 levels plotted against the E/E' average ratio. GDF-15 levels are presented as box (25th percentile, median, 75th percentile), and whiskers plots and upper outliers are presented as black dots ($p < 0.001$ for trend by Jonckheere-Tepstra). GDF-15 - growth differentiation factor-15.

Table 3 - Parameters of cardiac assessment according to GDF-15 levels

	GDF-15 1 st quartile	GDF-15 2 nd quartile	GDF-15 3 rd quartile	GDF-15 4 th quartile	p-value ^a
Systolic function					
Ejection fraction (%)	65 (62-70)	63 (59-68)	65 (61-72)	70 (64-73)	0.021*
Smax (cm/s)	6.60 (5.70-7.15)	5.90 (5.30-7.00)	5.65 (5.00-6.50)	6.10 (5.10-6.90)	0.283
GLS (-,%)	19.0 (21.3-16.9)	18.9 (21.4-16.6)	18.9 (20.5-17.5)	19.0 (22.0-17.1)	0.956
LV geometry					
LVEDD (mm)	43 (41-47)	45 (41-49)	44 (39-50)	44 (41-48)	0.851
LVMi (g/m ²)	79 (64-96)	96 (72-108)	83.1 (71-127)	92 (67-117)	0.326
RWT	0.50 (0.40-0.60)	0.49 (0.44-0.62)	0.54 (0.45-0.63)	0.51 (0.45-0.69)	0.145
Diastolic function					
LA- Index (ml/m ²)	27 (25-30)	29 (24-34)	34 (28-40)	36 (30-46)	<0.001*
E (cm/s)	60 (60-70)	70 (60-70)	60 (50-70)	70 (60-90)	0.047*
A (cm/s)	70 (60-80)	80 (70-90)	80 (70-90)	80 (65-95)	0.129
E/A ratio	0.9 (0.8-0.9)	0.9 (0.8-1.0)	0.8 (0.7-1.2)	0.9 (0.7-1.2)	0.978
E' septal (cm/s)	6.8 (5.3-7.9)	6.1 (5.3-7.6)	5.8 (4.8-6.5)	5.7 (4.1-6.2)	0.002*
E' lateral (cm/s)	9.9 (8.2-10.9)	8.2 (6.9-10.5)	7.6 (7.0-8.6)	7.3 (5.6-8.8)	<0.001*
Average E'	8.3 (7.0-9.6)	7.3 (6.1-8.7)	6.8 (6.0-7.7)	6.3 (5.0-7.3)	<0.001*
E/E' septal ratio	8.8 (8.0-11.4)	10.5 (8.5-12.7)	11.4 (8.3-14.1)	14.5 (11.5-17.1)	<0.001*
E/E' average ratio	7.4 (6.7-9.3)	9.0 (7.4-10.5)	9.4 (7.5-11.3)	12.1 (10.1-15.4)	<0.001*
Laboratory					
NT-pro BNP (pg/ml)	98 (64-189)	120 (90-253)	183 (65-502)	406 (117-773)	<0.001*

* Statistically significant ($p < 0.05$). ^aJonckheere-Terpstra test. A - late diastolic transmitral inflow velocity, EF - ejection fraction. GLS - global longitudinal strain, LA - left atrial, E - early diastolic transmitral inflow velocity, E' septal - early diastolic tissue Doppler velocity septal, E' lateral - early diastolic tissue Doppler velocity lateral, LV - left ventricular, LVEDD - left ventricular end diastolic diameter, LVM - left ventricular muscle mass index, NT-pro BNP - amino-terminal pro-B-type natriuretic peptide, RWT - relative wall thickness. Smax - systolic velocity. The growth differentiation factor-15 (GDF-15) levels in the first quartile ranged from 0.37 - 0.93ng/ml, in the second quartile from 0.93 - 1.28 ng/ml, in the third quartile from 1.28 - 2.02 ng/ml and in the fourth quartile from 2.02 - 3.60 ng/ml ($p = 0.005$ in the Jonckheere-Terpstra-Test).

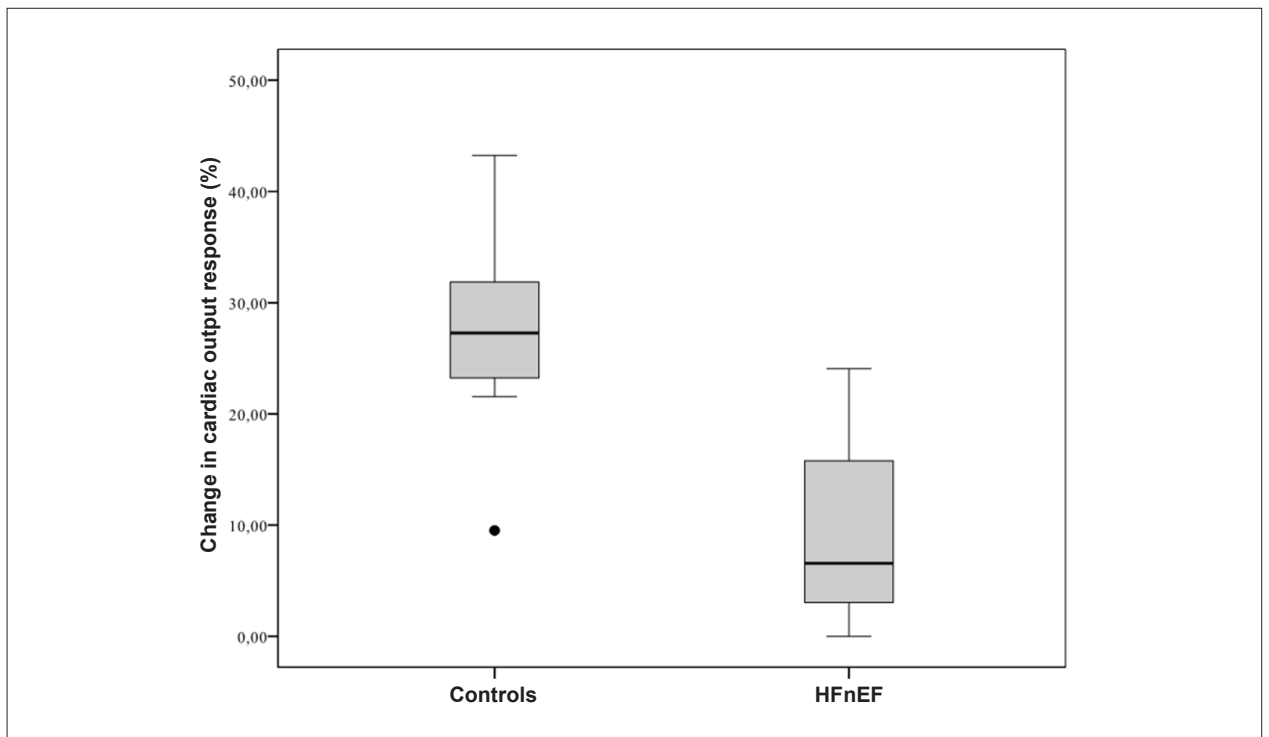


Figure 3a - Change in cardiac output response from upright to supine position. The y-axis shows the change in the cardiac output index by changes in body position from upright to supine measured by inert gas rebreathing techniques. Cardiac output index is presented as box (25th percentile, median, 75th percentile), and whiskers plots, upper outliers are presented as black dots. HFnEF - heart failure with normal ejection fraction.

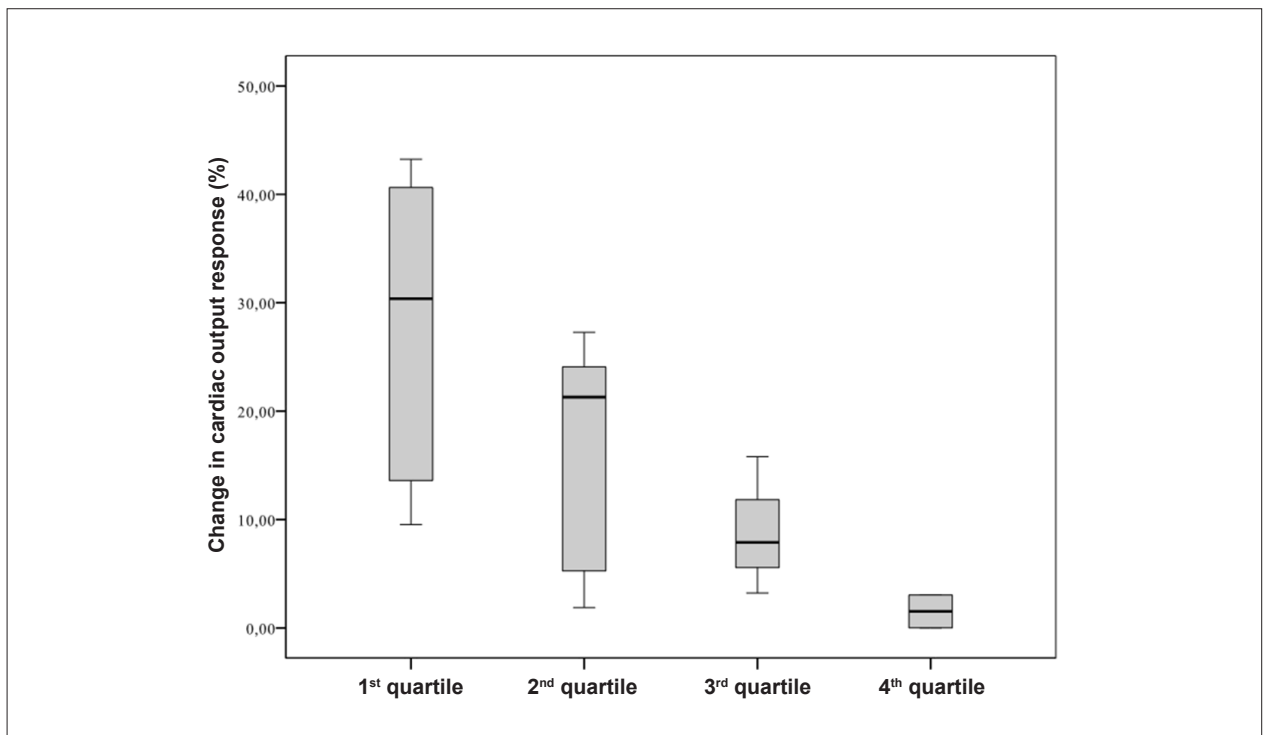


Figure 3b - Change in cardiac output response from upright to supine position stratified according to growth differentiation factor-15 quartiles. The y-axis shows the change in the cardiac output index caused by changes in body position from upright to supine measured non-invasively by inert gas rebreathing techniques. Cardiac output index is presented as box (25th percentile, median, 75th percentile), and whiskers plots, upper outliers are presented as black dots. The growth differentiation factor-15 (GDF-15) levels in the first quartile ranged from 0.33 - 0.83ng/ml, in the second quartile from 0.83 - 1.13 ng/ml, in the third quartile from 1.13 - 1.54 ng/ml and in the fourth quartile from 1.54 - 2.03 ng/ml ($p = 0.005$ in the Jonckheere-Terpstra-Test).

symptomatic status of patients with heart failure³¹. Consistent with the fact that GDF-15 expression is activated in response to stress²⁷, our data showed a significant negative correlation between the magnitude of COi response to the changes in body position from upright to supine, which can be considered a model of preload change. The association of a reduced cardiac output response to preload stress and increased GDF-15 levels confirmed the hypothesis that the adaptive response of GDF-expression is countermanded by the severity of the underlying disease. This is further reinforced particularly by the correlation of GDF-15 and the LAi, a parameter that indicates severe diastolic function abnormalities over a longer period of time. An increased LAi without concomitant mitral valve disease reflects an enduring cumulative effect of different contributors to the development of LVDD and is therefore less vulnerable to intermittent changes in preload (e.g. volume status) or afterload (e.g. blood pressure). Acute changes in these conditions during echocardiographic diagnosis might lead to misclassifications of diastolic function abnormalities.

The finding of an association between GDF-15 and LVDD is in line with a recent study of Stahrenberg et al³² that assessed the relationship in a community based trial. Nevertheless, there are some concerns about methodological issues that might have influenced the results presented in this interesting study. Most important, there was no general screening for the presence of CAD with either noninvasive stress testing or coronary angiography. Since CAD has been shown to be associated both with elevated GDF-15 levels^{28,33} and LVDD³⁴, the lack of information on coronary morphology is a potential source of bias towards increased plasma levels of GDF-15 and toward an increased prevalence and/or severity of LVDD. In accordance with the uncertainties about the presence of CAD, the E/E' ratio was not averaged based on medial and lateral velocities, as recommended by the current guidelines¹⁰. Because some of the patients likely had regional wall motion abnormalities, tethering effects might have been a possible source of bias. Moreover, patients were not screened with an oral glucose tolerance test for undetected diabetes, which have been associated both with increased GDF-15 levels^{35,36} and the presence and severity of LVDD, especially in subjects with newly detected diabetes on oGTT³⁷.

In contrast, the present study followed the current guidelines for the diagnosis of LVDD in subjects well characterized for the degree of abnormality in plasma glucose levels and coronary morphology. Therefore, we were able to

reinforce the data of Stahrenberg et al³². in a different cohort of patients with known coronary morphology and metabolic status. Furthermore, we extended the findings to a functional hemodynamic parameter, as we were able to demonstrate an association of GDF-15 levels with an impairment of the hemodynamic response to different preload conditions.

Limitations

The number of patients, especially with normal DF in our cohort, was relatively small, resulting in a limited statistical power. Furthermore, the rates of CAD and cardiovascular risk factors were high in this study population. Therefore, the present results may not readily represent the general population. In addition, patients with HF_{NEF} were older than those with normal diastolic function, a potential source of bias towards increased plasma levels of GDF-15. Nevertheless, association between LVDD and GDF-15 remains significant after adjustment for CAD, age, glucose metabolism and hypertension as covariates into multivariate regression models. Although we based the diagnosis of LVDD on current guidelines which have recently been published, their clinical value has yet to be prospectively validated. Furthermore, the rate of patients on antihypertensive treatment was higher in the HF_{NEF} group. Nevertheless, a significant relation of any of these drugs to the increased GDF-15 plasma levels has never been identified. Lastly, our cross sectional study design does not permit any conclusions on causality.

Conclusion

GDF-15 is a novel promising biomarker in HF_{NEF} that is elevated in subjects with either mild or moderate to severe LVDD regardless of the presence of CAD or other established risk factors frequently associated with HF_{NEF}. Considering the fact that GDF-15 appears to have cardioprotective functions, we hypothesize that higher levels of GDF-15 may reflect an adaptive response that is outweighed by the severity of the underlying disease. Incorporating a biomarker to aid in diagnosis, and risk prediction in HF_{NEF} may enhance the ability to accurately and early identify patients at greatest risk for heart failure-related complications. Better characterization of patients with HF_{NEF} could allow a more effective use of specific therapeutic and prevention strategies. Further studies are needed to determine the value of GDF-15 for diagnosis and therapy monitoring in diastolic heart failure.

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