European Journal of Heart Failure (2010) **12**, 1309–1316
doi:10.1093/eurjhf/hfq151

The novel biomarker growth differentiation factor 15 in heart failure with normal ejection fraction

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Received 12 April 2010; revised 6 June 2010; accepted 11 June 2010; online publish-ahead-of-print 13 September 2010

Aims Heart failure with normal ejection fraction (HFnEF) is an important clinical entity that remains incompletely understood. The novel biomarker growth differentiation factor 15 (GDF-15) is elevated in systolic heart failure (HFrEF) and is predictive of an adverse outcome. We investigated the clinical relevance of GDF-15 plasma levels in HFnEF.

Methods and results A subgroup of patients from the ongoing DIAST-CHF observational trial, with a history of chronic heart failure (CHF) or positive Framingham criteria at presentation, was selected. Patients were classified as having either HFrEF ($n = 86$) or HFnEF ($n = 142$) and compared with healthy elderly controls ($n = 188$) from the same cohort. Growth differentiation factor 15 levels in HFnEF were significantly higher than in controls and similar to those in HFrEF. In multivariate analysis, factors significantly associated with GDF-15 levels were age, sex, estimated glomerular filtration rate (eGFR), presence of HFrEF and HFnEF. Growth differentiation factor 15 correlated with multiple echocardiographic markers of diastolic function and was associated with 6 min walk test performance and SF-36 physical score on multivariate analysis in all patients. When using a classification for HFnEF that did not employ N-terminal pro brain natriuretic peptide (NT-proBNP) as a diagnostic criterion, the diagnostic properties of GDF-15 for detecting HFnEF tended to be superior to those of NT-proBNP, and a combination significantly improved diagnostic accuracy.

Conclusion Growth differentiation factor 15 is elevated in HFnEF to a similar degree as in HFrEF. It is independently associated with impairment in exercise capacity and in physical components of quality of life. Diagnostic precision of GDF-15 is at least as good as that of NT-proBNP and combining both markers improves diagnostic accuracy.

Keywords Heart failure • Diastolic heart failure • Biological markers • Growth differentiation factor 15 • Diagnosis • Cross-sectional studies

Introduction

Chronic heart failure (CHF) is an ongoing epidemic of growing dimensions.[1] Around 50% of patients with the clinical syndrome of heart failure have a normal left ventricular ejection fraction (LVEF).[2,3] These cases are termed 'heart failure with normal ejection fraction' (or HFnEF) and left ventricular diastolic dysfunction is believed to be the prominent aetiology.[4] Once hospitalized for heart failure, the prognosis of HFnEF is as grim as systolic heart failure (HFrEF).[2,3] Randomized trials in the search for specific

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therapeutic interventions have been few and notoriously unsuccessful.[5,6]

Biomarkers are increasingly used in CHF for diagnostic and prognostic purposes. Some markers, e.g. natriuretic peptides, have been introduced into clinical practice, but a growing number of novel markers is under investigation.[7] Growth differentiation factor 15 is a distant member of the TGF-ß superfamily. While it is not expressed in normal hearts, increased expression of GDF-15 has been demonstrated in animal models of dilative and hypertrophic cardiomyopathy, in load-induced cardiac hypertrophy[8] and in infarcted myocardium.[9] In these models, GDF-15 attenuated a reduction in fractional shortening[8] and protected the heart from cardiac hypertrophy[8] and ischaemia–reperfusion injury.[9] It has been suggested to be a downstream marker indicative of different myocardial stress pathways.[10] Promising data have led to a proposition to use GDF-15 for risk stratification in acute coronary syndromes[11,13] or pulmonary embolism.[14] Growth differentiation factor 15 is elevated and indicative of prognosis in HFrEF.[10,15]

The aim of our study was to evaluate the clinical relevance of GDF-15 plasma levels in an HFnEF population. We compared this population with HFrEF patients and healthy elderly controls from the same cohort.

Methods

Subjects

In the ongoing non-interventional DIAST-CHF trial, which is part of the nationwide German Competence Network Heart Failure, 1935 participants aged 50–85 years were recruited in 2004 and 2005 with at least one risk factor for HFnEF (defined as history of hypertension, diabetes mellitus, sleep apnoea syndrome or atherosclerotic disease) or established CHF. Participants were referred by a network of primary care physicians and inclusion criteria verified at the screening visit. As a population-based trial, the only exclusion criteria were unwillingness to participate or inability for logistic reasons. Participants underwent a comprehensive non-invasive diagnostic workup at baseline, including echocardiography. Diagnosis of CHF was made either based on the history or on the presence of at least one major and two minor Framingham diagnostic criteria[16] at presentation. Patients were classified retrospectively according to echocardiographic measurements to have HFrEF when LVEF was $\leq$50%, or HFnEF when LVEF was $>$50% and diagnostic criteria for HFnEF as recommended by the European Society of Cardiology[4] were met. A group of apparently healthy elderly subjects were included in DIAST-CHF as a reference group and followed the same procedures as the main cohort. These subjects were used as controls for comparative purposes and for assessing discriminatory properties of GDF-15 to detect HFnEF. As it turned out that GDF-15 might be a valuable marker for the presence of HFnEF, we used a second classification for HFnEF that did not employ N-terminal pro brain natriuretic peptide (NT-proBNP) as a diagnostic criterion for comparative purposes: all CHF patients with an LVEF $>$50% that fulfilled echocardiographic criteria indicative of elevated filling pressures as recommended by the American Society of Echocardiography (ASE)[17] were classified as $HFnEF_{New}$. For clarity of presentation, we termed the conventionally classified group $HFnEF_{ESC}$. While individual echocardiographic parameters used for $HFnEF_{ESC}$ and $HFnEF_{New}$ classification had been prospectively assessed according to the study protocol at baseline except for retrograde A wave duration in the pulmonary veins, final classification was performed retrospectively for the purpose of this study.

DIAST-CHF complies with the Declaration of Helsinki, the protocol was approved by the responsible ethics committee and all patients gave written informed consent.

Procedures

All participants had their medical history, physical examination and an ECG taken. In addition, a standardized 6-min walk test[18] was performed. As a general estimate of quality of life, the widely used and validated SF-36[19] was used. Blood samples were drawn after at least 15 min rest in the prone position 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® analyser (all Roche Diagnostics GmbH, Mannheim, Germany) by an investigator blinded to patient characteristics.

Echocardiography was performed on a Hewlett-Packard Sonos 5500 (Hewlett-Packard, Andover, MA, USA) according to the guidelines of the ASE current at the time of data collection, including measurement of peak velocities of early (*E*) and late (*A*) diastolic mitral inflow and early (*é*) and late (*á*) tissue Doppler velocity waves at the lateral mitral annulus, E wave deceleration time and peak systolic (*S*) and diastolic (*D*) pulmonary vein flow velocity. All examinations were performed by physicians experienced in the technique. All individual parameters used for the classification and grading of diastolic dysfunction were obtained successfully in $>$95% of patients. Randomly chosen echo examinations were reviewed by the echo core laboratory of the German Competence Network Heart Failure at the University of Essen for quality assurance.

Calculations and statistical analyses

Left ventricular mass index (LVMI) was calculated by the Devereux formula[20] indexed to body surface area. Left atrial volume index (LAVI) was calculated by the ellipsoid model.[21] For evaluation of diastolic function, we calculated the ratios *E*/*A*, *E*/*é*, *é*/*á*, and *S*/*D*. Diastolic dysfunction was graded as follows: normal diastolic function (1 $\leq$*E*/*A*, *E*/*é* $<$10, S/D $\geq$1, *E*/*A* with Valsalva manoeuvre $\geq$1), mild diastolic dysfunction (*E*/*A* $<$1), moderate diastolic dysfunction (1$\leq$ *E*/*A* $<$2 and one of the following: E/é $\geq$10, S/D $<$1, *E*/*A* Valsalva $<$1), and severe diastolic dysfunction (*E*/*A* $\geq$2 and one of the following: *E*/*é* $\geq$10, S/D $<$1).

Descriptive statistics were performed stratified by group. Data are expressed as median (interquartile range) for continuous variables. Absolute numbers (percentage) are given for categorical variables. Log-transformed values were used for some analyses as appropriate. For non-parametric tests of differences between groups we used the Kruskal–Wallis test for continuous variables and χ^2 test for categorical variables. Growth differentiation factor 15 levels were compared across different grades of diastolic dysfunction by the non-parametric Jonckheere–Terpstra test. To investigate relations between traits, bivariate Pearsons correlation coefficients were calculated. General linear models with conditional inclusion of candidate variables were used to unravel multivariate relationships. Receiver operating characteristic (ROC) curves were constructed for discrimination between controls and subjects with $HFnEF_{ESC}$ or $HFnEF_{New}$ and sensitivity, specificity, and odds ratios HFnEF were calculated. The areas under the curve (AUC) were compared by the method of Hanley and McNeil.[22] *P*-values $<$0.05 were considered significant.

Statistical analyses were performed with PASW Statistics 18.0 software.

Results

Of all patients with the clinical diagnosis of CHF, 86 had HFrEF and 142 $HFnEF_{ESC}$ while 115 had an LVEF >50% but did not meet ESC criteria for HFnEF. One hundred and eighty eight subjects were included as healthy controls. Characteristics of the cohort are shown in *Table 1*. Patients with HFrEF and $HFnEF_{ESC}$ were comparable with regard to age, NT-proBNP and 6 min walk distance. Patients with both HFrEF and $HFnEF_{ESC}$ were older than controls.

Growth differentiation factor 15 was significantly elevated in $HFnEF_{ESC}$ as compared with controls [1.66 (1.26; 2.34) vs. 0.90 (0.74; 1.09) ng/mL, $P < 0.001$] and was not significantly different from HFrEF [1.81 (1.37; 2.65) ng/mL] (*Figure 1*). 79.5% and 79.7% of patients in HFrEF and $HFnEF_{ESC}$, respectively, had values >1.20 ng/mL, which is the reported upper limit of normal,[15] while 14% of controls had values >1.20 ng/mL.

In the whole study sample, higher log(GDF-15) levels were strongly associated with higher age, lower estimated glomerular filtration rate (eGFR), higher NT-proBNP, shorter 6 min walk distance and lower SF-36 physical score, but also moderately with several echocardiographic parameters indicative of systolic and diastolic function (see Supplementary material online, Table S1). Prominent among correlations with echocardiographic parameters of diastolic function were those with increased LAVI, LVMI, *E*/é, decreased é and é/á. Jonckheere–Terpstra test unravelled significantly increasing GDF-15 levels across grades of diastolic dysfunction ($P < 0.001$, *Figure 2*). As differing algorithms for grading the severity of diastolic dysfunction have been used over the past years and guidelines published in the meantime stress the relevance of *E*/*é* values (with cut-offs that deviate from the one we used for our grading scheme), *Figure 3* additionally shows significantly increasing GDF-15 levels across three strata of *E*/é. Finally, we observed a strong association with estimated right ventricular systolic pressure.

When excluding patients with HFrEF, most correlations remained significant and retained their respective strength of association. Interestingly, however, the associations with parameters indicative of systolic function, i.e. LVEF and left ventricular end-systolic and end-diastolic volume, were not significantly associated with GDF-15 levels after exclusion of HFrEF. In contrast, the association with *E*/*é* appeared to be of a more continuous nature (*Figure 4*).

Table 1 Clinical characteristics

	$HFnEF_{ESC}$	HFrEF	Controls	*P*-value*
Age (years)	73 (66; 78)	71 (66; 75)	56 (52; 63)#	<0.0005
Female gender	91 (64)	15 (17)#	124 (66)	<0.0005
BMI (kg/m^2)	30.1 (26.7; 34.1)	29.1 (26.1; 32.7)	25.3 (22.9; 28.4)#	<0.0005
Systolic blood pressure (mmHg)	147 (130; 164)	138 (122; 150)#	127 (119; 137)#	<0.0005
Diastolic blood pressure (mmHg)	80 (70; 90)	80 (70; 85)	78 (71; 85)	0.447
Heart rate (L/min)	66 (61; 74)	69 (62; 76)	73 (65; 79)#	<0.0005
6 min walk distance (m)	431 (346; 500)	463 (400; 532)	584 (560; 604)#	<0.0005
SF-36 physical function	50 (25; 70)	65 (35; 80)#	90 (83; 100)#	<0.0005
Diabetes mellitus (%)	43 (30)	32 (37)	0 (0)#	<0.0005
Hypertension (%)	132 (93)	78 (91)	1 (1)#	<0.0005
Hyperlipidaemia (%)	75 (53)	47 (55)	0(0)#	<0.0005
Coronary artery disease (%)	49 (35)	45 (52)#	0 (0)#	<0.0005
Atrial fibrillation (%)	35 (25)	23 (27)	1 (1)#	<0.0005
ACE inhibitor (%)	69 (49)	58 (67)#	1 (1)#	<0.0005
AT1 receptor blocker (%)	41 (29)	16 (19)	0 (0)#	<0.0005
Aldosterone antagonists (%)	7 (5)	12 (14)#	0 (0)#	<0.0005
β-Blocker (%)	87 (61)	64 (74)	1 (1)#	<0.0005
Thiazide diuretic (%)	69 (49)	35 (41)	2 (1)#	<0.0005
Loop diuretic (%)	51 (36)	41 (48)	0 (0)#	<0.0005
Digitalis glycoside (%)	21 (15)	21 (24)	0 (0)#	<0.0005
Statin (%)	57 (40)	41 (48)	0 (0)#	<0.0005
Acetyl salicylic acid (%)	58 (41)	43 (50)	4 (2)#	<0.0005
GDF-15 (ng/mL)	1.66 (1.26; 2.34)	1.81 (1.37; 2.65)	0.90 (0.7; 1.09)#	<0.0005
NT-proBNP (ng/L)	326 (133; 634)	422 (148; 912)	63.9 (39.2; 112.0)#	<0.0005
Estimated GFR ($mL/min/1.73\ m^2$)	60 (49; 70)	61 (50; 76)	80 (71; 93)#	<0.0005
LVEF (%)	60 (56; 65)	45 (36; 48)#	61 (56; 66)	<0.0005
E/*é*	11.6 (9.2; 14.5)	10.4 (7.6; 13.3)	6.9 (5.9; 8.5)#	<0.0005

*P-value for difference across all groups by Kruskal–Wallis or χ^2 test, as appropriate.
#$P < 0.05$ vs. $HFnEF_{ESC}$ by Bonferroni-adjusted Mann–Whitney *U* or χ^2 test, as appropriate.

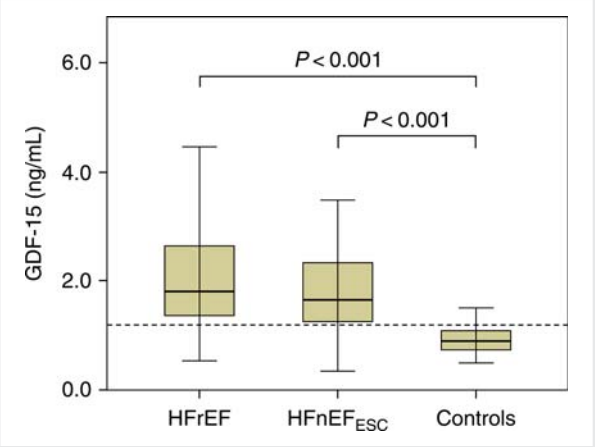

Figure 1 Growth differentiation factor 15 levels in systolic heart failure, HFnEF$_{ESC}$ and controls. Broken line denotes recommended cut-off of 1.20 ng/mL.

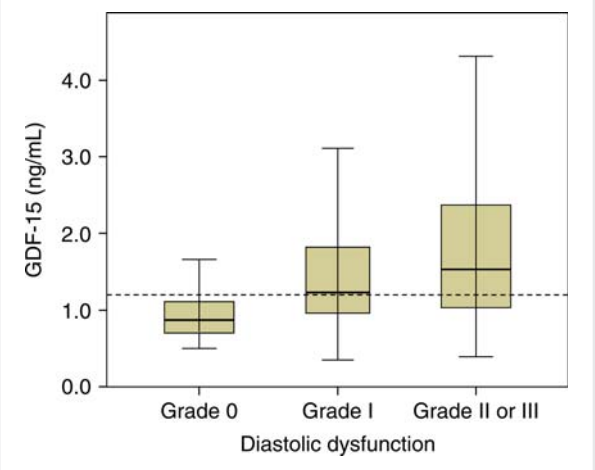

Figure 2 Growth differentiation factor 15 levels across grades of diastolic dysfunction. Grades 2 and 3 were grouped, as only very few subjects had Grade 3 diastolic dysfunction, $P < 0.001$ for trend by Jonckheere–Terpstra. Broken line denotes recommended cut-off of 1.20 ng/mL.

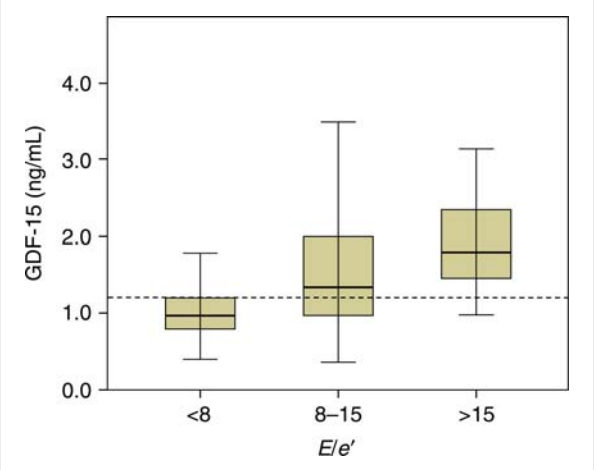

Figure 3 Growth differentiation factor 15 levels across three strata of *E/é*, $P < 0.001$ for trend by Jonckheere–Terpstra. Broken line denotes recommended cut-off of 1.20 ng/mL.

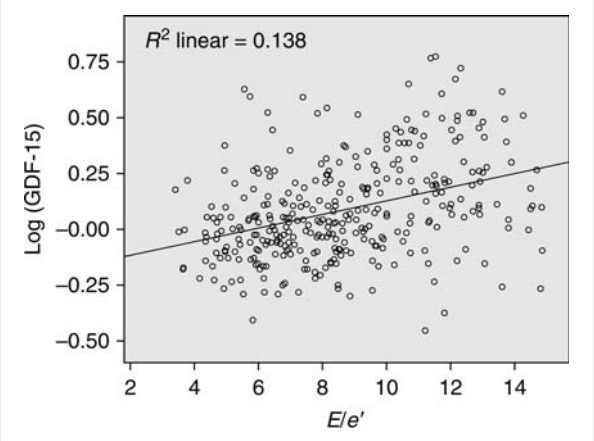

Figure 4 Scatterplot for *E/é* and log (growth differentiation factor 15) showing a moderately strong linear correlation within the normal to high-normal range of *E/é*.

In a multivariate general linear model with log(GDF-15) as dependent variable, higher age, male gender, lower eGFR, presence of HFrEF and presence of HFnEF were identified as variables significantly predictive of higher GDF-15 levels, while body mass index (BMI), systolic blood pressure, LVEF, *E/é*, LAVI, LVMI index and presence of coronary artery disease (CAD) did not add significantly to the model (adjusted $r^2 = 0.577$ for overall model).

Because GDF-15 appeared to be strongly associated with 6 min walk distance and SF-36 physical score, we used these parameters as the respective dependent variable in general linear models. Including BMI, age, gender, eGFR, CAD, HFrEF, HFnEF, *E/é*, LAVI, LVMI, LVEF, and the common logarithms of GDF-15 and NT-proBNP as covariates, GDF-15 remained significantly ($P = 0.01$) and inversely associated with 6 min walk distance (adjusted $r^2 = 0.524$ for overall model). Similarly, the inverse association of GDF-15 with SF-36 physical score just barely missed significance ($P = 0.052$) in a multivariate model with the same covariates (adjusted $r^2 = 0.281$ for overall model). Surprisingly, NT-proBNP as a covariate did not reach significance in these models.

As NT-proBNP is considered a valuable biomarker for the diagnosis of HFnEF$_{ESC}$,[4] we compared GDF-15 with NT-proBNP for the discrimination of HFnEF$_{ESC}$ from healthy controls. ROC curves for both markers were very similar (*Figure 5A*) with an AUC of 0.882 (0.842; 0.922) for NT-proBNP and 0.891 (0.850; 0.932) for GDF-15 ($P = 0.37$), while the combination of both markers exhibited a significantly larger AUC of 0.942 (0.912; 0.972) compared with NT-proBNP ($P < 0.01$) or GDF-15 ($P < 0.05$) alone. For cut-off levels of 1.16 ng/mL (GDF-15) and 199 ng/L (NT-proBNP), the respective sum of sensitivity and specificity was maximal. A cut-off value of 1.20 ng/mL has been

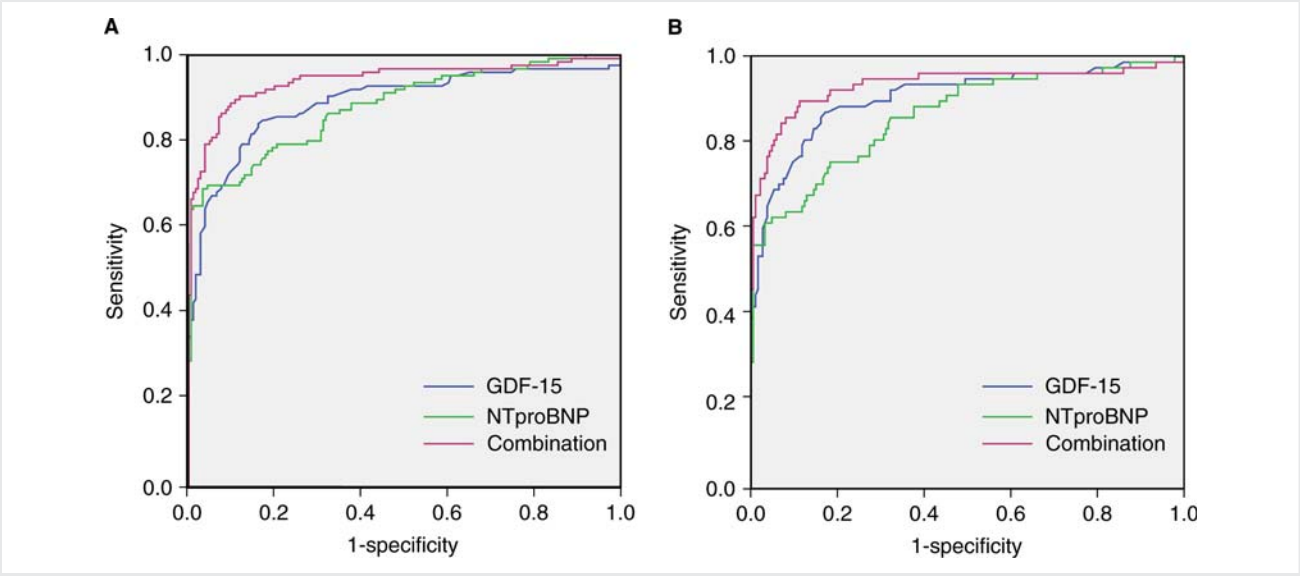

Figure 5 ROC curves for growth differentiation factor 15, NT-proBNP or their combination for discrimination of (*A*) $HFnEF_{ESC}$ from controls or (*B*) $HFnEF_{New}$ from controls.

proposed for GDF-15 for a diagnosis of HFrEF.[15] At this value, sensitivity reached 81.7% and specificity 85.5% with an odds ratio (OR) of 18.1 for having $HFnEF_{ESC}$. Sinilarly, an NT-proBNP >220 ng/L is recommended for a positive diagnosis of $HFnEF_{ESC}$,[4] which gives a sensitivity of 65.1%, a specificity of 96.8% and an OR of 46, respectively.

Because the utilization of NT-proBNP in the ESC algorithm for the diagnosis of $HFnEF_{ESC}$ would be expected to give this marker an advantage over GDF-15, we used a different scheme to classify subjects as $HFnEF_{New,}$ which did not employ NT-proBNP (see the section Methods). Eighty-five subjects had $HFnEF_{New}$. Overall, 49.3% of $HFnEF_{ESC}$ subjects also fulfilled the $HFnEF_{New}$ criteria, while 82.4% of $HFnEF_{New}$ subjects had $HFnEF_{ESC.}$ Six minute walk distance was 418 m (332 m; 504 m) in $HFnEF_{New}$. These figures are consistent with the notion that echocardiographic criteria for $HFnEF_{New}$ indicate elevated filling pressures at the time of echocardiography and may therefore select a more severely affected subgroup of HFnEF patients.

Figure 5B illustrates that the AUC of GDF-15 for detecting $HFnEF_{New}$ was clearly higher than for NT-proBNP [0.904 (0.857; 0.951) vs. 0.859 (0.805; 0.913)], although this difference did not reach statistical significance ($P = 0.10$). The combination of both markers resulted in an AUC of 0.935 (0.892; 0.977), which was significantly higher than for NT-proBNP alone ($P < 0.01$) but not for GDF-15 alone ($P = 0.12$). Optimal cut-off levels in the above sense for the individual markers were 1.16 ng/mL for GDF-15 and 200.7 ng/L for NT-proBNP. The combined presence of a GDF-15 >1.16 ng/mL and NT-proBNP >200.7 ng/L resulted in a sensitivity of 56.6% and a specificity of 98.9% for detecting $HFnEF_{New}$. For the proposed cut-off values of 220 and 120 ng/L for NT-proBNP, we identified analogous values for GDF-15 with equal sensitivity (specificity) and compared the corresponding specificity (or sensitivity, respectively) at these values. Classification at the identified cut-offs was better for GDF-15 than for NT-proBNP (*Table 2*).

Table 2 Comparative diagnostic properties of growth differentiation factor 15 and NT-proBNP for $HFnEF_{New}$

	NT-proBNP	GDF-15
Specificity fixed		
Cut-off value	220 ng/L[a]	1.51 ng/mL
Sensitivity (%)	55	61
Specificity (%)	97	97
Precision (%)	84	86
Odds ratio	36.7	47.8
Sensitivity fixed		
Cut-off value	120 ng/L[a]	1.28 ng/mL
Sensitivity (%)	74	74
Specificity (%)	80	90
Precision (%)	78	86
Odds ratio	10.9	27.6

[a]Recommended by ESC guidelines[4] to rule in or out $HFnEF_{ESC}$.

Discussion

Our study has the following three main findings.

(i) Growth differentiation factor 15 plasma levels are elevated in $HFnEF_{ESC}$ patients and comparable to HFrEF.
(ii) Growth differentiation factor 15 levels correlate with multiple echocardiographic markers of diastolic function and are independently associated with a worse 6 min walk test performance as well as a lower SF-36 physical score.
(iii) Growth differentiation factor 15 is at least as good as NT-proBNP for the detection of HFnEF and the combination of both markers is better than NT-proBNP alone.

The absolute values of GDF-15 in our cohort are in accordance with the published data: GDF-15 concentrations in HFrEF are significantly higher than in healthy elderly controls and the proportion of patients above the proposed cut-off of 1.20 ng/mL as well as the median value are comparable to data reported by Kempf *et al.*[10] This is in spite of our cohort being comparatively stable outpatients, as evidenced by a median LVEF of 45% and 6 min walk distance of 463 m. In our healthy control group, 86% of individuals had a GDF-15 value 1.20 ng/mL, similarly close to published data showing 1.20 ng/mL to be the 90th percentile for healthy elderly controls.[15] In $HFnEF_{ESC}$, GDF-15 was higher than in controls but not significantly lower than in HFrEF. In multivariate analysis, presence of $HFnEF_{ESC}$ (as well as HFrEF) was independently associated with a higher GDF-15 level. Whether GDF-15 levels in HFnEF are indicative of an adverse prognosis, as has been described for HFrEF and other cardiovascular diseases, has not been investigated thus far.

Growth differentiation factor 15 is considered a relatively global and non-specific marker of risk, as it is also strongly elevated in other conditions such as malignant or haematological diseases.[23,24] On the other hand, GDF-15 has recently been shown to be predictive of the presence of any cardiovascular disease, additive to an established clinical risk score in an elderly population-based cohort.[25] It is therefore noteworthy that we found rather strong—considering the epidemiological setting—associations of GDF-15 with several echocardiographic parameters, strengthening the usefulness of GDF-15 as an indicator of cardiac status. Moreover, it is interesting that a significant correlation between GDF-15 and structural and functional indicators of diastolic function such as LVMI, LAVI or *E*/*é* was observed. Also, GDF-15 increased with impairment in diastolic function. These data suggest that GDF-15 might be a marker indicative to some extent of diastolic function. Increased GDF-15 expression has been shown in animal models of hypertrophic cardiomyopathy and load-induced left ventricular hypertrophy,[8] thus providing experimental support for our observations. Oxidative stress through uncoupling of cardiac nitric oxide synthase has recently been shown to induce diastolic dysfunction in an animal model[26] while GDF-15 expression has been shown to be increased in cardiomyocytes by oxidative stress.[9,27] In this respect, GDF-15 levels may also partly represent an underlying pathophysiological mechanism for diastolic dysfunction. Our data also suggest that GDF-15 may potentially be an indicator of disease severity in HFnEF, given its reasonable properties for detecting patients with elevated filling pressures (see below) and correlations with parameters indicative of acutely or chronically elevated filling pressures (i.e. *E*/*é* and LAVI) and backwards failure (i.e. estimated right ventricular systolic pressure). The observation that higher GDF-15 levels were independently associated with physical impairment on multivariate analysis as assessed by 6 min walk distance and the SF-36 physical function scale supports this theory.

When we compared the discriminatory ability of GDF-15 for differentiating $HFnEF_{ESC}$ from healthy controls to that of NT-proBNP, to indirectly assess its diagnostic properties, we found it quite remarkable that the properties of GDF-15 were very similar to those of NT-proBNP. The inclusion of NT-proBNP for the diagnosis of $HFnEF_{ESC}$ would imply a strong methodological bias towards a better discriminatory ability of NT-proBNP. We therefore used a second classification (termed $HFnEF_{New}$)—which was independent from NT-proBNP—to identify CHF patients with normal LVEF and elevated left ventricular filling pressures according to the guideline-recommended echocardiographic criteria.[17] Discriminatory properties of GDF-15 for differentiating these $HFnEF_{New}$ patients from controls clearly tended to be superior to those of NT-proBNP. While exact numbers for AUC, sensitivity and specificity cannot be relied upon for clinical purposes due to the design of our study, the direct comparison suggests that GDF-15 might in fact be an alternative to NT-proBNP, although further validation in other cohorts will be necessary. Combining both biomarkers may be even more attractive[7] because GDF-15 may be indicative of pathophysiological processes different from NT-proBNP, such as inflammation.[23] In fact, the AUC for the combination of both markers was significantly higher than for NT-proBNP for both $HFnEF_{ESC}$ and $HFnEF_{New}$, although the combination was not significantly superior to GDF-15 for the latter. Therefore, future trials should specifically address whether the combination of NT-proBNP and GDF-15 for the diagnosis of HFnEF really gives incremental value or whether one of the two markers can be used in isolation. Ideally, these trials would validate both markers prospectively against invasive haemodynamic data as a gold standard, which would also offer the opportunity to compare their values against currently recommended echocardiographic criteria.

Considering the potential future use of GDF-15 as a marker in HFnEF it may be reassuring that the optimal discriminatory cut-off in our cohort of 1.16 ng/L (both for $HFnEF_{ESC}$ as well as $HFnEF_{New}$) is very close to the published value of 1.20 ng/L for HFrEF. Similarly, our observation of an optimal cut-off value for NT-proBNP of 200.7 ng/L to diagnose $HFnEF_{New}$ and the high specificity of 97% at the recommended cut-off of 220 ng/L actually validates current ESC recommendations for the use of natriuretic peptides in the diagnosis of $HFnEF_{ESC}$. These values were largely derived from two small invasive studies.[28,29] Our data show that the discriminatory properties of NT-proBNP are very similar in our cohort of patients with non-invasive, i.e. echocardiographic, evidence of elevated filling pressures, underlining the usefulness of NT-proBNP[30] as recommended currently, until novel markers such as GDF-15 or others[31] have been further validated individually or in combination.

Although GDF-15 may be superior to NT-proBNP for the diagnosis of $HFnEF_{New}$ (and similar for the diagnosis of $HFnEF_{ESC}$), its diagnostic properties are far from perfect. We would therefore consider the search for a biomarker for HFnEF to be ongoing. Also, considering the use of such a biomarker in the clinical setting, it would optimally distinguish HFnEF from HFrEF and other causes of exertional dyspnoea, of which the former is neither true for GDF-15 nor for NT-proBNP while the latter cannot be tested in our cohort.

With regards to limitations of our study, we would like to stress that the diagnostic properties reported are solely for comparative purposes. Differentiating apparently healthy elderly individuals from patients presenting with the syndrome of heart failure using additional biomarker testing is rarely a clinical issue. The number

of CHF patients in our cohort was relatively small and analyses are therefore of limited statistical power. On the other hand, all patients were comprehensively characterized by echocardiography, making a classification of $HFnEF_{ESC}$ or $HFnEF_{New}$ possible. Our interpretation that GDF-15 is a marker which is at least as good as NT-proBNP is formally somewhat imprecise, as there were no criteria for non-inferiority prospectively defined. Although an invasive confirmation and quantification of diastolic dysfunction would have been preferable, such an approach would not have been feasible in a medium-scale population-based study like DIAST-CHF. It is striking that of all the patients with a history or signs and symptoms of CHF and with an LVEF >50%, only 55.4% met the criteria for the diagnosis of $HFnEF_{ESC}$. While beyond the scope of this paper, the study of the remaining patients with CHF and LVEF >50% will help to facilitate a better understanding of HFnEF and the value of the ESC criteria. Preliminary analyses suggest that patients meeting ESC criteria are considerably more symptomatic, suggesting that these criteria are in fact more specific for the identification of truly symptomatic HFnEF.

Conclusions

The novel cardiovascular risk marker GDF-15 is elevated in patients with HFnEF. Growth differentiation factor 15 levels correlate with echocardiographic markers of diastolic function and elevated filling pressures. They are independently associated with exercise capacity and physical aspects of quality of life. Discriminatory properties for differentiating HFnEF from healthy individuals are at least as good as those of NT-proBNP. These results merit further investigation of the value of GDF-15 for diagnosis, prognostication and therapy guidance in diastolic heart failure.

Supplementary material

Supplementary material is available at *European Journal of Heart Failure* online.

Acknowledgements

Test kits for GDF-15 were kindly provided by Roche Diagnostics GmbH, Mannheim, Germany.

Conflict of interest: none declared.

Funding

This work was supported by grants from the German Federal Ministry of Education and Research (German Heart Failure Network, TP 7 (FKZ 01GI0205), including a scholarship to R.S., and clinical trial programme ALDO-DHF (FKZ 01KG0506). Funding to pay the Open Access publication charges for this article was provided by the University of Goettingen.

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