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

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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 (HFsF) 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
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-β 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 hypertrophy8 and in infarcted myocardium.9 In these models, GDF-15 attenuated a reduction in fractional shortening9 and protected the heart from cardiac hypertrophy9 and ischaemia–reperfusion injury.10 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 syndromes11,13 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 HFnEF 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 criteria16 at presentation. Patients were classified retrospectively according to echocardiographic measurements to have HFnEF when LVEF was ≤50%, or HFnEF when LVEF was >50% and diagnostic criteria for HFnEF as recommended by the European Society of Cardiology4 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 HFnEFNew. For clarity of presentation, we termed the conventionally classified group HFnEFESC. While individual echocardiographic parameters used for HFnEFESC and HFnEFNew 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.

Calculations and statistical analyses

Left ventricular mass index (LVMi) was calculated by the Devereux formula20 indexed to body surface area. Left atrial volume index (LAVI) was calculated by the ellipsoid model20. For evaluation of diastolic function, we calculated the ratios E/A, E/e´, e´/a, and S/D. Diastolic dysfunction was graded as follows: normal diastolic function (1 ≤ E/A, E/e´ < 10, S/D ≥ 1, E/A with Valsalva manoeuvre ≥ 1), mild diastolic dysfunction (E/A < 1), moderate diastolic dysfunction (1 ≤ E/A < 2 and one of the following: E/e´ ≥ 10, S/D < 1, E/A Valsalva < 1), and severe diastolic dysfunction (E/A ≥ 2 and one of the following: E/e´ ≥ 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 χ² 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 Pearson 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 HFnEFESC or HFnEFNew 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 HFnEFESC 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 HFnEFESC were comparable with regard to age, NT-proBNP and 6 min walk distance. Patients with both HFrEF and HFnEFESC were older than controls.

Growth differentiation factor 15 was significantly elevated in HFnEFESC as compared with controls [1.66 (1.26; 2.34) vs. 0.90 (0.74; 1.09) ng/mL, P(0.0005)] 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 HFnEFESC, 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 sample study, 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/e’, decreased e’ and e’/a’. 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/e’ 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/e’.

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/e’ appeared to be of a more continuous nature (Figure 4).

Table 1  Clinical characteristics

|                | HFnEFESC | 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/m²)    | 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)* | 0.447    |
| Diastolic blood pressure (mmHg) | 80 (70;90) | 80 (70;85) | 78 (71;85) | <0.0005  |
| 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 m²) | 60 (49;70) | 61 (50;76) | 80 (71;93)* | <0.0005  |
| LVEF (%) | 60 (56;65) | 45 (36;48)* | 61 (56;66) | <0.0005  |
| E/e’ | 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 χ² test, as appropriate.
*P < 0.05 vs. HFnEFESC by Bonferroni-adjusted Mann–Whitney U or χ² test, as appropriate.
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/e´, 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/e´, 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, 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
proposed for GDF-15 for a diagnosis of HFrEF. At this value, sensitivity reached 81.7% and specificity 85.5% with an odds ratio (OR) of 18.1 for having HFnEF ESC. Similarly, an NT-proBNP >220 ng/L is recommended for a positive diagnosis of HFnEF ESC, 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 New subjects also fulfilled the HFnEF ESC 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* | 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* | 1.28 ng/mL |
| Sensitivity (%)     | 74        | 74     |
| Specificity (%)     | 80        | 90     |
| Precision (%)       | 78        | 86     |
| Odds ratio          | 10.9      | 27.6   |

*Recommended by ESC guidelines 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. 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. In HFrEF ESC, GDF-15 was higher than in controls but not significantly lower than in HFrEF. In multivariate analysis, presence of HFnEFESC (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. Although GDF-15 may be superior to NT-proBNP for the diagnosis of HFnEFESC, 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 HFnEFESC as well as HFnEFNew) 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 HFnEFESC and HFnEFNew 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 HFnEFESC. These values were largely derived from two small invasive studies. 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 as recommended currently, until novel markers such as GDF-15 or others have been further validated individually or in combination.

Although GDF-15 may be superior to NT-proBNP for the diagnosis of HFnEFESC (and similar for the diagnosis of HFnEFESC), 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 HFpEF or HFpEFnew 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 HFpEFESC. 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 HFpEF 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 HFpEF.

Conclusions
The novel cardiovascular risk marker GDF-15 is elevated in patients with HFpEF. 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 HFpEF 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, prognosis and therapy guidance in diastolic heart failure.

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

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