Heart Failure

Prognostic Utility of Growth Differentiation Factor-15 in Patients With Chronic Heart Failure

Tibor Kempf, MD,* Stephan von Haehling, MD,†‡ Timo Peter, MS,* Tim Allhoff, MS,* Mariantonietta Cicoira, MD, PtID,§ Wolfram Doehner, MD, PtID,† Piotr Ponikowski, MD,‖ Gerasimos S. Filippatos, MD,# Piotr Rozentryt, MD,** Helmut Drexler, MD,* Stefan D. Anker, MD, PtID,†‡ Kai C. Wollert, MD*

Hannover and Berlin, Germany; London, United Kingdom; Verona, Italy; Wroclaw and Zabrze, Poland; and Athens, Greece

Objectives
We explored the prognostic utility of growth differentiation factor (GDF)-15 in patients with chronic heart failure (CHF).

Background
Growth differentiation factor-15 is a stress-responsive member of the transforming growth factor-β cytokine superfamily. It has recently been observed that patients with CHF have increased circulating levels of GDF-15. The relations of GDF-15 to other biomarkers and to mortality in CHF have never been studied.

Methods
Circulating levels of GDF-15 were determined by immunoradiometric assay in 455 patients with CHF with a median left ventricular ejection fraction (LVEF) of 32% (interquartile range 25% to 39%).

Results
The median GDF-15 level was 1,949 ng/l (interquartile range 1,194 to 3,577); 74.9% of the patients presented with GDF-15 levels >1,200 ng/l, the upper limit of normal in healthy elderly individuals. The GDF-15 levels were closely related to New York Heart Association (NYHA) functional class and to amino-terminal pro–B-type natriuretic peptide (NT-proBNP). The risk of death during follow-up increased with increasing quartiles of GDF-15. Mortality rates at 48 months were 10.0%, 9.4%, 33.4%, and 56.2% in the respective quartiles (p < 0.001). After adjustment for clinical variables and established biomarkers of adverse prognosis, including NT-proBNP, renal dysfunction, anemia, and hyperuricemia, GDF-15 remained an independent predictor of mortality (adjusted hazard ratio for 1U increase in LVEF 2.26; 95% confidence interval 1.52 to 3.37; p < 0.001). Growth differentiation factor 15 provided prognostic information in clinically relevant patient subgroups (defined according to age, body mass index, heart failure etiology, concomitant medical therapy, renal function, and the levels of hemoglobin and uric acid) and added prognostic information to NYHA functional class, LVEF, and NT-proBNP.

Conclusions
Growth differentiation factor 15 is a new biomarker of the risk of death in patients with CHF that provides prognostic information beyond established clinical and biochemical markers. (J Am Coll Cardiol 2007;50:1054–60)

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Risk stratification is important in patients with chronic heart failure (CHF) and enables informed decisions about treatment and end-of-life care. Clinical parameters, such as advanced age, higher New York Heart Association (NYHA) functional class, reduced left ventricular ejection fraction (LVEF), lower body mass index, renal dysfunction, and anemia have all been associated with poor outcomes in CHF (1–3). Moreover, metabolic markers, such as uric acid, and neurohormonal markers, including amino-terminal pro–B-type natriuretic peptide (NT-proBNP), provide prognostic information (4,5).
is strongly up-regulated in cardiac myocytes by various stressors, including reactive oxygen species, nitrosative stress, and inflammatory cytokines (7). Whereas GDF-15 is not normally expressed in the myocardium, GDF-15 expression is induced in the heart in response to experimental pressure overload and in mouse models of dilated cardiomyopathy (8). Studies in GDF-15 gene-targeted mice have shown that GDF-15 promotes antia apoptotic, antihypertrophic, and antiremodeling effects in the heart (7,8). Using a new immunoradiometric assay, we have recently observed that patients with CHF have increased circulating levels of GDF-15 (9).

Considering that GDF-15 may be a marker of multiple stress pathways in the heart, we hypothesized that elevated levels of GDF-15 provide prognostic information in CHF. The objective of the present study was to explore the prognostic utility of GDF-15 in patients with CHF in the context of clinical characteristics and established prognostic markers.

Methods

Study population and follow-up. We studied a cohort of 455 outpatients with CHF enrolled in Athens (Greece; n = 51), London (United Kingdom; n = 89), Wroclaw/Zabre (Poland; n = 95), and Verona (Italy; n = 220). All of the patients participated in projects designed to investigate novel biomarkers in CHF and provided written informed consent. The diagnosis of CHF was based on symptoms, clinical signs, and evidence of left ventricular enlargement and systolic functional impairment by echocardiography or radionuclide or contrast ventriculography. All patients had a history of CHF for at least 6 months and were stable on medication for at least 4 weeks before blood sampling. Patients with myocardial infarction within the preceding 12 weeks, known inflammatory or malignant disease, or creatinine levels >400 μmol/l were excluded. After blood sampling, patients were followed by outpatient assessments and telephone contact for a median of 40 months (interquartile range 14 to 78 months). Survival status was censored on May 25, 2005 (May 31, 2006, in the Italian cohort). No patient was lost to follow-up. The primary end point of the study was all-cause mortality. Information on the cause of death was available in the Italian cohort. Nine patients undergoing heart transplantation were censored at the time of the event. The institutional committees on human research approved the protocol at all participating study sites.

GDF-15 immunoradiometric assay and other laboratory analyses. Serum samples were drawn after >10 min of rest in a semirecumbent position for assessment of GDF-15 and other biomarkers. The GDF-15 concentrations were determined by immunoradiometric assay as previously described (9). All GDF-15 measurements were performed at Hannover Medical School by investigators that were not aware of patients' characteristics and outcomes. The NT-proBNP levels were determined by a chemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany). Creatinine, hemoglobin, and uric acid measurements were performed at the participating study centers using standard laboratory techniques. Creatinine clearance was calculated according to the Cockcroft and Gault equation.

Statistical analyses. Data are expressed as absolute number (percentage), median (interquartile range), or mean ± standard deviation as appropriate. The Kolmogorov–Smirnov test was used to test for a normal distribution of continuous variables. Continuous variables were compared by Mann–Whitney test. Comparisons between strata of patients were performed by Kruskal–Wallis test or analysis of variance. Proportions were compared by using the chi-square test. Multiple linear regression analysis was applied to identify factors that were independently associated with GDF-15. Simple and multiple Cox regression analyses were used to assess prognostic associations. Data on body mass index and creatinine clearance were available in 407 and 409 patients, respectively; we therefore performed 2 sets of prespecified analyses, one that included these 2 parameters and one that did not. Variables that were not normally distributed (GDF-15, NT-proBNP, creatinine, uric acid) were transformed to their natural logarithm for all regression analyses. Kaplan–Meier plots were used to illustrate the timing of events during follow-up in relation to GDF-15 levels, and statistical assessment was performed by simple Cox regression analysis. For additional comparison of the prognostic values of GDF-15, NT-proBNP, creatinine, hemoglobin, and uric acid, receiver–operating characteristic (ROC) curves were generated and c-statistics calculated. All analyses were performed with StatView 5.0.1 (SAS Institute Inc., Cary, North Carolina), MedCalc 8.2.0.3 (MedCalc Software, Mariakerke, Belgium), or SAS 9.1 (SAS Institute).

Results

GDF-15 levels and mortality in chronic heart failure. The heart failure cohort consisted of 455 patients (90.5% men) with a median age of 64 years (interquartile range 57 to 71 years). Baseline characteristics are summarized in Table 1. The median GDF-15 level was 1,949 (1,194 to 3,577) ng/l; 74.9% of the patients presented with GDF-15 levels above 1,200 ng/l, the upper limit of normal in apparently healthy elderly individuals (9). Because the upper limit of normal corresponded to the lower quartile boundary in patients, the patient material
was stratified in quartiles (rounded cut-off limits 1,200, 2,000, and 3,600 ng/l) when related to outcome. The GDF-15 levels were closely related to NYHA functional class and NT-proBNP (Fig. 1).

Out of 455 patients, 117 (25.7%) died during follow-up. In the overall patient cohort, the 12-, 24-, and 48-month mortality rates were 9.9%, 16.5%, and 24.6%, respectively. There was a graded relationship between the level of GDF-15 at study entry and the risk of death during follow-up. The GDF-15 levels within the first and second quartiles were associated with similar outcomes. The GDF-15 levels above the median (2,000 ng/l), however,

### Table 1

<table>
<thead>
<tr>
<th>Quartiles of GDF-15 (ng/l)</th>
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<tbody>
<tr>
<td>All Patients (n = 455)</td>
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<tr>
<td>≤1,200 (n = 114)</td>
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<tr>
<td>1.201 to 2,000 (n = 121)</td>
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<tr>
<td>2.001 to 3,600 (n = 107)</td>
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<tr>
<td>&gt;3,600 (n = 113)</td>
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</table>

#### Clinical characteristics

- **Age (yrs)**: 64 (57–71) 58 (51–62) 65 (57–70) 69 (60–73) 68 (62–74)
- **Male gender**: 412 (90.5) 104 (91.2) 107 (88.4) 102 (95.3) 99 (87.6)
- **BMI (kg/m²)**: 25.9 (23.5–29.0) 26.2 (24.2–29.3) 26.9 (24.1–29.0) 25.2 (23.5–29.4) 24.1 (21.9–28.2)
- **Ischemic etiology**: 308 (67.7) 62 (54.4) 82 (67.8) 82 (76.6) 82 (72.6)
- **NYHA functional class**: 2.5 ± 0.8 2.1 ± 0.7 2.3 ± 0.7 2.5 ± 0.7 3.1 ± 0.8
- **LVEF (%)**: 43 (9.5) 23 (20.2) 12 (9.9) 6 (5.6) 2 (1.8)
- **NT-proBNP (ng/l)**: 801 (306–2308) 351 (162–606) 492 (238–1093) 1,020 (522–2408) 3,520 (1283–6768)
- **LVEF (%)**: 100.0 (86.6–126.0) 90 (81–99) 95 (84–110) 109 (93–132) 136 (96–161)
- **Creatinine (mol/l)**: 70.0 (51.2–89.8) 89 (77–103) 78 (58–95) 56 (48–75) 46 (35–68)
- **Hemoglobin (g/dl)**: 13.9 (12.8–14.8) 14.2 (13.5–15.0) 14.0 (13.2–14.8) 13.9 (13.0–14.7) 12.9 (11.6–14.4)
- **Creatinine clearance (ml/min)**: 400 (338–482) 340 (300–418) 370 (300–450) 430 (370–500) 482 (379–589)

#### Medication at study entry

- **ACE inhibitor/ARB**: 422 (92.7) 114 (100) 116 (95.9) 99 (92.5) 93 (82.3)
- **Beta-blocker**: 258 (58.6) 83 (80.6) 71 (60.7) 57 (54.8) 47 (42.3)
- **Diuretic**: 361 (79.3) 82 (71.9) 96 (79.3) 93 (86.9) 90 (79.6)
- **Spironolactone**: 108 (23.7) 23 (20.2) 25 (20.7) 31 (29.0) 29 (25.7)

Data from 455 patients are reported as number (percentage), median (interquartile range), or mean ± SD. Data available from *407, †442, ‡449, §442, and *440 patients. The relationships between GDF-15 and gender, etiology, and baseline medications were assessed by the chi-square test. The relationship between GDF-15 and NYHA functional class was assessed by analysis of variance. All other relationships were tested by Kruskal-Wallis test.

ACE = angiotensin-converting enzyme; ARB = angiotensin receptor blocker; BMI = body mass index; Crea Cl = creatinine clearance; GDF = growth differentiation factor; LVEF = left ventricular ejection fraction; NT-proBNP = amino-terminal pro-B-type natriuretic peptide; NYHA = New York Heart Association.
Association of GDF-15 with clinical and biochemical factors. Increasing quartiles of GDF-15 were associated with age, a lower body mass index, ischemic etiology, higher NYHA functional class, and a lower LVEF (Table 1). Moreover, increased levels of GDF-15 were associated with higher levels of NT-proBNP and uric acid, lower levels of hemoglobin, and reduced renal function (increased creatinine, reduced creatinine clearance) (Table 1). Patients with elevated levels of GDF-15 were less likely to be currently treated with angiotensin-converting enzyme inhibitors/angiotensin receptor blockers or with beta-blockers (Table 1). By multiple linear regression analysis using the natural logarithm of GDF-15 as the dependent variable, age (p = 0.046), NYHA functional class (p < 0.001), creatinine (p < 0.001), uric acid (p = 0.011), NT-proBNP (p < 0.001), and LVEF (p < 0.001) were independently related to GDF-15. The \( r^2 \) value of this model was 0.58.

GDF-15 in the context of other markers of increased mortality. By simple Cox-regression analysis, GDF-15, advanced age, higher NYHA functional class, reduced LVEF, increased levels of NT-proBNP, creatinine, and uric acid, and reduced hemoglobin concentrations were associated with an increased risk of death during follow-up (Table 2). By multiple Cox regression analysis, GDF-15 (p < 0.001) and LVEF (p < 0.001) emerged as the only independent predictors of all-cause mortality (Table 2). When body mass index and creatinine clearance were included in the model, GDF-15 (p < 0.001), LVEF (p < 0.001), and NT-proBNP (p = 0.013) independently predicted all-cause mortality. Results were unchanged when using stratification of GDF-15 levels in quartiles. The ROC curve analyses further illustrated that GDF-15 is a strong indicator of increased 1-year mortality, with a c-statistic of 0.78, which was not significantly different (p = 0.65) from the c-statistic of NT-proBNP (0.79) but significantly (p < 0.01) greater than the c-statistics of creatinine (0.64), hemoglobin (0.64), and uric acid (0.60). Combination with GDF-15 did not improve the c-statistic of NT-proBNP and GDF-15 were not normally distributed and were therefore transformed to their natural logarithms. HRs refer to an increase of 1 U in the Ln scale in these variables.

### Table 2. Simple and Multiple Cox Regression Analysis for All-Cause Mortality

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Simple Model</th>
<th>p Value</th>
<th>Multiple Model</th>
<th>p Value</th>
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</thead>
<tbody>
<tr>
<td>Age (per 10 yrs)</td>
<td>1.46 (1.23-1.80)</td>
<td>&lt;0.001</td>
<td>1.16 (0.94-1.43)</td>
<td>0.17</td>
</tr>
<tr>
<td>Male gender</td>
<td>1.55 (0.72-3.33)</td>
<td>0.26</td>
<td>1.74 (0.78-3.91)</td>
<td>0.18</td>
</tr>
<tr>
<td>Ischemic etiology</td>
<td>1.40 (0.94-2.09)</td>
<td>0.10</td>
<td>0.98 (0.65-1.50)</td>
<td>0.94</td>
</tr>
<tr>
<td>NYHA functional class (per class)</td>
<td>2.11 (1.65-2.70)</td>
<td>&lt;0.001</td>
<td>1.12 (0.83-1.50)</td>
<td>0.46</td>
</tr>
<tr>
<td>LVEF (per 10% decrease)</td>
<td>1.77 (1.45-2.16)</td>
<td>&lt;0.001</td>
<td>1.65 (1.31-2.07)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ln NT-proBNP</td>
<td>1.89 (1.64-2.18)</td>
<td>&lt;0.001</td>
<td>1.17 (0.96-1.43)</td>
<td>0.13</td>
</tr>
<tr>
<td>Ln creatinine</td>
<td>4.50 (2.72-7.47)</td>
<td>&lt;0.001</td>
<td>1.55 (0.70-3.41)</td>
<td>0.28</td>
</tr>
<tr>
<td>Hb (per 1 g/dl decrease)</td>
<td>1.21 (1.08-1.36)</td>
<td>0.009</td>
<td>1.05 (0.93-1.19)</td>
<td>0.41</td>
</tr>
<tr>
<td>Ln uric acid</td>
<td>5.93 (2.91-12.10)</td>
<td>&lt;0.001</td>
<td>0.96 (0.49-1.90)</td>
<td>0.92</td>
</tr>
<tr>
<td>Ln GDF-15</td>
<td>3.09 (2.43-3.93)</td>
<td>&lt;0.001</td>
<td>2.26 (1.52-3.37)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Complete data from 428 patients and all variables from the simple model were included in the multiple model. NT-proBNP, creatinine, uric acid, and GDF-15 were not normally distributed and were therefore transformed to their natural logarithms. HRs refer to an increase of 1 U in the Ln scale in these variables. CI = confidence interval; Hb = hemoglobin; HR = hazard ratio; Ln = natural logarithm; other abbreviations as in Table 1.
The best GDF-15 level for predicting 1-year mortality was 2,729 ng/l (sensitivity 75.0%, specificity 70.9%).

Prognostic utility of GDF-15 in different patient subgroups. The prognostic value of GDF-15 was assessed in several patient subgroups (Fig. 3). The ROC curve-derived GDF-15 level of >2,729 ng/l was associated with an increased risk of death in patient subgroups defined according to age, gender (trend in women only), body mass index, heart failure etiology, renal function, medical therapy, and the presence or absence of anemia or hyperuricemia.

In clinical practice, NYHA functional class and LVEF, and perhaps NT-proBNP (or BNP), are commonly used for risk stratification in patients with stable CHF (10); GDF-15 added significant prognostic information to these established risk markers (Fig. 4).
Discussion

The present study identifies GDF-15 as a biomarker of raised mortality in patients with CHF that provides independent prognostic information beyond established clinical and biochemical markers.

The GDF-15 levels were significantly increased in patients with CHF and displayed a graded relationship to all-cause mortality. By multiple Cox regression analysis, GDF-15 and LVEF emerged as the strongest independent predictors of mortality. Growth differentiation factor 15 provided prognostic information in clinically relevant patient subgroups defined according to age, body mass index, heart failure etiology, renal function, and the presence or absence of anemia or hyperuricemia. Importantly, GDF-15 added prognostic information to NYHA functional class and LVEF. Addition of GDF-15 did not improve the c-statistic of NT-proBNP. The c-statistic is regarded, however, as a somewhat insensitive measure of model fit (11). In fact, GDF-15 allowed stratifying patients with low or high NT-proBNP levels into subgroups with markedly different mortality rates.

The GDF-15 levels were independently related to age, NYHA functional class, impaired renal function, uric acid levels, a possible readout for metabolic abnormalities in CHF (5), and NT-proBNP, a marker of myocyte stretch, and hypertrophy (12), indicating that GDF-15 integrates several important clinical and biochemical indicators of a poor prognosis in CHF. Together, these variables explained only part of the variation in the circulating GDF-15 levels, highlighting that additional, as yet undefined factors have an impact on GDF-15. Given the pathobiology of GDF-15 (8), and our observation that increasing quartiles of GDF-15 were associated with reduced LVEF, it was somewhat surprising that GDF-15 was related to increased LVEF by multiple linear regression analysis. It is possible that other factors, such as NYHA functional class or NT-proBNP, carried the information provided by reduced LVEF in the multiple model. The relationship between GDF-15 and LVEF needs to be further explored in larger patient populations.

Experimental studies suggest that GDF-15 levels may provide insight into a distinct pathophysiologic axis. It has been shown that GDF-15 expression significantly increases in the murine heart after various forms of stress, including ischemia-reperfusion injury and pressure overload (7,8). Furthermore, transgenic mice with dilated cardiomyopathy, caused by overexpression of calcineurin or myocyte enhancer factor 2C, display significant increases in GDF-15 expression levels in the heart (8). In these animal models, GDF-15 promotes protective effects by inhibiting apoptosis, hypertrophy, and adverse remodeling in the injured heart (7,8). The situation with GDF-15 is somewhat reminiscent of BNP, which is secreted from the heart in response to ischemia or increases in wall stress, which is thought to promote salutary effects in animal models and which also provides prognostic information in patients with CHF (12).

Although recent studies have highlighted important functional roles of GDF-15 in the heart and have defined, for the first time, a functional role of GDF-15 in vivo (7,8), it needs to be pointed out that GDF-15 is not a cardiac-specific cytokine. Other cell types besides cardiomyocytes can produce GDF-15 in response to toxics or environmental stress (13,14) and may possibly contribute to elevated levels of GDF-15 in patients with CHF. Elevated circulating levels of GDF-15 have been reported during pregnancy and in patients with certain malignancies (15,16). Accordingly, GDF-15 cannot be used for the diagnosis of heart failure. However, like other important biomarkers in CHF that are not cardiac specific (e.g., renal dysfunction, anemia, hyperuricemia), measurement of GDF-15 can provide significant prognostic information and pathophysiologic insight in patients that have been diagnosed with CHF using established criteria. Therefore, GDF-15 levels need to be interpreted in the context of cardiac-specific markers, such as LVEF or NT-proBNP, to arrive at an overall prognostic assessment.

Conclusions

Growth differentiation factor 15 is a new biomarker of the risk of death in patients with CHF. Although GDF-15 levels greater than the median (2,000 ng/l) or >2,729 ng/l (best ROC curve-derived cut-off level) were useful for risk stratification in the present patient population, forthcoming studies need to further define appropriate cut-off levels for GDF-15 in CHF. Before considering implementation in clinical practice, it will be important to see if GDF-15 levels track changes in clinical status and whether GDF-15 levels can help with therapeutic decision making.

Reprint requests and correspondence: Prof. Dr. Kai C. Wollert, Abt. Kardiologie und Angiologie, Medizinische Hochschule Hannover, Carl-Neuberg Str. 1, 30625 Hannover, Germany. E-mail: wollert.kai@mh-hannover.de.

REFERENCES


