Growth-differentiation factor-15 improves risk stratification in ST-segment elevation myocardial infarction

Tibor Kempf†, Erik Björklund2†, Sylvia Olofsson2, Bertil Lindahl2, Tim Allhoff1, Timo Peter1, Jörn Tongers1, Kai C. Wollert1*, and Lars Wallentin2*

1Department of Cardiology and Angiology, Hannover Medical School, Carl-Neuberg Str. 1, 30625 Hannover, Germany; and 2Department of Cardiology and Uppsala Clinical Research Center, University of Uppsala, University Hospital, 75185 Uppsala, Sweden

Received 20 March 2007; revised 28 August 2007; accepted 24 September 2007; online publish-ahead-of-print 31 October 2007

Aims Growth-differentiation factor-15 (GDF-15) is a transforming growth factor-β-related cytokine that is induced in the heart following ischaemia-reperfusion injury. We explored the prognostic utility of GDF-15 in patients with ST-segment elevation myocardial infarction (STEMI) receiving fibrinolytic therapy.

Methods and results Circulating levels of GDF-15 were determined by an immunoradiometric assay in 741 STEMI patients who were included in the Assessment of the Safety and Efficacy of a New Thrombolytic (ASSENT)-2 and ASSENT-plus trials. About 72.7% of the patients presented with GDF-15 levels >1200 ng/L, the upper limit of normal in apparently healthy elderly individuals. Increased levels of GDF-15 were associated with a higher risk of death during 1-year follow-up. Mortality rates at 1 year were 2.1, 5.0, and 14.0% in patients with GDF-15 levels <1200, 1200–1800, and >1800 ng/L, respectively (P < 0.001). GDF-15 remained an independent predictor of mortality after adjustment for clinical variables, troponin T, and N-terminal pro-B-type natriuretic peptide (NT-proBNP). GDF-15 provided prognostic information in clinically relevant patient subgroups, defined according to age, gender, cardiovascular risk factors, haemodynamic status, and the TIMI risk score. Moreover, GDF-15 added prognostic information to the established biomarkers of adverse prognosis in STEMI, troponin T, and NT-proBNP.

Conclusion GDF-15 is a new biomarker in STEMI that provides prognostic information beyond established clinical and biochemical markers.

Introduction

In-hospital and long-term mortality rates vary considerably across the spectrum of patients with acute ST-segment elevation myocardial infarction (STEMI), who receive fibrinolytic therapy.1 Early and continuous risk stratification is therefore important to enable informed decisions about tailoring of treatment and allocation of clinical resources. Advanced age, lower systolic blood pressure, elevated heart rate, and higher Killip class are important baseline factors associated with increased mortality rates.2 These and other clinical variables have been combined into the TIMI risk score, which may serve as a tool for the risk assessment at the bedside.3,4 In addition, elevated biomarker levels at presentation, including cardiac troponin T (cTnT) and N-terminal pro-B-type natriuretic peptide (NT-proBNP), have been shown to provide complementary prognostic information on STEMI.5–7 GDF-15 is a member of the transforming growth factor-β (TGF-β) cytokine superfamily.8 GDF-15 has recently been identified as a gene that is up-regulated in cardiac myocytes by simulated ischaemia, reactive oxygen species, and pro-inflammatory cytokines, suggesting that GDF-15 may be a marker of multiple stress pathways in the heart.9,10 Although GDF-15 is not normally expressed in the adult myocardium, GDF-15 is strongly induced in the heart during experimental ischaemia and reperfusion injury.9 We have recently shown that circulating levels of GDF-15 are elevated and independently related to all-cause mortality in patients with non-ST-elevation acute coronary syndrome (NSTE-ACS).11

To explore whether GDF-15 can provide prognostic information in patients with STEMI, circulating levels of GDF-15 and its relations to clinical characteristics, other biomarkers, and mortality were studied in patients with
GDF-15 improves risk stratification in STEMI

STEIM from the Assessment of the Safety and Efficacy of a New Thrombolytic (ASSENT)-2 and ASSENT-plus trials.

Methods

Patients and study design

The present investigation is a substudy from the ASSENT-2 and ASSENT-plus trials. ASSENT-2 was a world-wide, multicentre trial comparing tenecteplase with front-loaded alteplase. ASSENT-plus recruited patients in Scandinavia and the USA and compared the efficacy and safety of dalteparin with unfractionated heparin as an adjunct to alteplase. The protocols and main results of the two trials have been published. Inclusion criteria in both trials were symptoms of acute myocardial infarction within 6 h of onset, ST-elevation ≥0.1 mV in two or more limb leads, ≥0.2 mV in two or more contiguous precordial leads, or left bundle branch block. Major exclusion criteria included known cerebrovascular disease, uncontrolled hypertension, serum creatinine concentration >150 μmol/L (only in ASSENT-plus), any substantial trauma, or increased risk of severe bleeding. Plasma samples were available from patients enrolled at selected Swedish hospitals in ASSENT-2 (n = 546) and ASSENT-plus (n = 195). The primary endpoint of the present biomarker study was mortality during 1 year, which was evaluated by patient records and telephone contacts. The TIMI risk score, which has been developed as a tool for mortality risk prediction in fibrinolytic-eligible patients with STEMI, was calculated as previously described. Instead of ‘history of diabetes, hypertension, or angina’, which represents one risk feature in the TIMI score, we used ‘history of diabetes or hypertension’ because information on previous angina was not available. On the basis of the TIMI score, patients were classified as low risk (0–3 points) or high risk (4–14 points), as described previously.

Blood sampling and laboratory analyses

Venous blood samples were collected into citrate tubes immediately before the start of thrombolytic and anticoagulation treatment and after 90 min. In ASSENT-plus, additional blood samples were obtained after 24 h, 52 h, and 5 days. After centrifugation, plasma samples were stored frozen at −70°C until further analyses. The concentration of GDF-15 was determined by a new immunoradiometric assay, as described earlier. All biomarker measurements were performed by investigators who were unaware of patients’ characteristics and outcome. GDF-15 levels at individual time points did not differ significantly between the randomized treatment groups in ASSENT-2 or ASSENT-plus and were therefore combined. Patients were stratified on the basis of the two pre-specified GDF-15 cut-off levels, 1200 and 1800 ng/L. As previously shown, 1200 ng/L corresponds to the upper limit of normal in apparently healthy, elderly Swedish individuals. In our previous study in patients with NSTE-ACS, 1200 and 1800 ng/L corresponded to the lower and upper tertile boundaries and allowed for an identification of patients at low (<1200 ng/L), intermediate (between 1200 and 1800 ng/L), or high risk (≥1800 ng/L). cTnT was analysed with a third-generation assay on Elecsys 2010 (Roche Diagnostics), with a detection limit of 0.01 μg/L. A concentration of 0.1 μg/L was used for patient stratification based on previous evaluations of cTnT at presentation and prognosis in STEMI, this cut-off level should not be confused with the cut-off concentration used for diagnosis of myocardial infarction. NT-proBNP was determined with a sandwich immunoassay on Elecsys 2010; the cut-off level of 1000 ng/L was used based on the previous evaluations of NT-proBNP for risk stratification in STEMI.

ST-segment resolution

A subgroup of patients (n = 470) without bundle branch block was monitored for 24 h after presentation by continuous vectorcardiography or by continuous 12-lead ECG for the assessment of ST-segment resolution, as described earlier. Sixty minutes after the start of the recordings, patients were classified according to ST-segment resolution from maximal ST-elevation (<50% ST-segment resolution vs. ≥50% ST-segment resolution).

Statistical analyses

Data are presented as percentages or median (interquartile range), as appropriate. Spearman’s correlation coefficients were calculated to evaluate the relations between the levels of GDF-15 and baseline characteristics. Multiple linear regression was used to evaluate the association of GDF-15 as the dependent variable with other predictors. The Kaplan–Meier method was used to illustrate the timing of events during 1 year in relation to the pre-specified strata of GDF-15 (<1200, 1200–1800 and >1800 ng/L), and statistical assessment was performed using the log-rank test. The differences in proportions in outcome events in the different strata of GDF-15 levels were judged by Fisher’s exact test. Simple Cox-regression analysis was used to identify predictors of death during 1 year. All variables were then tested in a multiple Cox-regression analysis. The Wilcoxon signed-rank test was used to compare the changes in GDF-15 within the patient group over time. All P-values are reported two-sided. The alpha-level is 0.05. No adjustments for multiplicity were made, as the results are to be considered exploratory. All data analyses were performed using the SAS 9.1 statistical program.

Results

Baseline characteristics and growth-differentiation factor-15 levels at presentation

The combined patient sample consisted of 221 women (29.8%) and 520 men (70.2%), with a median age of 67 years (interquartile range, 58–74). Baseline characteristics are presented in Table 1. The median GDF-15 level at presentation was 1635 ng/L (interquartile range, 1164–2309). Two hundred and two patients (27.3%) presented with GDF-15 levels <1200 ng/L, the upper limit of normal in apparently healthy elderly individuals; GDF-15 levels were moderately elevated (between 1200 and 1800 ng/L) in 222 patients (30.0%) and highly elevated (>1800 ng/L) in 317 patients (42.8%).

Relations between growth-differentiation factor-15 levels and clinical and biochemical factors

Increasing levels of GDF-15 at presentation were associated with increased age, diabetes, and a history of previous myocardial infarction (Table 2). GDF-15 levels were also related to reduced systolic blood pressure, elevated heart rate, Killip class >1, NT-proBNP levels, and a cTnT level at presentation >0.1 μg/L (Table 2). By multiple regression analysis that included all patients’ characteristics shown in Table 2 and trial (ASSENT-2 vs. ASSENT-plus), and used the natural logarithm of GDF-15 as the dependent variable, GDF-15 was independently associated with age (P < 0.001), male gender (P = 0.027), diabetes (P < 0.001), current smoking (P < 0.001), reduced systolic blood pressure (P < 0.001), elevated heart rate (P < 0.001), Killip class >1 (P = 0.013), NT-proBNP (P < 0.001), and a cTnT level at presentation >0.1 μg/L (P = 0.011). R² value of the model was 0.32.
Growth-differentiation factor-15 levels and mortality

GDF-15 levels at presentation were significantly related to the risk of death during 1-year follow-up (Figure 1). Patients with GDF-15 levels <1200 ng/L had a low 1-year mortality rate of 2.1%. Patients with GDF-15 levels between 1200 and 1800 ng/L had an intermediate 1-year mortality rate of 5.0%, whereas those with GDF-15 levels >1800 ng/L had a very high 1-year mortality rate of 14.0% (P < 0.001).

Differences in mortality were observed early after the index event (Figure 1) and were highly significant after 30 days (Table 3). Receiver operating characteristic curve analysis further illustrated that GDF-15 is a strong indicator of mortality with a c-statistic of 0.75 [95% confidence interval (CI), 0.68–0.83]. For comparison, the c-statistics for NT-proBNP and cTnT were 0.79 (95% CI, 0.71–0.87) and 0.67 (95% CI, 0.59–0.75), respectively.

Growth-differentiation factor-15 in the context of other markers of increased mortality

Several established markers of increased patient risk, including reduced systolic blood pressure, elevated heart rate, Killip class >1, NT-proBNP, and a cTnT level at presentation >0.1 μg/L, were associated with an increased mortality during 1 year by simple Cox-regression analysis (Table 4). Using multiple Cox-regression analysis, only GDF-15 (P = 0.005), reduced systolic blood pressure (P = 0.007), and age [estimated hazard ratio per 5-year increase, 1.35 (95% CI, 1.08–1.70); P = 0.009] emerged as independent predictors of mortality (Table 4). Inclusion of peak CK-MB as an additional variable into the multiple Cox-regression analysis did not change the overall results (i.e. GDF-15, reduced systolic blood pressure, and age emerged as the only independent predictors). GDF-15 remained independently related to mortality when including in-hospital
β-blocker and ACE-inhibitor therapy as additional variables into the multiple Cox-regression analysis (data on in-hospital treatment were available only in ASSENT-2).

Prognostic utility of growth-differentiation factor-15 in different patient subgroups

The prognostic utility of GDF-15 was explored across the clinical spectrum of patients by using the pre-specified GDF-15 cut-off level of 1800 ng/L. As shown in Figure 2, a GDF-15 level >1800 ng/L was associated with a significantly increased risk of death in most investigated subgroups.

**Growth-differentiation factor-15 adds prognostic information to cardiac troponin T, N-terminal pro-B-type natriuretic peptide, and the TIMI risk score**

A GDF-15 level >1800 ng/L was associated with a 2.2-fold and a 4.8-fold higher risk of death during 1 year in patients with an NT-proBNP level ≤1000 or >1000 ng/L, respectively (Figure 2). Similarly, a GDF-15 level >1800 ng/L was associated with a 3.1-fold and a 5.0-fold higher risk of death during 1 year in patients presenting with a cTnT level ≤0.1 or >0.1 μg/L, respectively. Moreover, a GDF-15 level >1800 ng/L was associated with a 4.9-fold higher risk of death during 1 year in patients with a TIMI score >3. Patients presenting with a TIMI score 0–3 had a low 1-year mortality rate of 3.0% (13 deaths in 431 patients), and GDF-15 did not add significant prognostic information in the low-risk subgroup (Figure 2).

**Temporal evolution of growth-differentiation factor-15 levels in ST-segment elevation myocardial infarction**

After presentation, GDF-15 levels remained elevated for at least 5 days (Figure 3). In the combined patient population, 72.7, 88.2, 76.0, 76.8, and 69.0% of the patients had GDF-15 levels above the upper limit of normal (1200 ng/L) at presentation, after 90 min, 24 h, 52 h, and after 5 days, respectively. GDF-15 levels measured 90 min after the start of randomized treatment were significantly higher when compared with the values at presentation; the levels increased from 1661 ng/L (interquartile range, 1187–2367) to 2179 ng/L (1470–3262) in ASSENT-plus and from 1563 ng/L (1105–2148) to 3009 ng/L (1789–4554) in ASSENT-plus (Figure 3). As shown in ASSENT-plus, GDF-15 levels at 24 and 52 h were still slightly higher when compared with the values at presentation (Figure 3).

To explore whether the peak in circulating GDF-15 concentrations at 90 min was related to successful reperfusion, the rise in GDF-15 from baseline to 90 min was re-evaluated in 462 patients, in whom paired baseline and 90 min plasma samples and continuous vectorcardiography or 12-lead ECG recordings were available. However, GDF-15 levels increased to a similar extent after 90 min in patients with or without ST-segment resolution (P = 0.28), arguing against a reperfusion-induced rise in circulating GDF-15 levels.

GDF-15 levels at 90 min (n = 711) provided significant prognostic information, with 1-year mortality rates of 1.2 and 8.4% in patients with GDF-15 levels <1200 or ≥1200 ng/L, respectively (P = 0.014). Similar trends were observed at 24 h, 52 h, or after 5 days, although the numbers of available samples at these later time points were too small to allow definitive conclusions (data not shown).

**Discussion**

This study demonstrates, for the first time, that GDF-15 is a biomarker of raised mortality in patients with acute STEMI that adds independent prognostic information to established clinical and biochemical markers. Our data show that patients who present with a GDF-15 concentration within the normal range (<1200 ng/L) have a rather good prognosis with a 1-year mortality rate of 2.1%. Conversely, elevated levels of GDF-15 are indicative of a worse prognosis, with 1-year mortality rates of 5.0 and 14.0% in patients with moderately elevated (between 1200 and 1800 ng/L) or markedly elevated levels of GDF-15 (>1800 ng/L), respectively. Mortality curves showed a very early separation, indicating that measurement of GDF-15 may enable risk stratification...
Patients with STEMI in the present study and an independent prognostic marker also in patients with coronary syndrome, we have recently identified GDF-15 as even broader role for GDF-15 as a prognosticator in acute status, and TIMI risk score at presentation. Highlighting an relationship between the GDF-15 level and mortality risk three strata. In fact, there appeared to be a continuous that mortality risk is homogeneous within each of these strata. Although our data suggest that 1200 and 1800 ng/L are related to an increase in the cardiovascular mortality. This parallels our findings in NSTE-ACS, in which GDF-15 provided prognostic information on clinically relevant patient subgroups defined according to age, gender, delay time, cardiovascular risk factors, haemodynamic status, and TIMI risk score at presentation. Highlighting an even broader role for GDF-15 as a prognosticator in acute coronary syndrome, we have recently identified GDF-15 as an independent prognostic marker also in patients with NSTE-ACS. Patients with STEMI in the present study and patients with NSTE-ACS in our previous report presented with similar levels of GDF-15 in our study is related to an increase in the cardiovascular mortality. Although our data suggest that 1200 and 1800 ng/L are useful cut-points for risk stratification, this does not imply that mortality risk is homogeneous within each of these three strata. In fact, there appeared to be a continuous relationship between the GDF-15 level and mortality risk as shown by the multiple Cox-regression analysis. GDF-15 provided prognostic information in NSTE-ACS patients with STEMI, as patients presenting already with elevated levels of troponin are known to have a 2–3-fold higher risk of death during follow-up. As shown in the present study, risk stratification based on cTnT on admission was significantly improved by adding GDF-15. In STEMI, cTnT levels are related to symptom duration, and most patients have elevated levels at least 5 days after presentation in our study, which is consistent with studies in mice showing that GDF-15 expression is controlled by oxidative/nitrosative stress and pro-inflammatory cytokine-mediated pathways. Circulating levels of GDF-15 may therefore provide insight into a distinct pathophysiological process in patients with STEMI. Circulating levels of GDF-15 remained increased for at least 5 days after presentation in our study, which is consistent with studies in mice showing that GDF-15 expression levels in the heart remain elevated for at least 1 week after an episode of myocardial ischaemia. There was a transient peak in GDF-15 levels 90 min after the start of randomized treatment, which was observed in patients with or without successful reperfusion as judged by ST-segment

### Table 4 Cox-regression analyses for mortality during 1 year in relation to risk markers at presentation

<table>
<thead>
<tr>
<th>Risk Marker</th>
<th>Simple Model</th>
<th>Multiple Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP (per 10 mmHg decrease)</td>
<td>1.22 (1.09–1.37)</td>
<td>1.22 (1.06–1.40)</td>
</tr>
<tr>
<td>Heart rate (per 10 b.p.m.)</td>
<td>1.37 (1.23–1.53)</td>
<td>1.14 (0.96–1.35)</td>
</tr>
<tr>
<td>Killip class &gt; 1</td>
<td>2.65 (1.49–4.72)</td>
<td>1.21 (0.58–2.51)</td>
</tr>
<tr>
<td>NT-proBNP</td>
<td>2.89 (2.23–3.75)</td>
<td>1.35 (0.90–2.01)</td>
</tr>
<tr>
<td>cTnT (&gt; vs. ≤ 0.1 µg/L)</td>
<td>3.13 (1.88–5.23)</td>
<td>1.55 (0.76–3.18)</td>
</tr>
<tr>
<td>GDF-15</td>
<td>2.14 (1.82–2.52)</td>
<td>1.55 (1.14–2.11)</td>
</tr>
</tbody>
</table>

The multiple model is adjusted for age, gender, delay time, current smoking, hypertension, diabetes mellitus, history of myocardial infarction, and trial (ASSENT-2 vs. ASSENT-plus). CI, confidence interval; BP, blood pressure.

<table>
<thead>
<tr>
<th>Risk Marker</th>
<th>Estimated hazard ratio (95% CI)</th>
<th>P-value</th>
<th>Estimated hazard ratio (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP (per 10 mmHg decrease)</td>
<td>1.22 (1.09–1.37)</td>
<td>0.001</td>
<td>1.22 (1.06–1.40)</td>
<td>0.007</td>
</tr>
<tr>
<td>Heart rate (per 10 b.p.m.)</td>
<td>1.37 (1.23–1.53)</td>
<td>&lt;0.001</td>
<td>1.14 (0.96–1.35)</td>
<td>0.13</td>
</tr>
<tr>
<td>Killip class &gt; 1</td>
<td>2.65 (1.49–4.72)</td>
<td>0.001</td>
<td>1.21 (0.58–2.51)</td>
<td>0.61</td>
</tr>
<tr>
<td>NT-proBNP</td>
<td>2.89 (2.23–3.75)</td>
<td>&lt;0.001</td>
<td>1.35 (0.90–2.01)</td>
<td>0.15</td>
</tr>
<tr>
<td>cTnT (&gt; vs. ≤ 0.1 µg/L)</td>
<td>3.13 (1.88–5.23)</td>
<td>&lt;0.001</td>
<td>1.55 (0.76–3.18)</td>
<td>0.23</td>
</tr>
<tr>
<td>GDF-15</td>
<td>2.14 (1.82–2.52)</td>
<td>&lt;0.001</td>
<td>1.55 (1.14–2.11)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

These variables were not normally distributed and transformed to their natural logarithms before analysis.

Hazard ratios refer to 1 SD in the natural logarithmic scale.
GDF-15 improves risk stratification in STEMI

resolution at 60 min.\textsuperscript{24,25} Washout of GDF-15 from the reperfused myocardium is therefore unlikely to explain this peak. The early rise in circulating GDF-15 levels may be related to increased synthesis of GDF-15 during the early hours of a myocardial infarction, or, possibly, to thrombolytic therapy and plasmin-mediated cleavage and release of GDF-15 from extracellular proGDF-15 stores, a mechanism that is operative in the processing of TGF-\(\beta\).\textsuperscript{26,27} In any case, measurement of GDF-15 levels at 90 min provided prognostic information, similar to the levels at presentation.

In conclusion, GDF-15 is a new and independent biomarker of the risk of death in patients with STEMI. As this is the first presentation of results on the prognostic importance of GDF-15 in STEMI, these results need to be further explored in forthcoming studies before considering implementation in clinical practice. These studies need to define the prognostic value and clinical utility of GDF-15 when compared with more established biomarkers, such as the cardiac troponins and NT-proBNP. It will be especially important to see whether GDF-15 levels can help with patient management decisions.

![Risk of death during 1 year associated with a growth-differentiation factor-15 level >1800 ng/L at presentation in subgroups of 730 patients with ST-segment elevation myocardial infarction from ASSENT-2 or ASSENT-plus. The number of patients, mortality rates [% (n)], estimated hazard ratios, and 95% confidence interval are shown. P-values refer to the comparison between the event rates in patients with growth-differentiation factor-15 levels >1800 vs. \(\leq\)1800 ng/L in each stratum. Data have been adjusted for trial (ASSENT-2 vs. ASSENT-plus). For analyses according to age, delay time, systolic blood pressure (BP), and heart rate, the median values of the entire study sample were used to create subgroups of comparable size. *Hazard ratios could not be calculated in the ASSENT-plus cohort (there were zero deaths in 121 patients with GDF-15 levels >1800 ng/L and eight deaths in 74 patients with GDF-15 levels \(\leq\)1800 ng/L) and in diabetic patients (there were zero deaths in 29 patients with GDF-15 levels >1800 ng/L and nine deaths in 52 patients with GDF-15 levels \(\leq\)1800 ng/L).](image-url)
Conflict of interest: K.C.W., T.K., and L.W. have filed a patent and have a contract with Roche Diagnostics to develop a commercial assay for GDF-15 used for diagnosis and prognosis in cardiovascular disease.

Funding

This work was supported by grants from the Swedish Heart-Lung Foundation to L.W. and the German Ministry of Education and Research to K.C.W. (BMBF, BioChancePlus Program).

References


