Growth Differentiation Factor-15 in the Early Diagnosis and Risk Stratification of Patients with Acute Chest Pain

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BACKGROUND: Growth differentiation factor-15 (GDF-15) is a stress-responsive marker that might aid in the early diagnosis and risk stratification of patients with suspected acute myocardial infarction (AMI).

METHODS: In a prospective, international multicenter study, GDF-15, high-sensitivity cardiac troponin T (hs-cTnT), and B-type natriuretic peptide (BNP) were measured in 646 unselected patients presenting to the emergency department with acute chest pain. The final diagnosis was adjudicated by 2 independent cardiologists. The primary prognostic end point was all-cause mortality during a median follow-up of 26 months.

RESULTS: AMI was the adjudicated final diagnosis in 115 patients (18%). GDF-15 concentrations at presentation were significantly higher in AMI patients compared to patients with other diagnoses. The diagnostic accuracy of GDF-15 at presentation for the diagnosis of AMI as quantified by the area under the ROC curve (AUC) was lower (AUC 0.69, 95% CI 0.64–0.74) compared to hs-cTnT (AUC 0.96, 95% CI 0.94–0.98, P < 0.001) and BNP (AUC 0.74, 95% CI 0.69–0.80, P = 0.02). A total of 55 deaths occurred during follow-up. GDF-15 predicted all-cause mortality independently of and more accurately than hs-cTnT [AUC 0.85 (95% CI 0.81–0.90) vs 0.77 (95% CI 0.72–0.83), P = 0.002] and BNP (AUC 0.75, 95% CI 0.68–0.82, P = 0.007). Net reclassification improvement was 0.15 (P = 0.01), and the absolute integrated discrimination improvement was 0.07, yielding a relative integrated discrimination improvement of 0.36 (P = 0.07).

CONCLUSIONS: GDF-15 predicts all-cause mortality in unselected patients with acute chest pain independently of and more accurately than hs-cTnT and BNP. However, GDF-15 does not seem to help in the early diagnosis of AMI.

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Chest pain accounts for up to 5% to 10% of the consultations in emergency departments (ED)6 (1). However, only 10% to 25% of the patients presenting with chest pain benefit from hospital admission and aggressive treatment (2). Rapid and accurate identification of acute myocardial infarction (AMI) is critical for the initiation of effective evidence-based medical treatment and management but is still an unmet clinical need (3, 4).

To date, clinical assessment, electrocardiogram (ECG), and measurement of cardiac markers form the cornerstones of the diagnosis of AMI (5). Unfortunately, both the ECG and current cardiac markers have important limitations. Reliable markers for short- as well as long-term risk stratification of a heterogeneous group of patients with chest pain are limited. In patients with acute coronary syndrome (ACS), cardiac troponin is the most established marker for prediction of short- and long-term adverse cardiac events (2, 6–9). B-type natriuretic peptide (BNP), a quantitative marker of hemodynamic cardiac stress, has emerged as a prognostic marker for short- and long-term mortality in patients with ACS (10, 11).

Growth differentiation factor-15 (GDF-15) is a stress-responsive member of the transforming growth factor beta cytokine superfamily and is weakly produced in most tissues, including the heart. In the heart, GDF-15 increases in response to stress associated with tissue injury or inflammation (for example, myocardial ischemia) (12, 13). The induction of GDF-15 in cardi-
omyocytes in the setting of myocardial ischemia and reperfusion suggests that it might be a protective factor after AMI. This hypothesis was supported by a mouse model showing GDF-15–deficient mice developed greater infarct size and more cardiomyocyte apoptosis after simulated ischemia/reperfusion in vivo (12). GDF-15 concentrations are independently related to age, smoking, diabetes, impaired renal function, C-reactive protein, and BNP, suggesting that GDF-15 is a general biomarker of cardiovascular risk and inflammation (14, 15). Initial pilot studies have shown that GDF-15 may provide powerful prognostic information in multiple cardiovascular disorders (14–20). The diagnostic and prognostic performance of GDF-15 in unselected patients presenting with acute chest pain to the ED is unknown, particularly when compared with high-sensitivity cardiac troponin T (hs-cTnT) and BNP.

We performed a large multicenter study to examine the diagnostic and prognostic performance of GDF-15 in unselected patients presenting to the ED with acute chest pain.

**Methods**

**STUDY DESIGN AND POPULATION**

APACE (Advantageous Predictors of Acute Coronary Syndrome Evaluation) is an ongoing prospective international multicenter study designed and coordinated by the University Hospital Basel, Switzerland (21–23). From April 2006 to May 2008, a total of 689 consecutive patients presenting to the ED with symptoms suggestive of AMI such as acute chest pain and angina pectoris were recruited. GDF-15 and hs-cTnT values at presentation were available in 646 (93%) of these patients. Patients with terminal kidney failure requiring dialysis were excluded. The study was carried out according to the principles of the Declaration of Helsinki and approved by the local ethics committees. Written informed consent was obtained from all patients.

**ROUTINE CLINICAL ASSESSMENT**

All patients underwent an initial clinical assessment including clinical history, physical examination, 12-lead ECG, pulse oximetry, standard blood tests, and chest radiography. Standard cardiac troponin as determined by the local hospital assays, the MB fraction of creatine kinase, and myoglobin were measured at presentation, and thereafter as long as clinically indicated. Treatment of patients was left at the discretion of the attending physicians.

**ADJUDICATED FINAL DIAGNOSIS**

Final diagnoses were adjudicated centrally by 2 independent cardiologists after review of all available medical records (including patient history, physical examination, results of laboratory and radiologic testing, ECG, echocardiography, cardiac exercise test, and coronary angiography) pertaining to the patient from the time of ED presentation to 60-day follow-up. Cases were reviewed and adjudicated in conjunction with a third cardiologist in situations of diagnostic disagreement.

AMI was defined as recommended in current guidelines (5, 24). In brief, AMI was diagnosed when there was evidence of myocardial necrosis in a clinical setting consistent with myocardial ischemia. Cardiac necrosis was diagnosed by at least 1 value of the local cardiac troponin above the 99th percentile (or above the 10% imprecision value if not fulfilled at the 99th percentile) (5, 24–26). A significant rise and/or fall was defined as a change of at least 30% of the 99th percentile (or the 10% CV level) within 6–9 h (5, 24–26). The following local standard cardiac troponin assays were used for the adjudication of the final diagnosis: Abbott Axsym cTnI ADV, Beckmann Coulter Accu cTnI, and Roche cTnT fourth generation. All 3 are well-validated current standard cardiac troponin assays with comparable performance in the diagnosis of AMI (25, 26). Unstable angina was diagnosed in cases of cardiac troponin concentrations within reference intervals and typical angina at rest, deterioration of a previously stable angina, positive cardiac exercise testing, or cardiac catheterization with coronary arteries found to have stenosis ≥70% and in ambiguous cases in which follow-up information revealed AMI or a sudden unexpected cardiac death within 60 days. Furthermore, predefined diagnostic categories included cardiac but not coronary symptoms (e.g., perimyocarditis, tachyarrhythmias) and noncardiac symptoms. If AMI was excluded in the ED but no sufficient further diagnostic procedures were performed for conclusive diagnosis, symptoms were classified to be of unknown origin.

**BIOCHEMICAL ANALYSIS**

At presentation to the ED patient blood samples were collected in serum tubes for determination of GDF-15 and hs-cTnT and in EDTA-plasma for determination of BNP. After centrifugation, samples were frozen at −80 °C until assayed in a blinded fashion in a dedicated core laboratory.

GDF-15 was measured by using a precommercial sandwich immunoassay based on the enhanced chemiluminescence immunoassay principle. The assay used a polyclonal biotinylated goat capture antibody and a monoclonal detection antibody linked to a ruthenium complex. According to the manufacturer the lower limit of detection of the GDF-15 assay was <90 ng/L and the between-run imprecision of the assay was 2.8% (CV) at a concentration of 480 ng/L. The assay was
standardized to the immunoradiometric GDF-15 assay described by Kempf et al., with reported median GDF-15 concentrations in healthy, elderly individuals of 762 ng/L [interquartile range (IQR) 600–959 ng/L] (27). On the basis of previously established and published cutoff concentrations, patients were stratified according to 2 prespecified GDF-15 cutoffs, 1200 and 1800 ng/L (17).

hs-cTnT was measured on the Elecsys 2010 (Roche Diagnostics). For hs-cTnT the limit of blank and limit of detection have been determined to be 3 and 5 ng/L, respectively; an imprecision corresponding to 10% CV was reported at 12 ng/L and the 99th-percentile of a healthy reference population at 14 ng/L (28).

BNP was measured by using the AxSYM BNP assay (Abbott Laboratories) (29). The analytical range for the AxSYM assay as reported by the manufacturer extends from 15–20 000 pg/mL. Glomerular filtration rate was calculated by using the abbreviated Modification of Diet in Renal Disease formula (30).

**Results**

**Characteristics of Patients**

Baseline characteristics of the 646 patients enrolled are shown in Table 1. The adjudicated final diagnosis was AMI in 115 patients (18%) (30% ST-elevation MI, 70% non–ST-elevation MI), unstable angina in 98 (15%), cardiac symptoms of origin other than coronary artery disease (CAD) in 83 (13%), noncardiac symptoms in 298 (46%), and symptoms of unknown origin in 52 (8%). The overall median value at presentation of GDF-15 was 1225 ng/L (IQR 798–2013), of hs-cTnT 7.8 ng/L (IQR 3.4–23.6), and of BNP 77 pg/mL (IQR 26–211). Baseline characteristics according to GDF-15 concentrations at presentation are displayed in Table 1 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol58/issue2.
coronary disease, median GDF-15 values were 1450 ng/L (IQR 974–2410 ng/L), which was lower compared to patients with AMI (P = 0.05) and similar compared to unstable angina patients (P = 0.30). There were no significant differences regarding GDF-15-values in patients with ST-elevation MI and non-ST-elevation MI (P = 0.55).

GDF-15 FOR THE DIAGNOSIS OF AMI
The diagnostic accuracy of GDF-15 at presentation for the diagnosis of AMI as quantified by the AUC was 0.69 (95% CI 0.64–0.74), which was lower compared to hs-cTnT at presentation (AUC 0.96, 95% CI 0.94–0.98, P < 0.001 for comparison). BNP was available in 513 of the 646 patients and had a higher diagnostic accuracy than GDF-15 (AUC 0.74, 95% CI 0.69–0.80, P = 0.02 for comparison). The additional use of GDF-15 in combination with hs-cTnT did not increase the diagnostic accuracy provided by hs-cTnT alone (data not shown).

GDF-15 FOR THE PREDICTION OF ALL-CAUSE DEATH
During a median (IQR) follow-up time in survivors of 26 (5) months, there were 55 deaths in the whole cohort. Median GDF-15 values in deceased patients (3070 ng/L, IQR 1900–5210) were significantly higher compared to those in survivors (1140 ng/L, IQR 775–1823, P < 0.001). Cumulative 24-month all-cause mortality rates were 0.7%, 6.3%, and 21.1% in patients with low (<1200 ng/L), moderately increased (1200–1800 ng/L), and markedly increased (>1800 ng/L) concentr-
Concentrations of GDF-15 at presentation (log rank $P < 0.001$, Fig. 2 A). The same predictive value of GDF-15 concentrations for all-cause mortality was observed in Kaplan–Meier analyses for the important subgroups of patients with baseline hs-cTnT below the 99th percentile (troponin-negative patients, $P < 0.001$), patients with AMI ($P < 0.001$), with nonischemic cardiac chest pain ($P = 0.008$), and with noncardiac chest pain ($P < 0.001$), but not for patients with unstable angina ($P = 0.20$).

Assessing the prognostic value for the prediction of all-cause death by ROC-curve analysis showed a significantly higher AUC for presentation values of GDF-15 (AUC 0.85, 95% CI 0.81–0.90) compared to both hs-cTnT (AUC 0.77, 95% CI 0.72–0.83, $P = 0.002$) and BNP (AUC 0.75, 95% CI 0.68–0.82, $P = 0.007$). A value of 1375 ng/L emerged as the best prognostic cutoff value of GDF-15 to predict death, with a sensitivity of 95% and a specificity of 61%. All 3 markers showed highest accuracy in predicting midterm mortality (180 days of follow up, AUC for GDF-15 0.90, for hs-cTnT 0.82, for BNP 0.83, Fig. 3). At 360, 540, and 720 days, the prognostic performance of GDF-15 was significantly better compared to hs-cTnT and BNP.

According to multivariable Cox proportional hazard analysis GDF-15 at presentation significantly predicted all-cause mortality independently of other predictors including hs-cTnT and BNP (Table 2). Reclassification tables for predicting death according to age, cardiovascular risk factors, and presentation values of hs-cTnT and BNP (model 1) and the additional use of GDF-15 (model 2) yielded a net reclassification improvement of 0.15 ($P = 0.01$; see online Supplemental Table 2). The absolute integrated discrimination improvement was 0.07, yielding a relative integrated discrimination improvement of 0.36 ($P = 0.07$).

**GDF-15 for the Prediction of Cardiovascular Death**

Of the 55 deceased patients, 34 (62%) died from cardiovascular causes, 10 (18%) from cancer, 10 (18%) from infectious diseases, and 1 (2%) from suicide. Cumulative 24-month cardiovascular mortality rates were 0.7%, 4.5%, and 13.3% in patients with low (<1200 ng/L), moderately increased (1200–1800 ng/L), and markedly increased (>1800 ng/L) concentrations of GDF-15 at presentation (log rank $P < 0.001$, Fig. 2B).

In ROC analysis, GDF-15 was prognostically superior to hs-cTnT (AUC 0.81 vs 0.75, $P = 0.04$ for comparison) and similar to BNP (AUC 0.77, $P = 0.35$ for comparison). In univariate Cox–proportional hazard analysis, concentrations of GDF-15 predicted cardiovascular death ($P < 0.001$), as did BNP ($P < 0.001$) and hs-cTnT ($P = 0.05$). In multivariable analysis, however, none of the 3 markers was significant (all $P$ values $>0.05$), and only age ($P = 0.001$) and history of CAD ($P = 0.01$) remained significant predictors of cardiovascular mortality.
GDF-15 FOR THE PREDICTION OF DEATH OR AMI IN CHEST PAIN PATIENTS WITHOUT AMI

In patients without AMI at the initial presentation (n = 531, 82%), there were 30 (5.6%) deaths and 35 (6.6%) first AMIs during follow-up, resulting in a composite end point of death or AMI in 52 patients (9.8%). Median GDF-15 concentrations of patients reaching the composite end point were significantly higher compared to those in patients who did not (median 1800 ng/L, IQR 1190–3070 ng/L vs median 1045 ng/L, IQR 739–1730 ng/L, P < 0.001) (Fig. 4). Whereas only 3.6% of chest pain patients without AMI with low GDF-15 concentrations (<1200 ng/L) reached the combined end point of death or AMI, 12.3% with moderately increased concentrations (1200–1800 ng/L) and 16.8% with markedly increased GDF-15 concentrations (>1800 ng/L, P < 0.001) reached the combined end point.

Analysis of diagnostic subgroups revealed that concentrations of GDF-15 predicted the combined end point of death or AMI during follow-up in patients with nonischemic cardiac chest pain (P = 0.008) and noncardiac chest pain (P < 0.001), but not in patients with unstable angina (P = 0.54).

In ROC analysis, the 3 markers performed similarly (AUC 0.73 for GDF-15, 0.72 for hs-cTnT, and 0.67 for BNP; P > 0.05 for all comparisons). Although all 3 markers were significant predictors in univariate Cox proportional hazards analysis, only GDF-15 (P = 0.005) and hs-cTnT (P = 0.02), but not BNP (P = 0.72), remained significant predictors after multivariable adjustment.

Discussion

In this prospective, international multicenter study of 646 consecutive patients presenting to the ED with acute chest pain, we examined the diagnostic and prognostic value of GDF-15. We report 3 major findings:

First, GDF-15 concentrations at presentation were significantly higher in patients with AMI compared to patients with other diagnoses. The diagnostic accuracy of GDF-15 at presentation for the diagnosis of AMI, however, was markedly lower compared to hs-cTnT.
Second, GDF-15 at presentation was a powerful predictor of all-cause death and cardiovascular death in all patients, and a powerful predictor of death or AMI in patients without AMI at the initial presentation. Importantly, these associations also held in the subgroups of patients with AMI, nonischemic cardiac chest pain, and noncardiac chest pain. Third, the prognostic value of GDF-15 for adverse outcomes was more accurate than, and independent of, hs-cTnT and BNP.

These results are of clinical importance: GDF-15 constitutes a strong, valuable, and independent prognostic indicator in unselected patients with acute chest pain regardless of the underlying disease.

**PROGNOSTIC VALUE OF GDF-15**

Reliable markers for short- as well as long-term risk stratification of a heterogeneous group of patients presenting with acute chest pain remain an unmet need. One of the validated bedside risk stratification tools is the TIMI (Thrombolysis in Myocardial Infarction) risk score that estimates the risk of death and MI and the need for urgent coronary revascularization in patients with ACS (7). According to the findings of 2 recent studies, the TIMI risk score may also be useful in an unselected cohort of patients with chest pain (7, 8). Although cardiac troponin is the most well-established marker for prediction of short- and long-term adverse cardiac events in patients with ACS (2, 6–9), an increased cardiac troponin concentration implies that cardiac necrosis has already occurred. An ideal prognostic marker would identify patients at an earlier stage, when preventive or therapeutic measures might influence the course of the disease. In the heart, GDF-15 concentrations increase in response to stress associated with tissue injury or inflammation, and myocardial ischemia seems to be such a stressor (12, 13). Results of earlier studies demonstrated a powerful predictive value of GDF-15 in patients with various cardiovascular disorders, including acute chest pain (14–20). Wollert et al. showed that increasing concentrations of GDF-15 were associated with an increasing risk of
death at 1 year (AUC 0.76, mortality rates 1.5%, 5.0%, 14.1% for patients with GDF-15 concentrations <1200 ng/L, 1200–1800 ng/L, and >1800 ng/L) in 2081 patients presenting with non–ST-elevation ACS (17). These findings were later confirmed in a coronary care unit cohort of 479 patients (20). Posthoc analysis from the FRISC-II (Fast Revascularization during InStability in Coronary artery disease II) trial suggested that increased GDF-15 concentrations also might aid in the selection of patients for the invasive strategy (16). This finding, however, must be studied prospectively, and the modification of GDF-15 by cardiovascular drugs or treatments must be better investigated.

The major limitation of these studies was a rather selected patient population. In this respect, our study extends the evidence on GDF-15 to an all-comers ED chest pain population. Furthermore, our study is the first to include a high-sensitivity troponin assay for comparison with GDF-15. In our study GDF-15 at presentation predicted all-cause mortality independently of and more accurately than both hs-cTnT (AUC 0.85 vs AUC 0.77, P = 0.002) and BNP (AUC 0.85 vs 0.75, P = 0.006). It is of particular importance to note that the prognostic role of GDF-15 was maintained in our study for the subgroup of “hs-cTnT-negative” chest pain patients (presentation concentrations of hs-cTnT below the 99th percentile).

**Diagnostic Value of GDF-15**

GDF-15 is not expressed in the heart under normal physiological conditions, but has been shown to be strongly induced in the myocardium after ischemic injury (12). This inducibility suggested that GDF-15 could be a valuable marker for the diagnosis of AMI. To our knowledge, this is the first study to prospectively analyze the performance of GDF-15 in the diagnosis of AMI in comparison to the novel hs-cTnT. Our data clearly showed that GDF-15 concentrations do not have added diagnostic utility over that provided by hs-cTnT.

Our data provide further support to the concept of using a multiple-marker approach in patients with acute chest pain. GDF-15 provided additional prognostic value when used in combination with hs-cTnT, whereas previous studies had suggested that copeptin, another novel biomarker, seemed to provide additional diagnostic value when used in combination with cTnT and cTnl (23, 34).

Potential limitations of the current study merit consideration. First, because this was a prospective observational study, we were unable to quantify exactly the clinical benefit associated with improved risk stratification. A further unknown was how to best avoid adverse outcome in patients with acute chest pain identified to be at increased risk by increased concentrations of GDF-15. Second, given that escalating GDF-15 concentrations were linked to factors also linked to increased morbidity and mortality, residual confounding may be present in the reported data. Third, we cannot comment on the diagnostic and prognostic accuracy among patients with terminal kidney failure requiring dialysis, because such patients were excluded from our study. Fourth, because the final diagnosis was adjudicated according to cardiac troponin concentrations as suggested in the universal definition of AMI, there was a bias favoring hs-cTnT over the other biomarkers. Fifth, the total number of events was relatively small (n = 55 deaths). This limited statistical power, particularly for subgroup analyses.

In conclusion, GDF-15 predicts all-cause mortality in unselected patients with acute chest pain independently of and more accurately than hs-cTnT and BNP regardless of the adjudicated final diagnosis. It furthermore predicts cardiovascular mortality in all patients and the composite of death and AMI in chest pain patients without AMI at initial presentation. However, GDF-15 does not seem to help in the early diagnosis of AMI.

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GDF-15 for Diagnosis and Risk Stratification of Patients with Acute Chest Pain

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