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Growth differentiation factor-15 as a prognostic marker in patients with acute myocardial infarction

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Aims Our aim was to assess the long-term prognostic value of growth differentiation factor-15 (GDF-15) in patients post-acute myocardial infarction (AMI). Growth differentiation factor-15 is a member of the transforming growth factor β family. Growth differentiation factor-15 is expressed in the myocardium and upregulated due to 'stress' and has been shown to have antiapoptotic actions. Its role in the cardiovascular system however is not well defined. We were interested to see if GDF-15 could provide long-term prognostic value in post-AMI patients. We compared GDF-15 with N-terminal pro-B-type natriuretic peptide (NT-proBNP).

Methods and results We recruited 1142 consecutive post-AMI patients [820 men, median (range) age 67 (24–97) years] in a prospective study with a follow-up period of 505 (range 1–2837) days. Growth differentiation factor-15 levels increased with increasing Killip class ($P < 0.001$) and were correlated with NT-proBNP ($r = 0.47$, $P < 0.001$). Using a multivariable Cox proportional hazards model, log GDF-15 (HR 1.77), log NT-proBNP (HR 2.06), age (HR 1.03) Killip class above 1, (HR 1.62), use of beta-blockers (HR 0.54) and past history of MI (HR 1.44) were significant independent predictors of death or heart failure (HF). Predictors of death were log NT-proBNP, log GDF-15, age, eGFR, past history of MI, use of beta-blockers, and use of ACE inhibitors or angiotensin receptor blockers. The C-statistic for GDF-15 for predicting death or HF at 1 year was 0.73 (95% CI: 0.70–0.76, $P < 0.001$) and was 0.76 (95% CI: 0.70–0.80, $P < 0.001$) for NT-proBNP. Combining these markers yielded an AUC of 0.81 (95% CI: 0.77–0.85), which exceeded that of GDF-15 ($P < 0.001$) and NT-proBNP ($P = 0.004$) alone. The Kaplan–Meier analysis revealed that those patients with above median GDF-15 and NT-proBNP had the highest event rate for death and HF (log rank 50.22, $P < 0.001$).

Conclusion Growth differentiation factor-15 is a new marker for predicting death and HF in post-AMI patients. GDF-15 provides prognostic information over and above clinical factors and the established biomarker NT-proBNP. Combined levels of GDF-15 with NT-proBNP can identify a high-risk group of patients.

Keywords Myocardial infarction • Heart failure • Biomarkers • N-terminal B-type natriuretic peptide • Prognosis • Growth differentiation factor-15

Introduction

Growth differentiation factor-15 (GDF-15) is a member of the transforming growth factor β cytokine family. Under normal circumstances, GDF-15 is not expressed in tissue; however, during 'stress', its levels have been shown to increase in a variety of tissues.[1–3] Cardiomyocytes express and secrete GDF-15 during periods of ischaemia and reperfusion,[4] suggesting that it may be a protective factor. Growth differentiation factor-15 is also an anti-hypertrophic regulating factor in the heart and GDF-15 gene-targeted mice have enhanced cardiac hypertrophic growth following pressure overload.[5] Growth differentiation factor-15 is generated as a propeptide, and after cleavage of the N-terminus, a dimeric protein is secreted.[6] In a nested case–control study,

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GDF-15 (also known as macrophage inhibitory cytokine-1) has been shown to be linked to adverse cardiovascular outcome.[7] Recently GDF-15 has been shown to be of independent prognostic value in predicting death in patients with both non-ST-elevation myocardial infarction (NSTEMI) and ST-elevation myocardial infarction (STEMI)[8,9] over and above clinical and biochemical markers including troponin and N-terminal pro-B-type natriuretic peptide (NT-proBNP). Both these studies were part of larger prospective randomized controlled trials.

We were interested to investigate whether GDF-15 alone or in combination with NT-proBNP would be of benefit in determining the long-term prognosis post-acute myocardial infarction (AMI), particularly for death and heart failure (HF) in prospectively recruited patients who are not part of any randomized control trial and whether a multimarker approach would improve risk stratification.

Methods

Study population

We studied 1142 consecutive AMI patients admitted to the Coronary Care Unit of the Leicester Royal Infirmary. Patients were recruited between 1 March 2000 and 31 July 2007. Twelve patients refused to give consent, and 18 patients were excluded due to various exclusion criteria (see subsequently). Acute myocardial infarction was diagnosed if a patient had a plasma creatine kinase-MB elevation greater than twice normal or cardiac troponin I level >0.1 ng/mL with at least one of the following, chest pain lasting >20 min or diagnostic serial electrocardiographic changes consisting of new pathological Q waves or ST-segment and T-wave changes. Acute myocardial infarction was subcategorized into STEMI or NSTEMI. We recorded details of pharmacological therapy prescribed during the index admission. The study complied with the Declaration of Helsinki and was approved by the local ethics committee; written informed consent was obtained from all patients. Exclusion criterion was known malignancy or surgery in the previous month, and these patients were not invited to participate. The estimated GFR (eGFR) was calculated from the simplified formula derived from the Modification of Diet in Renal Disease study, recently validated in patients with HF.[10] The last patient had 60 days follow-up.

Plasma samples

Blood samples were drawn on one occasion 3–5 days after the onset of chest pain for determination of plasma GDF-15 and NT-proBNP. A total of 826 patients were recruited on day 3, 240 on day 4, and 76 on day 5. After 15 min bed rest, 20 mL blood was collected into tubes containing EDTA and aprotinin. All plasma was stored at −70°C until assayed in a blinded fashion in a single batch.

N-terminal pro-B-type natriuretic peptide assay

Our NT-proBNP assay was based on a non-competitive assay as previously published.[11] Sheep antibodies were raised to the N-terminus of human NT-proBNP and monoclonal mouse antibodies were raised to the C-terminus. Samples or NT-proBNP standards were incubated in C-terminal IgG-coated wells with the biotinylated N-terminal antibody for 24 h at 4°C. Detection was with methyl-acridinium ester (MAE) labelled streptavidin on an MLX plate luminometer (Dynex Technologies Ltd, Worthing, UK). The lower limit of detection was 0.3 pmol/L. There was no cross-reactivity with atrial natriuretic peptide, BNP, or C-type natriuretic peptide. Owing to the different immunoreactivities and standards used in this assay compared with the Roche NT-proBNP Elecys assay, the equivalent value in the Roche assay (in ng/L) has been provided for different cut-off values in our assay in pmol/L: 763.5 (804); 1107.6 (1114); 569.9 (623).

Growth differentiation factor-15 assay

The GDF-15 assay was constructed using antibodies from R&D Systems, Abingdon, Oxfordshire, UK, which were also utilized in previous studies.[8,9] Mouse monoclonal antibodies (200 ng/100 μL) specific for GDF-15 were coated on ELISA plates overnight at room temperature. Following blocking of the plates with 10% foetal calf serum, 10 μL plasma samples or standards were pipetted into the wells with 100 μL of assay buffer. Plates were incubated overnight, and following washes the next day, 5 ng of biotinylated goat antibody specific for GDF-15 in 100 μL of assay buffer was pipetted into each well. Plates were incubated for 2 h and then bound biotinylated tracer antibody was detected using the MAE–streptavidin method as described for the NT-proBNP assay. The lower limit of detection was 2.55 pg/mL.

Endpoints

Our primary endpoint was death or HF. We also investigated death, hospitalization for HF, and recurrent AMI as individual secondary endpoints. Hospitalization for HF was defined as a hospital re-admission for which HF was the primary reason requiring treatment with high-dose diuretics, inotropes, or intravenous nitrate. Myocardial infarction (MI) was diagnosed on established criteria as described above.[12] Endpoints were obtained by reviewing the Office of National Statistics Registry and by contacting each patient. There was a minimum 60-day follow-up of all surviving patients.

Statistical analysis

Statistical analyses were performed on SPSS Version 14 (SPSS Inc., Chicago, IL, USA) and Stata version 10 (TX, USA). Comparisons of continuous variables were made using the Mann–Whitney *U* test. Proportions were compared by using the χ^2 test. Spearman's correlations were performed. The relationship of baseline variables with death and HF was assessed using Cox proportional hazards analysis by univariable and multivariable analysis. An epidemiological approach was taken and factors thought to be important for the endpoints were entered in multivariable analyses. The factors entered into the model were age, gender, previous history of AMI, HF, hypertension and diabetes mellitus, smoking history, territory of infarction, STEMI or NSTEMI, Killip class, eGFR, troponin I, therapy with ACE inhibitors, angiotensin receptor blockers and beta-blockers, NT-proBNP, and GDF-15. Multiple linear regression analysis was used to determine factors that were important in influencing GDF-15 levels. To compare the accuracy of NT-proBNP and GDF-15, receiver-operating characteristic (ROC) curves were generated at 1-year and the areas under the curves (AUCs) were calculated. Comparisons between ROC curves was by the method of Hanley and McNeil.[13] Kaplan–Meier cumulative survival curves were constructed and compared by the log-rank test and the log-rank test for trend. Normality of continuous variables was assessed by the Kolmogorov–Smirnov test. Levels of NT-proBNP and GDF-15 were normalized by $\log_{10}$ transformation. Thus, odds ratios and hazard ratios (HR) refer to a 10-fold rise in the levels of these markers. Hazards ratio and 95% confidence intervals for risk factors and significance level for χ^2 (likelihood ratio test) are given. Subgroup analysis according to presenting diagnosis was undertaken as an 'a priori' analysis. This was done to assess whether GDF-15 was an important prognosticator in both STEMI

and NSTEMI patients (interaction term was found to be significant). We sought the most appropriate functional form of the covariates in Cox models by plotting the martingale residuals from a null Cox model (with no covariates) against each of the covariates suspected of non-linear functional forms. Lowess line plots were linear for age, eGFR, log NT-proBNP and log GDF, indicating that these were the optimal functional form for the linearity assumption of the Cox models. Plots of the martingale residuals with these functional forms included in the model revealed no overall pattern, confirming the appropriateness of the forms chosen. In order to test the proportional hazard assumption in Cox models, we introduced a time-dependent covariate consisting of the product of the time variable with every covariate in the equation (introducing only one time-dependent covariate at any one time). None of these time-dependent covariates provided significant coefficients in any of the Cox models (*P*-values ranged from 0.232 to 0.828), thus upholding the proportional hazards assumption. In addition, plots of the scaled Schoenfeld residuals of covariates revealed no significant slope with time, confirming the proportionality hazard assumption.[14] A two-tailed *P*-value <0.05 was deemed to be statistically significant. Assuming an event rate of 15% and that the covariates predict up to 30% of the variance of the biomarker, a sample size of 1000 patients would be powered (0.911 at $P < 0.005$) to detect an HR of the biomarker of 1.5. All authors had full access to the data and take responsibility for its integrity and accuracy of the analysis. All authors have read and agree to the manuscript as written.

Results

Patient characteristics

Patient details are recorded in *Table 1*. Median length of follow-up was 505 (range 1–2837) days. The minimum length of follow-up for survivors was 60 days. During follow-up, 140 patients died, 113 were readmitted with HF, and there were 150 recurrent AMI. The 1-year mortality was 9.8%. There were 509 STEMI patients. No patient was lost to follow-up.

Growth differentiation factor-15

GDF-15 levels were available over three timepoints and the temporal relationship of GDF-15 was evaluated. No significant difference in levels were found during the three timepoints examined

Table 1 Characteristics of patients in the study and subdivided by ST-elevation myocardial infarction and non-ST-elevation myocardial infarction

	AMI patients	STEMI	NSTEMI	*P*-value *
Number	1142	509	633	
Age (in years)	67 (24–97)	64 (24–92)	70 (37–97)	<0.001
Male	820 (71.8)	387 (76.0)	433 (68.4)	<0.001
Previous medical history				
Angina pectoris (%)	270 (23.6)	66 (13.0)	204 (32.2)	<0.001
Myocardial infarction (%)	242 (21.2)	60 (11.8)	182 (28.8)	<0.001
Hypertension (%)	597 (52.3)	221 (43.4)	376 (59.4)	<0.001
Diabetes mellitus (%)	269 (23.6)	94 (18.5)	175 (27.6)	<0.001
Heart failure (%)	41 (3.6)	27 (5.3)	14 (2.2)	0.005
Current smokers/ex-smokers	507 (44.4)	302 (59.3)	205 (32.4)	<0.001
Revascularization (fibrinolysis)	340/509 (66.8)	340/509 (66.8)	N/A	N/A
Revascularization (PCI)	161 (14.1)	123 (24.2)	38 (6.0)	<0.001
Cardiogenic shock	15 (1.3)	12 (2.4)	3 (0.5)	0.005
Killip class on admission				<0.001
I	648 (56.7)	250 (49.1)	398 (62.9)	
II	349 (30.6)	194 (38.1)	155 (24.5)	
III	130 (11.4)	53 (10.4)	77 (12.2)	
IV	15 (1.3)	12 (2.4)	3 (0.5)	
Beta-blockers	910 (79.7)	438 (86.1)	472 (74.6)	<0.001
ACEi/ARB	899 (78.7)	444 (87.2)	455 (71.9)	<0.001
Troponin I (μg/L)	3.6 (0.05–150)	11.9 (0.06–150)	2.1 (0.05–67)	<0.001
CK-MB (IU/L)	729 (21–9523)	1055 (21–9523)	140 (22–7264)	<0.001
eGFR (mL/min/1.73 m^2 surface area)	66.2 (12.0–184.3)	68.7 (17.8–177.3)	63.3 (12.0–184.3)	<0.001
NT-proBNP (pmol/L)	763.5 (0.1–28886.8)	1107.6 (0.3–28886.8)	569.9 (0.1–24016.0)	<0.001
GDF-15 (ng/mL)	1.47 (0.24–31.86)	1.41 (0.30–26.6)	1.53 (0.24–31.86)	0.92

Values are medians (range) or numbers (percentage). eGFR, estimated glomerular filtration rate; STEMI, ST-segment elevation myocardial infarction; PCI, percutaneous coronary intervention; GDF-15, growth differentiation factor-15; NT-proBNP, N-terminal pro-B-type natriuretic peptide; ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; NA, not applicable.
*P-value for STEMI vs. NSTEMI.

($\chi^2 = 2.64$, $P = 0.29$, data not shown). There was no significant difference in GDF-15 levels between anterior or other site of AMI, STEMI vs. NSTEMI, and those with a prior history of HF. However there was a significantly higher level in females vs. males, patients with a prior history of AMI, hypertension, diabetes, and angina. GDF-15 levels were higher in patients with Killip class above 1 (data not shown). There was a grading of GDF-15 which was related to Killip class ($P < 0.001$, *Figure 1*). Plasma GDF-15 correlated with age ($r_s = 0.51$, $P < 0.001$), eGFR ($r_s = -0.47$, $P < 0.001$), and NT-proBNP (r_s=0.47, $P < 0.001$). Patients were stratified into GDF-15 above and below the median; the breakdown in demographics is shown in *Table 2*. GDF-15 levels were positively associated with age, female gender, NT-proBNP levels, beta-blocker use, Killip class, previous history of AMI, HF, angina, hypertension, and diabetes. GDF-15 levels were inversely correlated with eGFR, revascularization, and smoking history. On multiple linear regression analysis, age, gender, eGFR, NT-proBNP levels, use of beta-blockers, Killip class above 1, and prior history of diabetes independently influenced GDF-15 levels (data not shown).

Primary endpoints: growth differentiation factor-15 and N-terminal pro-B-type natriuretic peptide as predictors of death and heart failure

When clinical characteristics were entered into a Cox proportional hazards model (*Table 3*), GDF-15 (HR 1.77) and NT-proBNP (HR 2.06) together with age, past history of MI, Killip class >1, and use of beta-blockers independently predicted the primary endpoint. Past history of hypertension or diabetes were not predictors.

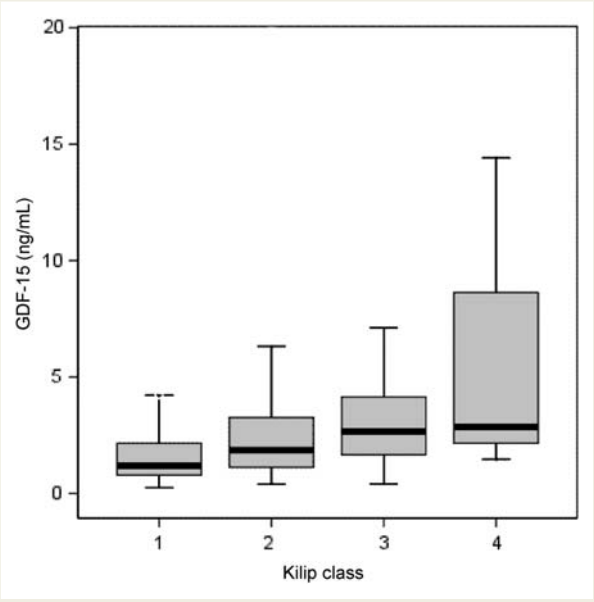


Figure 1 Box and whisper plots growth differentiation factor-15 levels according to Killip class, $P < 0.001$.

Table 2 Patient characteristics according to median growth differentiation factor-15

	GDF-15 <1.47 ng/mL	GDF-15 ≥1.47 ng/mL	*P*-value
Age (in years)	60 (32–95)	74 (24–97)	<0.001
Male	436 (76.4)	384 (67.3)	<0.001
Previous medical history			
Angina pectoris (%)	104 (18.2)	166 (29.1)	<0.001
Myocardial infarction (%)	90 (15.8)	152 (26.6)	<0.001
Hypertension (%)	261 (45.7)	336 (58.8)	<0.001
Diabetes mellitus (%)	93 (16.3)	176 (30.8)	<0.001
Heart failure (%)	14 (2.5)	27 (4.7)	0.044
Current smokers/ex-smokers (%)	295 (51.7)	212 (37.1)	<0.001
Revascularization (fibrinolysis) (%)	189 (33.1)	151 (26.4)	0.010
Revascularization (PCI) (%)	99 (17.3)	62 (10.9)	0.003
Killip class on admission			<0.001
I	388 (68.0)	260 (45.5)	
II	142 (24.9)	207 (36.3)	
III	32 (5.6)	98 (17.2)	
IV	6 (1.0)	9 (1.6)	
Beta-blockers (%)	478 (83.7)	432 (75.6)	<0.001
ACEi/ARB (%)	456 (79.9)	443 (77.6)	0.131
Troponin I (μg/L)	3.02 (0.06–150)	3.99 (0.06–150)	0.074
CK-MB (IU/L)	687 (21–6907)	815.5 (21–9523)	0.347
eGFR (mL/min/1.73 m^2 surface area)	73.1 (31.7–177.3)	57.8 (12.0–184.3)	<0.001
NT-proBNP (pmol/L)	361.8 (0.3–24016.0)	1723.6 (0.1–28886.8)	<0.001

Values are medians (range) or numbers (percentage).

Table 3 Cox regression analysis for death or heart failure post-acute myocardial infarction

	Univariable analysis		Multivariable analysis	
	Hazard ratio (95% CI)	*P*-value	Hazard ratio (95% CI)	*P*-value
Age	1.07 (1.06–1.08)	<0.001	1.03 (1.01–1.05)	0.006
Male	0.62 (0.47–0.81)	<0.001	0.91 (0.62–1.33)	0.62
Previous medical history				
AMI	2.18 (1.64–2.90)	<0.001	1.44 (1.00–2.08)	0.049
Heart failure	1.24 (0.64–2.43)	0.52	0.65 (0.26–1.63)	0.36
Hypertension	1.62 (1.24–2.12)	<0.001	1.09 (0.75–1.58)	0.65
Diabetes mellitus	1.77 (1.35–2.36)	<0.001	1.20 (0.83–1.73)	0.34
Smoking	0.59 (0.45–0.78)	<0.001	1.05 (0.69–1.58)	0.83
Anterior AMI	1.04 (0.79–1.36)	0.79	0.86 (0.60–1.63)	0.40
ST-elevation AMI	0.96 (0.74–1.26)	0.80	0.81 (0.51–1.27)	0.35
Killip class >1	3.52 (2.63–4.71)	<0.001	1.62 (1.11–2.35)	0.012
Log NT-proBNP	3.98 (3.14–5.14)	<0.001	2.06 (1.40–3.02)	<0.001
Log GDF-15	4.24 (3.21–5.62)	<0.001	1.77 (1.03–3.05)	0.039
eGFR	0.96 (0.95–0.97)	<0.001	0.99 (0.98–1.00)	0.11
Log troponin I	1.09 (0.89–1.33)	0.42	1.18 (0.92–1.52)	0.19
ACEi/ARB	0.77 (0.57–1.03)	0.074	0.92 (0.63–1.96)	0.69
Beta-blockers	0.43 (0.33–0.56)	<0.001	0.54 (0.38–0.77)	<0.001

The AUC ROC at 1 year for GDF-15 [0.73 (95% CI: 0.70–0.76)] and NT-proBNP [0.76 (95% CI: 0.70–0.80)] were similar and not statistically different ($P = 0.09$). The logistic model combining these markers yielded an AUC of 0.81 (95% CI: 0.77–0.85), which exceeded that of GDF-15 ($P < 0.001$) and NT-proBNP ($P = 0.004$) alone.

The Kaplan–Meier survival curves plotting median values of GDF-15 or NT-proBNP show that both GDF-15 and NT-proBNP are useful predictors of death or HF post-AMI.

Patients above the median for GDF-15 (above 1.47 ng/mL) had a significantly higher mortality than those below the median (log rank test 137.5, $P < 0.001$, *Figure 2*). In patients stratified by NT-proBNP (median 763 pmol/L), above and below median, GDF-15 gave additional information on death or HF in those patients who had an NT-proBNP level above the median (log rank test 50.22, $P < 0.001$, *Figure 3*). GDF-15 also had predictive power in patients with below median NT-proBNP (log rank test 19.63, $P < 0.001$). Patients can therefore be classified into low- (both markers below median), intermediate- (either marker above median), or high-risk (both markers above median) groups (log rank for trend 179.37, $P < 0.001$, *Figure 4*).

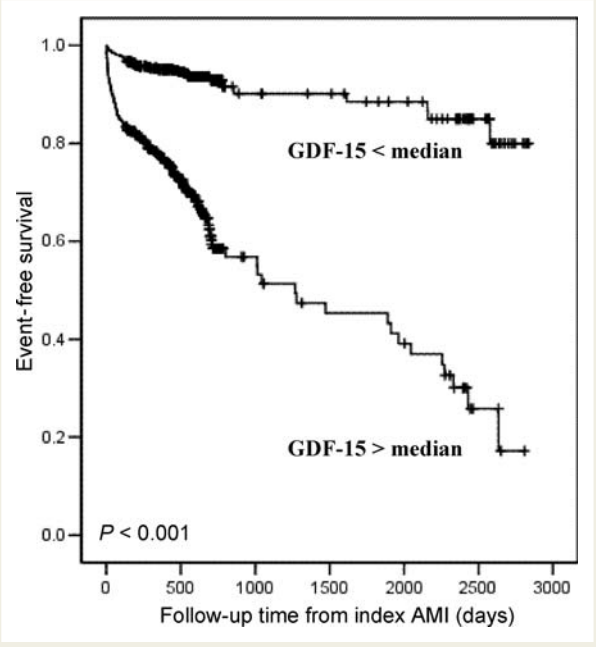


Figure 2 Kaplan–Meier survival curves event rate (death or heart failure) in patients grouped according to median levels of plasma growth differentiation factor-15.

Secondary endpoints: growth differentiation factor-15 and N-terminal pro-B-type natriuretic peptide as predictors of death, heart failure, or recurrent myocardial infarction as individual endpoints

Cox modelling revealed the following independent significant predictors for death: NT-proBNP, GDF-15, age, past history of MI, eGFR, use of beta-blockers, and use of ACE inhibitors/angiotensin receptor blockers.

Cox modelling revealed the following independent significant predictors for HF: NT-proBNP, GDF-15, Killip class above 1, and use of beta-blockers.

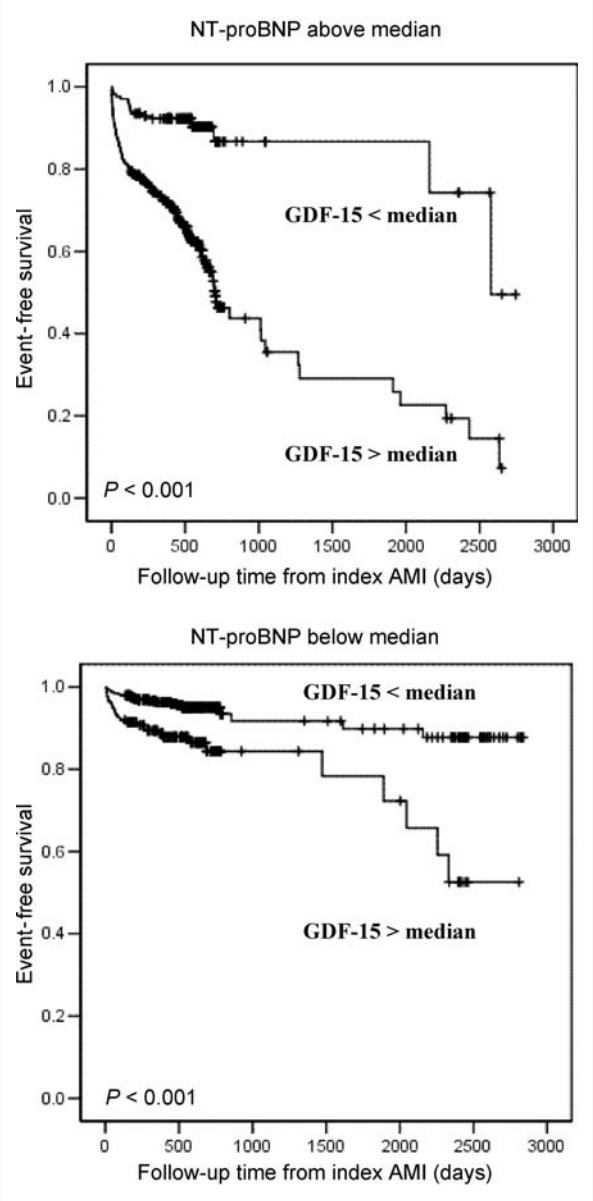


Figure 3 Kaplan–Meier survival curves event rate (death or heart failure) in patients with above or below median N-terminal pro-B-type natriuretic peptide grouped according to above or below median growth differentiation factor-15 levels.

For prediction of recurrent AMI, Cox modelling revealed only past history of MI or diabetes and presentation with STEMI as independent predictors (*Table 4*).

Growth differentiation factor-15 and N-terminal pro-B-type natriuretic peptide as predictors of death or heart failure in ST-elevation myocardial infarction and non-ST-elevation myocardial infarction

We examined the cohort of patients with presenting diagnosis of STEMI or NSTEMI.

In STEMI, only NT-proBNP, troponin I, and prior history of AMI were significant independent predictors. In NSTEMI, only NT-proBNP, GDF-15, age, Killip class >1, and use of beta-blockers were significant independent predictors of death or HF (*Table 5*).

Growth differentiation factor-15 and N-terminal pro-B-type natriuretic peptide as predictors of death in ST-elevation myocardial infarction and non-ST-elevation myocardial infarction

In STEMI, only NT-proBNP, troponin I, and prior history of AMI were significant independent predictors of death. In NSTEMI, only NT-proBNP, GDF-15, and age were significant independent predictors of death (*Table 6*).

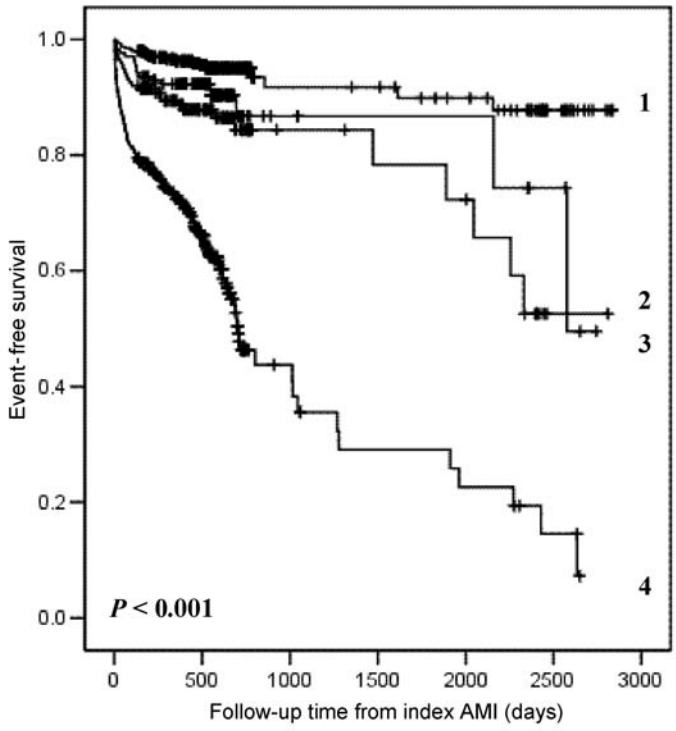
Discussion

Our data indicated that NT-proBNP and GDF-15 are powerful predictors of death and HF after AMI. The combination of markers gives added prognostic information above existing clinical characteristics, thus enabling patients to be stratified into low-, intermediate-, or high-risk groups.

Current tools available to the clinician enabling risk stratification after an AMI involve clinical factors and these have been well defined in various scoring systems such as TIMI and GRACE scores.[15,16] Biochemical markers are also useful and give prognostic information to the clinician after an AMI, including the well-established marker troponin[17] and newer markers which are now finding their way into clinical decision-making, such as BNP[18] or its more stable counterpart NT-proBNP.[19,20] Risk stratification at an early stage after AMI remains important and may be useful in helping to select treatment regimes for patients. There is some recent retrospective trial data to show that GDF-15 levels may be useful in determining the value of an early invasive strategy in patients with NSTEMI. This trial was part of the Fast Revascularization during InStability in Coronary artery disease II (FRISC-II).[21]

The ROC curve analysis indicated that NT-proBNP and GDF-15 were of similar accuracy in prediction of death or HF. Kaplan–Meier analysis revealed GDF-15 was useful irrespective of whether NT-proBNP was high or low. A raised GDF-15 and NT-proBNP was particularly useful in defining a high-risk group of patients. In multiple Cox regression analysis, both GDF-15 and NT-proBNP emerged as strong predictors of death or HF. Multimarker strategies are useful in that they can give information about the different pathways that are being activated after an acute coronary syndrome. Here we have shown that prognostic information can be gained by targeting the neurohormonal system with a pathway that is activated during ischaemia reperfusion.

There are similarities between GDF-15 and NT-proBNP, both showing elevated levels in females compared with males and a strong correlation with eGFR (a surrogate marker of renal function). There are however important differences in that GDF-15 is raised in patients who have diabetes and NT-proBNP is not; diabetes independently influences the levels of GDF-15.



Group	NT-proBNP	GDF-15
1	Below median	Below median
2	Above median	Below median
3	Below median	Above median
4	Above median	Above median

Figure 4 Combined Kaplan–Meier survival curve growth differentiation factor-15 levels (above or below median) predicting the primary endpoint of death or heart failure, in patients stratified by NT-proBNP (above or below median).

GDF-15 has been previously reported as being a strong predictor of adverse events in two therapeutic trials including patients with STEMI[8] (Assessment of the Safety and Efficacy of a New Thrombolytic (ASSENT)-2 and ASSENT-plus trials) and non-ST elevation acute coronary syndrome[9] (Global Utilization of Strategies to Open Occluded Arteries (GUSTO)-IV trial). In patients with NSTEMI, GDF-15 and NT-proBNP both emerged as independent predictor of death in Cox analysis. Interestingly, however, in STEMI, GDF-15 but not NT-proBNP was a significant independent predictor of mortality. This is in contrast to what we have found. For the prediction of death and HF, NT-proBNP was found to be a significant independent predictor in both STEMI and NSTEMI patients, GDF-15 however was only a significant predictor in NSTEMI patients. Similar results were found for the prediction of death. The difference in findings between our study and the aforementioned trials may in part be explained by the different cohort of patients in the studies and also, the timing of the plasma samples obtained. Previous studies have recruited patients from large randomized control trials, and patient selection may have influenced the analysis of the predictive value of biomarkers. Our cohort of patients in comparison was unselected. In the STEMI patients of ASSENT-2 and ASSENT-plus, the independent predictive value of GDF-15 was reported on blood samples obtained at presentation, whereas our findings of lack of independent predictive value of GDF-15 in the STEMI subgroup related to post-reperfusion blood samples.[8] However, our study suggests that both NT-proBNP and GDF-15 remain independent predictors of endpoints in the NSTEMI subgroup of patients, even when samples are obtained 3–5 days following admission. This re-inforces the data from GUSTO-IV[9] and FRISC-II[21] in NSTEMI patients in which GDF-15 levels were obtained on admission (GUSTO-IV $<$20 h, FRISC-II 27–54 h from admission), and suggests that in NSTEMI, GDF-15 retains its predictive value even with delayed blood samples. The follow-up of our patients is also longer than the previous studies that have investigated GDF-15 as a prognostic marker (maximum follow-up was 2 years in FRISC II[21]), suggesting that GDF-15 may be a useful marker both in early and late prognostication post-AMI.

There are some strengths and limitations that should be mentioned. This was a single-centre study involving two emergency admitting hospitals. However, on a positive note, we did not

employ strong exclusion criteria and would therefore argue that our population is in keeping with that which is routinely seen by clinicians around the world. We had a good weighting for STEMI and NSTEMI patients in our cohort and were able to investigate GDF-15 in the entire cohort. Our study did, however, employ blood samples in the recovery phase of AMI, and we would have missed the early deaths during the first 72 h.

Conclusion

This report shows that GDF-15 is a prognostic marker of death and HF in patients with AMI, independent of established conventional risk factors. A multimarker approach with GDF-15 and NT-proBNP is more informative than either marker alone and may be useful for risk stratification in AMI patients.

Table 4 Cox regression analysis for secondary endpoints of death, heart failure, or recurrent myocardial infarction

	Death		HF		Recurrent MI	
	Hazard ratio (95% CI)	*P*-value	Hazard ratio (95% CI)	*P*-value	Hazard ratio (95% CI)	*P*-value
Age	1.03 (1.01–1.05)	0.004	1.02 (0.99–1.04)	0.18	1.00 (0.98–1.02)	0.68
Male	0.92 (0.62–1.36)	0.67	0.87 (0.56–1.37)	0.55	1.00 (0.67–1.47)	0.98
Previous medical history						
AMI	1.62 (1.09–2.41)	0.016	1.24 (0.79–1.94)	0.35	1.51 (1.02–2.23)	0.04
Heart failure	1.11 (0.50–2.43)	0.80	0.54 (0.19–1.50)	0.24	0.65 (0.24–1.79)	0.41
Hypertension	0.92 (0.63–1.33)	0.66	1.37 (0.86–2.17)	0.18	1.35 (0.95–1.34)	0.10
Diabetes mellitus	1.12 (0.75–1.66)	0.58	1.19 (0.76–1.86)	0.44	1.48 (1.01–2.15)	0.04
Smoking	1.20 (0.80–1.81)	0.38	0.87 (0.54–1.81)	0.56	0.88 (0.55–1.18)	0.27
Anterior AMI	1.07 (0.74–1.57)	0.72	0.88 (0.57–1.37)	0.58	1.02 (0.77–1.44)	0.93
ST-elevation AMI	0.96 (0.63–1.47)	0.85	1.23 (0.76–1.99)	0.41	1.80 (1.22–2.66)	0.003
Killip class >1	1.26 (0.86–1.86)	0.24	3.26 (1.96–5.44)	<0.001	1.18 (0.82–2.17)	0.37
Log NT-proBNP	2.57 (1.74–3.79)	<0.001	1.64 (1.06–2.54)	0.027	1.09 (0.82–2.74)	0.55
Log GDF-15	1.83 (1.06–3.15)	0.03	1.61 (0.86–3.03)	0.039	1.27 (0.78–2.08)	0.34
eGFR	0.98 (0.97–0.99)	0.01	0.99 (0.98–1.00)	0.14	0.99 (0.98–1.01)	0.32
Log troponin I	1.19 (0.86–1.63)	0.29	1.32 (0.94–1.85)	0.10	1.11 (0.83–1.47)	0.48
ACEi/ARB	0.63 (0.43–0.92)	0.016	1.64 (0.95–2.83)	0.08	0.81 (0.54–1.21)	0.29
beta-blockers	0.57 (0.40–0.83)	0.003	0.51 (0.33–0.78)	0.002	1.22 (0.79–1.87)	0.38

Table 5 Cox regression analysis for death or heart failure post-acute myocardial infarction according to presenting diagnosis

	STEMI		NSTEMI	
	Hazard ratio (95% CI)	*P*-value	Hazard ratio (95% CI)	*P*-value
Age	1.05 (1.00–1.10)	0.06	1.03 (1.01–1.06)	0.02
Male	1.62 (0.71–3.67)	0.25	0.92 (0.59–1.44)	0.71
Previous medical history				
AMI	4.61 (2.13–9.95)	<0.001	1.21 (0.80–1.83)	0.37
Heart failure	0.51 (0.06–4.13)	0.53	0.68 (0.24–1.94)	0.47
Hypertension	0.62 (0.31–1.23)	0.17	1.33 (0.84–2.12)	0.23
Diabetes mellitus	2.23 (0.99–5.05)	0.05	1.17 (0.77–1.77)	0.47
Smoking	1.34 (0.61–2.96)	0.47	1.02 (0.60–1.72)	0.95
Anterior AMI	1.05 (0.64–1.72)	0.86	0.87(0.60–1.28)	0.48
Killip class >1	2.14 (0.95–4.83)	0.07	1.49 (0.97–2.31)	0.007
Log NT-proBNP	6.90 (2.53–18.84)	<0.001	1.97 (1.26–3.09)	0.003
Log GDF-15	1.00 (0.91–1.10)	0.99	2.07 (1.11–3.88)	0.023
eGFR	0.98 (0.95–1.00)	0.09	0.99 (0.98–1.01)	0.25
Log troponin I	1.88 (1.13–3.12)	0.02	0.97 (0.71–1.33)	0.87
ACEi/ARB	0.72 (0.28–1.88)	0.51	0.99 (0.64–1.54)	0.96
Beta-blockers	0.54 (0.24–1.22)	0.14	0.53 (0.36–0.79)	0.002

Table 6 Cox regression analysis for death post-acute myocardial infarction according to presenting diagnosis

	STEMI		NSTEMI	
	Hazard ratio (95% CI)	*P*-value	Hazard ratio (95% CI)	*P*-value
Age	1.04 (0.97–1.12)	0.22	1.04 (1.00–1.07)	0.029
Male	1.11 (0.34–1.64)	0.87	1.07 (0.58–1.94)	0.84
Previous medical history				
AMI	6.44 (2.12–19.55)	<0.001	1.26 (0.74–2.15)	0.40
Heart failure	0.94 (0.11–8.44)	0.96	1.04 (0.35–3.11)	0.94
Hypertension	0.85 (0.30–2.36)	0.75	1.16 (0.65–2.07)	0.62
Diabetes mellitus	0.78 (0.18–3.32)	0.75	0.99 (0.57–1.71)	0.97
Smoking	1.06 (0.34–3.31)	0.92	1.93 (0.98–3.62)	0.15
Killip class >1	0.62 (0.20–1.92)	0.41	1.16 (0.64–2.04)	0.60
Anterior AMI	0.89 (0.45–1.75)	0.73	0.99 (0.63–1.57)	0.97
Log NT-proBNP	4.83 (1.13–20.60)	0.033	1.83 (1.01–3.30)	0.046
Log GDF-15	2.98 (0.54–16.30)	0.21	2.18 (1.03–4.99)	0.035
eGFR	0.98 (0.93–1.02)	0.26	0.98 (0.97–1.00)	0.06
Log troponin I	2.56 (1.14–5.76)	0.024	0.95 (0.64–1.40)	0.79
ACEi/ARB	0.51 (0.13–1.99)	0.33	0.56 (0.33–0.95)	0.30
Beta-blockers	0.65 (0.20–2.15)	0.48	0.56 (0.33–0.95)	0.26

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