European Heart Journal (2016) **37**, 1325–1333
doi:10.1093/eurheartj/ehv491

Growth differentiation factor-15 level predicts major bleeding and cardiovascular events in patients with acute coronary syndromes: results from the PLATO study

Emil Hagström[1,2]*, Stefan K. James[1,2], Maria Bertilsson[2], Richard C. Becker[3], Anders Himmelmann[4], Steen Husted[5], Hugo A. Katus[6], Philippe Gabriel Steg[7,8,9,10], Robert F. Storey[11], Agneta Siegbahn[2,12], and Lars Wallentin[1,2], for the PLATO Investigators

[1]Department of Medical Sciences, Cardiology, Uppsala University, Uppsala, Sweden; [2]Uppsala Clinical Research Center, Uppsala, Sweden; [3]Division of Cardiovascular Health and Disease, Heart, Lung and Vascular Institute, University of Cincinnati College of Medicine, OH, USA; [4]AstraZeneca Research and Development, Mölndal, Sweden; [5]Medical Department, Hospital Unit West, Herning/Holstebro, Denmark; [6]Medizinishe Klinik, Universitätsklinikum Heidelberg, Heidelberg, Germany; [7]INSERM-Unité 1148, Paris, France; [8]Assistance Publique-Hôpitaux de Paris, Département Hospitalo-Universitaire FIRE, Hôpital Bichat, Paris, France; [9]Université Paris-Diderot, Sorbonne-Paris Cité, Paris, France; [10]NHLI Imperial College, ICMS, Royal Brompton Hospital, London, UK; [11]Department of Cardiovascular Science, University of Sheffield, Sheffield, UK; and [12]Department of Medical Sciences, Clinical Chemistry, Uppsala University, Uppsala, Sweden

Received 6 February 2015; revised 11 August 2015; accepted 27 August 2015; online publish-ahead-of-print 28 September 2015

See page 1334 for the editorial comment on this article (doi:10.1093/eurheartj/ehv638)

Aims Growth differentiation factor-15 (GDF-15) predicts death and composite cardiovascular (CV) events in patients with acute coronary syndrome (ACS). We investigated the independent associations between GDF-15 levels and major bleeding, the extent of coronary lesions and individual CV events in patients with ACS.

Methods and results Growth differentiation factor-15 was analysed at baseline ($n = 16\,876$) in patients with ACS randomized to ticagrelor or clopidogrel in the PLATO (PLATelet inhibition and patient Outcomes) trial. Growth differentiation factor-15 levels were related to extent of coronary artery disease (CAD) and to all types of non-coronary artery bypass grafting (CABG)-related major bleeding, spontaneous myocardial infarction (MI), stroke, and death during 12-month follow-up. In Cox proportional hazards models adjusting for established risk factors for CV disease and prognostic biomarkers (N-terminal pro B-type natriuretic peptide, cystatin C, high-sensitive C-reactive protein, and high-sensitive troponin T), 1 SD increase in ln GDF-15 was associated with increased risk of major bleeding with a hazard ratio (HR) 1.37 (95% confidence interval: 1.25–1.51) and with a similar increase in risk across different bleeding locations. For the same increase in ln GDF-15, the HR for the composite of CV death, spontaneous MI, and stroke was 1.29 (1.21–1.37), CV death 1.41 (1.30–1.53), all-cause death 1.41 (1.31–1.53), spontaneous MI 1.15 (1.05–1.26), and stroke 1.19 (1.01–1.42). The *C*-statistic improved for the prediction of CV death and non-CABG-related major bleeding when adding GDF-15 to established risk factors.

Conclusions In patients with ACS, higher levels of GDF-15 are associated with raised risks of all types of major non-CABG-related bleeding, spontaneous MI, and stroke as well as CV and total mortality and seem to improve risk stratification for CV-mortality and major bleeding beyond established risk factors.

Clinical Trial Registration www.clinicaltrials.gov; NCT00391872.

Keywords GDF-15 • Major bleeding • Mortality • Myocardial infarction • Cardiovascular risk factors

* Corresponding author. Tel: +46 186119500, Fax: +46 18506638, Email: emil.hagstrom@ucr.uu.se

Published on behalf of the European Society of Cardiology. All rights reserved. © The Author 2015. For permissions please email: journals.permissions@oup.com.

Downloaded from https://academic.oup.com/eurheartj/article-abstract/37/16/1325/1748693
by guest
on 09 November 2017

Introduction

Patients who have suffered from an acute coronary syndrome (ACS) are at high risk of recurrent cardiovascular (CV) morbidity and mortality. Patients with ACS are also at high risk for bleeding because of antithrombotic therapy, underlying CV disease and other co-morbidities. The increased risk of ischaemic events is related to a wide range of clinical risk factors and to biomarkers associated with CV disease and other co-morbidities such as the growth differentiation factor-15 (GDF-15).[1–5]

Multiple lines of evidence, from experimental and clinical studies, suggest that GDF-15 is associated with cellular oxidative stress, ischaemia, and strain although it is unknown if GDF-15 is causally involved in the pathological process leading to CV diseases or has a cellular protective function.[6–10] Furthermore, several studies report associations between increased levels of GDF-15 and higher prevalence of CV risk factors as well as prevalence of CV diseases and death.[11–15] Numerous risk factors predictive of CV events in patients with ACS are also prognostic for bleeding events, such as age and co-morbidities. Previously, GDF-15 has been reported to be associated with the risk of bleeding in patients with atrial fibrillation treated with oral anticoagulation.[16] However, no previous study has investigated whether the level of GDF-15 provides independent prognostic information concerning risk of bleeding as well as different types of CV events in patients with ACS receiving modern dual antiplatelet treatment and early revascularization. Therefore, the aim of this study was to investigate the GDF-15 level and its independent associations with major bleeding, extent of coronary artery disease (CAD), and specific CV outcomes in patients with ACS receiving dual antiplatelet treatment in the PLATO (PLATelet inhibition and patient Outcomes) trial.

Methods

Study population

The study is based on the PLATO trial of 18 624 patients with ST-elevation ACS (STE-ACS) or non-ST-elevation ACS (NSTE-ACS) randomized to either ticagrelor or clopidogrel in addition to established optimal medical or revascularization therapy.[17] Ticagrelor was given with a loading dose of 180 mg followed by 90 mg twice daily. Clopidogrel was given with a maintenance dose of 75 mg daily and clopidogrel-naïve patients received a loading dose of 300–600 mg. All patients received acetylsalicylic acid unless intolerant. The randomized treatment continued from 6 to 12 months with a median duration of 9.1 months. The study design and outcome variables have been published previously.[17,18]

Baseline characteristics, CV risk factors, previous medical history, and medication were recorded at baseline. Cardiovascular and bleeding events were recorded at discharge and at outpatient visits at 1, 3, 6, 9, and 12 months.

The PLATO trial was approved by regulatory authorities in all participating countries and by participating sites' institutional review boards. All participants provided written informed consent.

For laboratory evaluations and outcome assessments, see Supplementary material online.

Statistical analyses

Natural logarithmic (ln) transformations were performed for continuous variables with skewed distributions (all biomarkers). Baseline and in-hospital patient characteristics were compared across GDF-15 quartile groups. Continuous variables are presented as medians and interquartile ranges (IQRs), and groups compared using Kruskal–Wallis tests, categorical variables as counts and percentages, and groups compared using χ^2 tests. A multivariable linear model was used to assess the relationship between natural log-transformed GDF-15 (below denoted as GDF-15, unless stated otherwise) and baseline characteristics, CV risk factors, and biomarkers. Geometric means and ratios were calculated using the antilog of the model-adjusted means.

Growth differentiation factor-15 levels (original scale) were evaluated using descriptive statistics. The relation of baseline GDF-15 level to each clinical outcome is displayed in Kaplan–Meier curves and analysed with Cox proportional hazards models [hazard ratios (HRs) 95% confidence intervals (CIs)] with GDF-15 as continuous or categorical variable divided into quartiles. Hazard ratios are expressed per 1 SD increase in GDF-15 level or relative to the lowest quartile group, respectively. The following models were used for adjustment:

- **Model 1**: Randomized treatment and risk factors for recurrent CV disease [age, gender, previous myocardial infarction (MI), previous stroke, previous peripheral arterial disease (PAD), previous percutaneous coronary intervention (PCI), previous coronary artery bypass grafting (CABG), congestive heart failure, diabetes mellitus, chronic kidney disease (CKD), ST-depression on admission ECG, hypertension, smoking, body mass index (BMI), hypercholesterolaemia, antihypertensive medication, and lipid lowering medication].
- **Model 2**: Model 1 (excluding CKD) and prognostic biomarkers [hs-troponin T, cystatin C, c-reactive protein (CRP), N-terminal pro B-type natriuretic peptide (NT-proBNP)].

The functional form of the relationship between GDF-15 and outcomes was explored using cumulative sums of martingale residuals and restricted cubic splines.[19] The assumption of proportional hazards was assessed visually using log-cumulative hazard plots and by use of the cumulative sum of martingale residuals.[19]

The effects of GDF-15 levels on outcomes in relation to randomized treatment and ACS type, respectively, were evaluated using a Cox proportional hazards model that included GDF-15 transformed using restricted cubic splines and the treatment or ACS type (NSTE-ACS vs. STE-ACS), respectively, by GDF-15 interaction. In addition, we investigated the effect of GDF-15 on clinical outcomes in the subset of patients that underwent coronary angiography, taking into account the extent of CAD. The effects of GDF-15 levels on outcomes in relation to extent of CAD were analysed using a Cox proportional hazards model including extent of CAD, GDF-15 quartile group, and the CAD by GDF-15 interaction as independent variables. To assess the discriminatory ability of the models, Harrell's *C*-index was estimated. Models with plasma GDF-15 level added were compared with models without GDF-15 in terms of global model fit using likelihood ratio (LR) tests.

P-Values <0.05 from two-sided tests were considered statistically significant. The *P*-values were not adjusted for multiple comparisons, due to the exploratory nature of the present study. All analyses were performed at the Uppsala Clinical Research Center, Uppsala University, Uppsala, Sweden using SAS 9.3 (SAS Institute Inc., Cary, NC, USA).

Results

Baseline and in-hospital characteristics

A total of 16 876 patients were included with a median concentration of (IQR) GDF-15 at randomization of 1550 ng/L (1145–2219). With higher quartiles of GDF-15, the prevalence of hypertension, diabetes mellitus, and CKD increased, as did the proportion of

Downloaded from https://academic.oup.com/eurheartj/article-abstract/37/16/1325/1748693
by guest
on 09 November 2017

individuals with previous MI, PAD, revascularization, and congestive heart failure. Furthermore, the levels of NT-proBNP, cystatin C, CRP, and hs-troponin T, increased with higher quartiles of GDF-15 (*Table 1*). Also, higher GDF-15 levels were associated with increasing age, and the prevalence of several risk factors and comorbidities (see Supplementary material online, *Table S1*).

Coronary artery disease assessment

The proportions of patients with 0- and 1-vessel CAD were larger, 30 and 45%, respectively, in the lowest compared with 21 and 32% in the highest quartile of GDF-15 (*Table 1*). Patients with 3-vessel CAD, compared with 0- or 1-vessel CAD, had higher levels of GDF-15 (see Supplementary material online, *Table S1*).

Non-coronary artery bypass grafting-related major bleeding

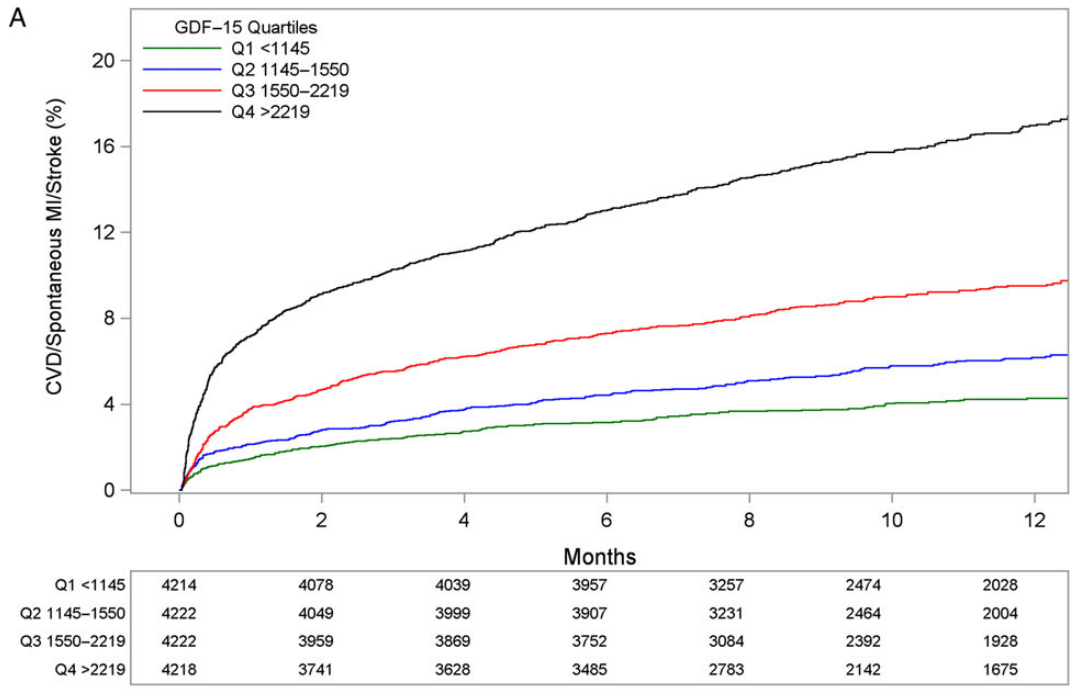
During follow-up, 607 patients had at least one non-CABG-related major bleeding. Higher levels of GDF-15 were associated with a higher risk of non-CABG-related bleeding. As illustrated in the Kaplan–Meier graphs, there were throughout follow-up, gradually increasing differences in bleeding rates between the GDF-15

Table 1 Baseline and in-hospital characteristics by growth differentiation factor-15 quartiles

Variable	Whole population	GDF-15 quartile				*P*-value
		Q1 (<1145 ng/L)	Q2 (1145–1550 ng/L)	Q3 (1550–2219 ng/L)	Q4 (>2219 ng/L)	
Number	16 876	4214	4222	4222	4218	
Demographics						
Age (years)	62 (54–71)	56 (49–62)	61 (54–68)	65 (56–72)	69 (60–76)	<0.0001
Female	4840 (28.7%)	997 (23.7%)	1173 (27.8%)	1240 (29.4%)	1430 (33.9%)	<0.0001
BMI (kg/m^2)	27.5 (24.8–30.5)	27.7 (25.1–30.4)	27.4 (24.8–30.4)	27.4 (24.7–30.5)	27.3 (24.4–30.5)	0.0003
Disease classification						
STE-ACS	6800 (40.3%)	1765 (41.9%)	1730 (41.0%)	1673 (39.6%)	1632 (38.7%)	0.0145
Medical history						
Current smoker	5994 (35.5%)	1587 (37.7%)	1620 (38.4%)	1500 (35.5%)	1287 (30.5%)	<0.0001
Dyslipidaemia	7945 (47.1%)	2064 (49.0%)	2009 (47.6%)	1924 (45.6%)	1948 (46.2%)	0.0085
Hypertension	11055 (65.5%)	2399 (56.9%)	2687 (63.6%)	2837 (67.2%)	3132 (74.3%)	<0.0001
Diabetes mellitus	4219 (25.0%)	550 (13.1%)	828 (19.6%)	1107 (26.2%)	1734 (41.1%)	<0.0001
Previous MI	3489 (20.7%)	675 (16.0%)	777 (18.4%)	923 (21.9%)	1114 (26.4%)	<0.0001
Previous congestive heart failure	977 (5.8%)	88 (2.1%)	148 (3.5%)	241 (5.7%)	500 (11.9%)	<0.0001
Previous PCI or CABG	3232 (19.2%)	705 (16.7%)	733 (17.4%)	797 (18.9%)	997 (23.6%)	<0.0001
Previous stroke or TIA	1103 (6.5%)	150 (3.6%)	216 (5.1%)	333 (7.9%)	404 (9.6%)	<0.0001
Previous PAD	1041 (6.2%)	137 (3.3%)	195 (4.6%)	270 (6.4%)	439 (10.4%)	<0.0001
CKD	708 (4.2%)	43 (1.0%)	65 (1.5%)	155 (3.7%)	445 (10.6%)	<0.0001
Coronary angiography						
0-vessel disease	1026 (7.5%)	309 (30.1%)	279 (27.2%)	220 (21.4%)	218 (21.3%)	<0.0001
1-vessel disease	5361 (39.2%)	1630 (44.8%)	1461 (41.4%)	1285 (38.0%)	985 (31.7%)	–
2-vessel disease	3891 (28.5%)	1034 (28.4%)	997 (28.2%)	944 (27.9%)	916 (29.4%)	–
3-vessel disease	3387 (24.8%)	662 (18.2%)	796 (22.5%)	936 (27.7%)	993 (31.9%)	–
Biochemical analyses						
GDF-15 (ng/L)	1550 (1145–2219)	946 (820–1049)	1333 (1235–1437)	1820 (1677–1999)	3046 (2530–4121)	–
Hs-Troponin T (ng/L)	182 (42.2–619)	144 (31.7–439)	160 (38.5–540)	195 (45.5–643)	261 (57.6–930)	<0.0001
NT-proBNP (pmol/L)	59.2 (19–185)	29.4 (11.4–77.2)	43.5 (15.9–118)	73.3 (23.2–197)	175 (47.9–529)	<0.0001
Cystatin C (mg/L)	0.83 (0.68–1.01)	0.71 (0.60–0.83)	0.78 (0.66–0.91)	0.87 (0.71–1.04)	1.05 (0.84–1.36)	<0.0001
eGFR (mL/min/1.73 m^2)	104 (79–120)	120 (104–120)	114 (92.0–120.0)	98.0 (76.0–120)	74.0 (51.0–103)	<0.0001
CRP (mg/L)	3.7 (1.6–9.5)	2.5 (1.2–5.6)	3.3 (1.5–7.6)	4.1 (1.9–9.9)	6.3 (2.4–20)	<0.0001

Values are medians (IQRs) and *n* (%) for categorical variables.
BMI, body mass index; MI, myocardial infarction; STE-ACS, ST-elevation acute coronary syndrome; PCI, percutaneous coronary intervention; CABG, coronary artery bypass grafting; TIA, transient ischaemic attack; PAD, peripheral arterial disease; hs-troponin T, high-sensitivity troponin T; NT-proBNP, N-terminal pro B-type natriuretic peptide; eGFR, estimated glomerular filtration rate; CKD, chronic kidney disease. CV, cardiovascular; CVD, cardiovascular disease.

Downloaded from https://academic.oup.com/eurheartj/article-abstract/37/16/1325/1748693
by guest
on 09 November 2017



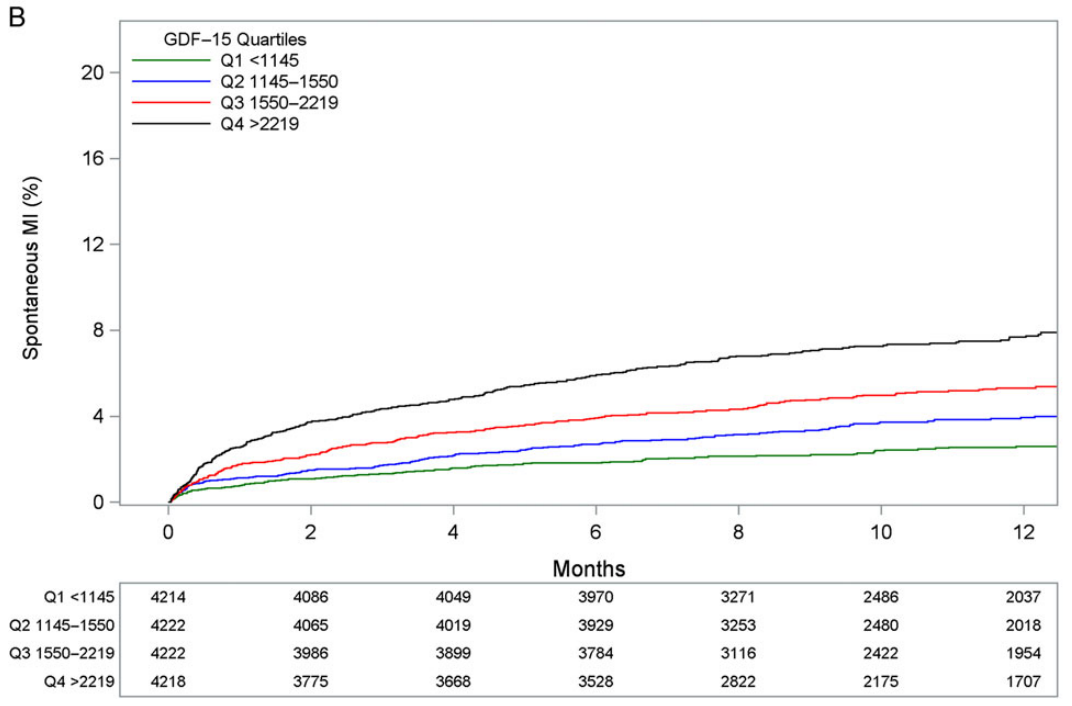


Figure 1 Kaplan–Meier estimated event rates of (*A*) the primary outcome (composite of cardiovascular death, spontaneous myocardial infarction, and stroke), (*B*) spontaneous myocardial infarction, (*C*) non-coronary artery bypass grafting-related major bleeding, (*D*) cardiovascular death, by quartiles of growth differentiation factor-15 (ng/L). Abbreviations as in *Table 1*.

quartiles (*Figure 1C* and Supplementary material online, *Table S2*). Higher levels of GDF-15 were independently associated with a raised risk of bleeding and were 2.17 (95% CI 1.64–2.88) and 1.78 (95% CI 1.29–2.45) times higher in the highest, compared with the lowest quartile, when adjusting either for clinical characteristics alone or for clinical characteristics and other prognostic biomarkers, respectively (*Table 2*). The spline graph verified a consistent increase in the rate of non-CABG related bleeding from 700 to 3500 ng/L of

Downloaded from https://academic.oup.com/eurheartj/article-abstract/37/16/1325/1748693
by guest
on 09 November 2017

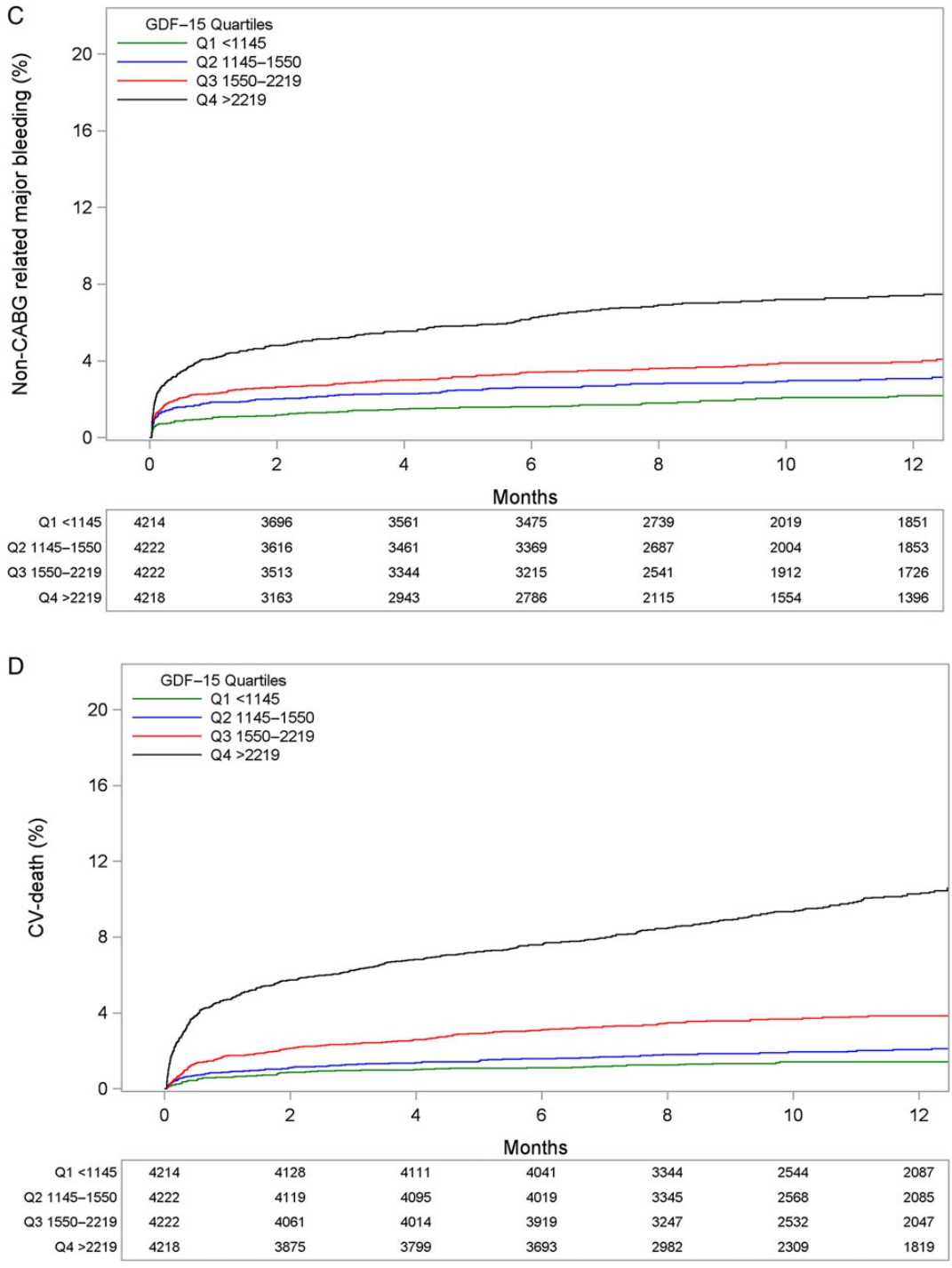


Figure 1 Continued.

GDF-15 (*Figure 2*). Higher level of GDF-15 was associated with higher rates of non-CABG related bleeding of all types and locations (see Supplementary material online, *Table S3*).

Primary outcome

During follow-up, 1446 patients suffered from the primary outcome (CV death, spontaneous MI, or stroke). Higher levels of GDF-15 at baseline were associated with a continuous increased risk of the primary outcome, and the estimates of event rates by quartiles showed gradually increasing differences between the event curves during follow-up (*Figures 1A*, *2* and Supplementary material online, *Table S2*). In adjusted models, higher quartiles of GDF-15 were associated with a gradually higher risk of the primary outcome (*Table 2*). Continuous GDF-15 level modelled in a spline graph suggested a linear

Downloaded from https://academic.oup.com/eurheartj/article-abstract/37/16/1325/1748693
by guest
on 09 November 2017

Table 2 Associations between growth differentiation factor-15 and outcome (n = 16 876)

Event	Model	Continuous GDF-15		GDF-15 quartile (Q1–Q4)				P-value*
		1 SD increase	P-value	Q1 (<1145 ng/L)	Q2 (1145–1550 ng/L)	Q3 (1550–2219 ng/L)	Q4 (≥2219 ng/L)	
Primary outcome	Model 1	1.44 (1.37–1.51)	<0.0001	Referent	1.23 (1.00–1.50)	1.70 (1.40–2.06)	2.52 (2.08–3.05)	<0.0001
	Model 2	1.29 (1.21–1.37)	<0.0001	Referent	1.21 (0.97–1.51)	1.54 (1.25–1.90)	1.83 (1.46–2.28)	<0.0001
Cardiovascular death	Model 1	1.70 (1.60–1.80)	<0.0001	Referent	1.19 (0.84–1.68)	1.92 (1.40–2.64)	3.96 (2.91–5.39)	<0.0001
	Model 2	1.41 (1.30–1.53)	<0.0001	Referent	1.04 (0.72–1.51)	1.48 (1.05–2.09)	2.01 (1.41–2.85)	<0.0001
All-cause death	Model 1	1.68 (1.58–1.78)	<0.0001	Referent	1.22 (0.88–1.69)	1.98 (1.46–2.68)	4.01 (2.99–5.38)	<0.0001
	Model 2	1.41 (1.31–1.53)	<0.0001	Referent	1.08 (0.76–1.53)	1.52 (1.09–2.10)	2.08 (1.49–2.91)	<0.0001
Spontaneous MI	Model 1	1.23 (1.14–1.32)	<0.0001	Referent	1.33 (1.02–1.72)	1.61 (1.25–2.07)	1.95 (1.51–2.53)	<0.0001
	Model 2	1.15 (1.05–1.26)	<0.0001	Referent	1.34 (1.02–1.77)	1.53 (1.16–2.01)	1.63 (1.21–2.20)	<0.0001
Procedure-related MI	Model 1	1.00 (0.87–1.15)	0.9767	Referent	0.88 (0.62–1.25)	1.06 (0.74–1.50)	1.06 (0.72–1.55)	0.7102
	Model 2	1.03 (0.87–1.21)	0.7623	Referent	0.96 (0.66–1.41)	1.24 (0.85–1.82)	1.23 (0.79–1.91)	0.4798
Stroke	Model 1	1.19 (1.04–1.38)	<0.0141	Referent	1.07 (0.63–1.81)	1.71 (1.05–2.78)	1.80 (1.09–2.98)	0.0231
	Model 2	1.19 (1.01–1.42)	<0.0434	Referent	1.19 (0.67–2.11)	1.87 (1.09–3.21)	1.84 (1.03–3.29)	0.0537
Non-CABG-related major bleeding	Model 1	1.37 (1.27–1.48)	<0.0001	Referent	1.23 (0.92–1.64)	1.40 (1.06–1.87)	2.17 (1.64–2.88)	<0.0001
	Model 2	1.37 (1.25–1.51)	<0.0001	Referent	1.09 (0.80–1.49)	1.28 (0.94–1.75)	1.78 (1.29–2.45)	0.0005

Estimates are HRs (95% CI). For continuous GDF-15, HRs are expressed per 1 SD GDF-15 increase.
Abbreviations as in *Table 1*.
*P-values for the effect of GDF-15.

increase for the primary outcome from GDF-15 levels ranging from ~1000 to 3500 ng/L (*Figure 2*). In Model 1, including extent of CAD, GDF-15 still predicted the primary outcome (HR 1.4, 95% CI 1.3–1.5) as well as its individual components [CV death (HR 1.7, 95% CI 1.6–1.8), spontaneous MI (HR 1.2, 95% CI 1.1–1.3), and non-CABG-related major bleeding (HR 1.4, 95% CI 1.3–1.5)], with similar results as models without inclusion of the extent of CAD. The association to stroke was attenuated (HR 1.2, 95% CI 0.99–1.4, $P = 0.0682$).

Cardiovascular and all-cause death

Cardiovascular and all-cause death occurred in 677 and 782 patients, respectively. Higher levels of GDF-15 were associated with a higher risk of CV and all-cause death, and the differences in estimated event rates continued to increase throughout follow-up (*Figure 1D* and Supplementary material online, *Figure S3A* and *Table S2*). Patients in quartile 4 had, compared with patients in quartile 1, 3.96 (95% CI 2.91–5.39) and 2.01 (95% CI 1.41–2.85), times higher risk for CV death when adjusting for clinical characteristics and for other prognostic biomarkers, respectively (*Table 2*). The spline graph showed a consistent increase in event rate for CV and all-cause death at GDF-15 levels from 1000 to ~4000 ng/L (*Figure 2*).

Spontaneous and procedure-related myocardial infarction

In the study, 742 patients suffered at least one spontaneous MI. Higher GDF-15 levels were associated with a higher risk of spontaneous MI. The differences in the estimated rates of spontaneous MI between the baseline GDF-15 quartiles gradually increased during the 12-month follow-up (*Figure 1B* and Supplementary material online, *Table S2*). Higher quartiles of GDF-15 were associated with a higher risk of spontaneous MI with 1.95 (95% CI 1.51–2.53) and 1.63 (95% CI 1.21–2.20) times increase of risk, respectively, in the highest compared with the lowest quartile using adjustment Models 1 and 2 (*Table 2*). The spline graph showed a consistent increase in event rate for spontaneous MI at GDF-15 levels ranging from 1000 to ~3500 ng/L (*Figure 2*). A total of 280 patients suffered a procedure-related MI. There was no significant association between the level of GDF-15 and the occurrence of procedure-related MI.

Stroke

A total of 207 patients had a stroke at least once during the study. Higher levels of GDF-15 were related to an elevated risk of stroke with a gradual increase in the difference in event rates between the GDF-15 quartiles during follow-up (see Supplementary material online, *Figure S3* and *Table S2*). In both adjusted Models 1 and 2, higher GDF-15 quartiles were associated with a higher risk of stroke with a 1.8 times higher risk in the highest when compared with the lowest quartile for both adjustment models (*Table 2*).

Interactions

There was no interaction detected between the GDF-15 level and the effect of randomized treatment on any outcome (see Supplementary material online, *Figure S1*). Furthermore, no interaction between GDF-15 by type of ACS (NSTE-ACS vs. STE-ACS) was

Downloaded from https://academic.oup.com/eurheartj/article-abstract/37/16/1325/1748693
by guest
on 09 November 2017

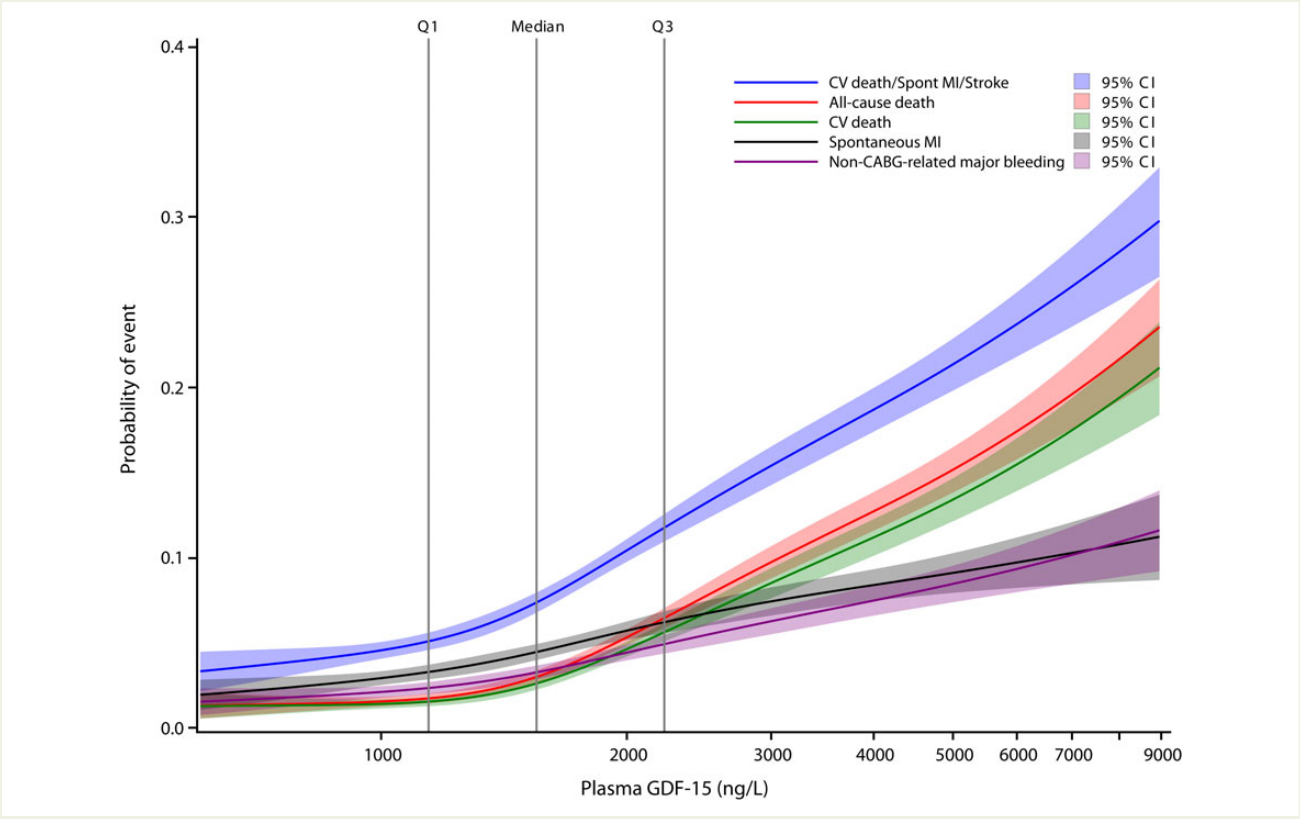


Figure 2 Twelve months event rate of the different outcomes. *Solid line*: event rate (with 95% confidence limits, shaded area) in relation to growth differentiation factor-15 transformed using restricted cubic splines with four knots. *X*-Axis is truncated at 1st and 99th percentiles. *Vertical lines*: quartile limits. Abbreviations as in *Table 1*.

observed for any outcome (see Supplementary material online, *Figure S2*).

The CAD category did not interact with the associations between the GDF-15 and the primary outcome ($P = 0.4695$) or any of the separate outcomes ($P \geq 0.1763$).

Prognostic value of growth differentiation factor-15

The model performance improved significantly for the prediction of the primary composite endpoint, CV death, all-cause death, and non-CABG-related major bleeding but not for stroke or MI (spontanous and procedure-related), when plasma GDF-15 was incorporated into Model 1 (see Supplementary material online, *Table S4*).

Discussion

In this study, we observed that the GDF-15 level is associated with all types of non-CABG-related major bleeding in patients with ACS treated with dual antiplatelet medication. The increased risk of major bleeding was independent of a wide range of other indicators of raised risk of bleeding such as age, gender, and comorbidities and also of other biomarkers indicating organ dysfunction. We also showed that, in patients with ACS, the GDF-15 level is independently associated with a raised risk of spontaneous MI but not with procedure-related MI. In addition, we observed that the GDF-15 level in patients with ACS is independently associated with a raised risk of stroke. Finally, we verified that the GDF-15 level is independently and strongly associated with an increased risk of CV and total mortality in ACS.

In line with previous studies of patients with ACS, this study showed that higher levels of GDF-15 predicted the composite of CV death, spontaneous MI, and stroke beyond established risk factors and newer biomarkers.[1,3–5,20–22] These previous findings were based on retrospective evaluations of smaller patient populations from a time when early revascularization and modern dual antiplatelet treatment were not standard of care. In the FRISC-2 study, GDF-15 predicted both short- and long-term risk of the composite of CV death and MI in non-revascularized patients,[2] whereas only late events were prognosticated in revascularized patients.[20] The present study corroborated the FRISC-2 findings that patients with elevated levels of GDF-15 had more severe CAD.[2] In comparison with the findings in previous trials, this present large scale trial provided clear evidence that GDF-15 is not only independently related to total and CV mortality but also to recurrent spontaneous MI and stroke. The lack of association between GDF-15 and procedure-related MI strengthens a possible pathophysiological link between elevated GDF-15 and the development of

Downloaded from https://academic.oup.com/eurheartj/article-abstract/37/16/1325/1748693
by guest
on 09 November 2017

atherosclerotic disease, as procedure-related MI most often appears to be a minor mechanically induced event without important long-term consequences.[22] We also observed that the associations between GDF-15 and disease development occur independent of intense platelet inhibition, early revascularization, and even extent of underlying CAD, as well as baseline characteristics, co-morbidities, and biomarkers associated with higher GDF-15 levels.

This study, for the first time in patients with ACS, showed that increasing levels of GDF-15 were independently associated with all types of bleeding complications during antiplatelet treatment. Reinforcing these associations are signs of causality with genetic effects on levels of GDF-15 and on non-CABG-related bleeding.[23] These findings are in line with our recent report that the level of GDF-15 is an independent risk factor for major bleeding in anticoagulated patients with atrial fibrillation.[16] The identification of a biomarker with a strong independent association with bleeding indicates an opportunity for an improved assessment of the balance between the risk of CV events and bleeding during antiplatelet treatments, which currently is associated with large difficulties when based on clinical variables alone.

In accordance with several previous studies of patients with ACS, GDF-15 was not only associated with prognosis, but also with a wide range of CV risk factors, such as age, male gender, smoking, diabetes mellitus, and comorbidities including myocardial and renal dysfunction as well as previous ischaemic events.[1–3,5,19,20] In agreement with previous studies, the level of GDF-15 was associated with several biomarkers indicating myocardial damage and dysfunction (troponins, NTpro-BNP), renal dysfunction (cystatin C), and inflammatory activity (CRP).[1–5,19,20] The increased C-indices and statistically significant LR tests for major bleeding, the composite ischaemic endpoint, and CV-death indicate that the addition of plasma GDF-15 to information from established cardiovascular risk factors, might represent an important improvement in risk stratification for several outcomes. If further validated and shown to influence selection of treatment, GDF-15 might be of clinical value in refining risk stratification and tailoring treatment of patients with ACS.

The underlying mechanisms explaining the independent associations between GDF-15 and CV disease and events are unknown. Part of the observed associations between GDF-15 and CV events probably is a reflection the burden of risk given its association with several CV risk factors, established CV disease and other biomarkers reflecting CV disease. In addition, GDF-15 seems to be a downstream marker of established cell stress, such as inflammation, oxidation, tissue injury, and tissue aging. The mechanism for the association between GDF-15 and the risk of bleeding may be that GDF-15 is expressed as a consequence of cellular stress and vulnerability, which might be related to a raised risk of bleeding due to external or internal trauma. There is also a potential for a specific mechanism as GDF-15 has been shown to have an inhibitory effect on platelet activation mediated via a mechanism similar to glycoprotein IIb/IIIa inhibition resulting in a lower ability to form thrombus.[24] Therefore, measurement of the GDF-15 level might provide unique information on underlying disease processes leading to a raised risk of severe events, e.g. fatal CV events and major bleeding during antithrombotic treatment.

Conclusions

In patients with ACS, higher levels of GDF-15 are associated with an increased risk of non-CABG-related major bleeding, a raised risk of recurrent spontaneous MI and stroke as well as with greater CV- and total mortality in models adjusting for established and newer risk factors. GDF-15 might in the future be a useful biomarker for tailoring antiplatelet treatment in patients with ACS. Furthermore, the association between GDF-15 and spontaneous MI, stroke and bleeding indicates a need for further investigations of possible direct effects of GDF-15 on the development and balance of these events in patients with ACS and other settings.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

Authors' contributions

M.B.: performed statistical analysis. S.K.J., R.C.B., A.H., S.H., H.A.K., P.G.S., R.F.S., A.S., L.W.: handled funding and supervision; E.H., S.K.J., R.F.S.: acquired the data. E.H., S.K.J., L.W.: drafted the manuscript. E.H., S.K.J., M.B., R.C.B., A.H., S.H., H.A.K., P.G.S., R.F.S., A.S., L.W., conceived and designed the research, and made critical revision of the manuscript for key intellectual content.

Acknowledgements

The complete list of PLATO investigators and main study committees has been published previously. We thank Ebba Bergman, PhD, Uppsala Clinical Research Center for editorial support.

Funding

This study was funded by AstraZeneca. Support for the analysis and interpretation of results and preparation of the manuscript was provided through funds to the Uppsala Clinical Research Center and Duke Clinical Research Institute as part of the Clinical Study Agreement. Roche Diagnostics supported the research by providing the pre-commercial assay of GDF-15 free of charge.

Conflict of interest: E.H.: institutional research grant from AstraZeneca, and AMGEN, Sanofi and honoraria from Sanofi, AMGEN, and Ariad. S.K.J.: institutional research grant from AstraZeneca, Terumo Inc., Medtronic, and Vascular Solutions; honoraria from The Medicines Company and AstraZeneca; and consultant/advisory board fees from AstraZeneca, Dachii Sanchio, Janssen, Medtronic, and Sanofi. M.B.: institutional research grant from AstraZeneca. R.C.B.: scientific advisory board member for Regado Biosciences, Daiichi-Sankyo, Portola, and Boehringer Ingelheim. A.H.: employee of AstraZeneca. S.H.: advisory board member for AstraZeneca, Bristol-Myers Squibb, Pfizer, and Bayer; research support from GlaxoSmithKline and Pfizer. H.A.K.: honoraria from AstraZeneca, Eli Lilly, GlaxoSmithKline, Roche, and Bayer; and holds a Troponin T Test Invention patent jointly with Roche and receives royalties for this patent. P.G.S.: fees from Amarin, AstraZeneca, Bayer, Bristol-Myers Squibb, Boehringer Ingelheim, Daiichi-Sankyo, GlaxoSmithKline, Merck, Novartis, Otsuka, Pfizer, Roche, Sanofi, Servier, The Medicines Company, and Vivus for steering committees, data monitoring committees, event committees, and consulting activities. P.G.S.s institution receives research grants from Sanofi and Servier; P.G.S. is a stockholder in Aterovax. R.F.S.: research grants from AstraZeneca and Merck; research support from

Downloaded from https://academic.oup.com/eurheartj/article-abstract/37/16/1325/1748693
by guest
on 09 November 2017

Accumetrics; honoraria from AstraZeneca, Accumetrics, and Medscape; consultancy fees from AstraZeneca, Correvio, Accumetrics, Sanofi-Aventis, Regeneron, PlaqueTec, Roche, and Daiichi-Sankyo; named by the company as an inventor on a patent pending related to discoveries made during the PEGASUS-TIMI 54 study but has no personal financial interest in this. A.S.: institutional research grants from AstraZeneca, Boehringer Ingelheim, and Bristol-Myers Squibb. L.W.: institutional research grants from AstraZeneca, Merck & Co, Boehringer Ingelheim, Bristol-Myers Squibb/Pfizer, Roche GlaxoSmithKline; consultant for Abbott, Merck & Co, Regado Biosciences, Athera Biotechnologies, Boehringer Ingelheim, AstraZeneca, GlaxoSmithKline, and Bristol-Myers Squibb/Pfizer; lecture fees from AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb/Pfizer, GlaxoSmithKline; honoraria from Boehringer Ingelheim, AstraZeneca, Bristol-Myers Squibb/Pfizer, GlaxoSmithKline; travel support from AstraZeneca, Bristol-Myers Squibb/Pfizer, and GlaxoSmithKline.

References

1. Kempf T, Björklund E, Olofsson S, Lindahl B, Allhoff T, Peter T, Tongers J, Wollert KC, Wallentin L. Growth-differentiation factor-15 improves risk stratification in ST-segment elevation myocardial infarction. *Eur Heart J* 2007;**28**:2858–2865.
2. Wollert KC, Kempf T, Lagerqvist B, Lindahl B, Olofsson S, Allhoff T, Peter T, Siegbahn A, Venge P, Drexler H, Wallentin L. Growth differentiation factor 15 for risk stratification and selection of an invasive treatment strategy in non ST-elevation acute coronary syndrome. *Circulation* 2007;**116**:1540–1548.
3. Wollert KC, Kempf T, Peter T, Olofsson S, James S, Johnston N, Lindahl B, Horn-Wichmann R, Brabant G, Simoons ML, Armstrong PW, Califf RM, Drexler H, Wallentin L. Prognostic value of growth-differentiation factor-15 in patients with non-ST-elevation acute coronary syndrome. *Circulation* 2007;**115**:962–971.
4. Widera C, Pencina MJ, Bobadilla M, Reimann I, Guba-Quint A, Marquardt I, Bethmann K, Korf-Klingebiel M, Kempf T, Lichtinghagen R, Katus HA, Giannitsis E, Wollert KC. Incremental prognostic value of biomarkers beyond the GRACE (Global Registry of Acute Coronary Events) Score and high-sensitivity cardiac troponin T in non-ST-elevation acute coronary syndrome. *Clin Chem* 2013;**59**:1497–1505.
5. Bonaca MP, Morrow DA, Braunwald E, Cannon CP, Jiang S, Breher S, Sabatine MS, Kempf T, Wallentin L, Wollert KC. Growth differentiation factor-15 and risk of recurrent events in patients stabilized after acute coronary syndrome: observations from PROVE IT-TIMI 22. *Arterioscler Thromb Vasc Biol* 2011;**31**:203–210.
6. Xu J, Kimball TR, Lorenz JN, Brown DA, Bauskin AR, Klevitsky R, Hewett TE, Breit SN, Molkentin JD. GDF15/MIC-1 functions as a protective and antihypertrophic factor released from the myocardium in association with SMAD protein activation. *Circ Res* 2006;**98**:342–350.
7. Schlittenhardt D, Schober A, Strelau J, Bonaterra GA, Schmiedt W, Unsicker K, Metz J, Kinscherf R. Involvement of growth differentiation factor-15/macrophage inhibitory cytokine-1 (GDF-15/MIC-1) in oxLDL-induced apoptosis of human macrophages in vitro and in arteriosclerotic lesions. *Cell Tissue Res* 2004;**318**:325–333.
8. Kempf T, Eden M, Strelau J, Naguib M, Willenbockel C, Tongers J, Heineke J, Kotlarz D, Xu J, Molkentin JD, Niessen HW, Drexler H, Wollert KC. The transforming growth factor-beta superfamily member growth-differentiation factor-15 protects the heart from ischemia/reperfusion injury. *Circ Res* 2006;**98**:351–360.
9. Kempf T, Zarbock A, Widera C, Butz S, Stadtmann A, Rossaint J, Bolomini-Vittori M, Korf-Klingebiel M, Napp LC, Hansen B, Kanwischer A, Bavendiek U, Beutel G, Hapke M, Sauer MG, Laudanna C, Hogg N, Vestweber D, Wollert KC. GDF-15 is an inhibitor of leukocyte integrin activation required for survival after myocardial infarction in mice. *Nat Med* 2011;**17**:581–588.
10. Heger J, Schiegnitz E, von Waldthausen D, Anwar MM, Piper HM, Euler G. Growth differentiation factor 15 acts anti-apoptotic and pro-hypertrophic in adult cardiomyocytes. *J Cell Physiol* 2010;**224**:120–126.
11. Lind L, Wallentin L, Kempf T, Tapken H, Quint A, Lindahl B, Olofsson S, Venge P, Larsson A, Hulthe J, Elmgren A, Wollert KC. Growth-differentiation factor-15 is an independent marker of cardiovascular dysfunction and disease in the elderly: results from the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) Study. *Eur Heart J* 2009;**30**:2346–2353.
12. Daniels LB, Clopton P, Laughlin GA, Maisel AS, Barrett-Connor E. Growth-differentiation factor-15 is a robust, independent predictor of 11-year mortality risk in community-dwelling older adults: the Rancho Bernardo Study. *Circulation* 2011;**123**:2101–2110.
13. Rohatgi A, Patel P, Das SR, Ayers CR, Khera A, Martinez-Rumayor A, Berry JD, McGuire DK, de Lemos JA. Association of growth differentiation factor-15 with coronary atherosclerosis and mortality in a young, multiethnic population: observations from the Dallas Heart Study. *Clin Chem* 2012;**58**:172–182.
14. Wallentin L, Zethelius B, Berglund L, Eggers KM, Lind L, Lindahl B, Wollert KC, Siegbahn A. GDF-15 for prognostication of cardiovascular and cancer morbidity and mortality in men. *PLoS ONE* 2013;**8**:e78797.
15. Eggers KM, Kempf T, Wallentin L, Wollert KC, Lind L. Change in growth differentiation factor 15 concentrations over time independently predicts mortality in community-dwelling elderly individuals. *Clin Chem* 2013;**59**:1091–1098.
16. Wallentin L, Hijazi Z, Andersson U, Alexander JH, De Caterina R, Hanna M, Horowitz JD, Hylek EM, Lopes RD, Asberg S, Granger CB, Siegbahn A. Growth differentiation factor 15, a marker of oxidative stress and inflammation, for risk assessment in patients with atrial fibrillation: insights from the Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation (ARISTOTLE). *Circulation* 2014;**130**:1847–1858.
17. Wallentin L, Becker RC, Budaj A, Cannon CP, Emanuelsson H, Held C, Horrow J, Husted S, James S, Katus H, Mahaffey KW, Scirica BM, Skene A, Steg PG, Storey RF, Harrington RA, Freij A, Thorsén M. Ticagrelor versus clopidogrel in patients with acute coronary syndromes. *N Engl J Med* 2009;**361**:1045–1057.
18. James S, Akerblom A, Cannon CP, Emanuelsson H, Husted S, Katus H, Skene A, Steg PG, Storey RF, Harrington R, Becker R, Wallentin L. Comparison of ticagrelor, the first reversible oral P2Y (12) receptor antagonist, with clopidogrel in patients with acute coronary syndromes: Rationale, design, and baseline characteristics of the PLATelet inhibition and patient Outcomes (PLATO) trial. *Am Heart J* 2009;**157**:599–605.
19. Lin DY, Wei LJ, Ying Z. Model-checking techniques based on cumulative residuals. *Biometrics* 2002;**58**:1–12.
20. Eggers KM, Kempf T, Lagerqvist B, Lindahl B, Olofsson S, Jantzen F, Peter T, Allhoff T, Siegbahn A, Venge P, Wollert KC, Wallentin L. Growth-differentiation factor-15 for long-term risk prediction in patients stabilized after an episode of non-ST-segment-elevation acute coronary syndrome. *Circ Cardiovasc Genet* 2010;**3**:88–96.
21. Khan SQ, Ng K, Dhillon O, Kelly D, Quinn P, Squire IB, Davies JE, Ng LL. Growth differentiation factor-15 as a prognostic marker in patients with acute myocardial infarction. *Eur Heart J* 2009;**30**:1057–1065.
22. Damman P, Wallentin L, Fox KAA, Windhausen F, Hirsch A, Clayton T, Pocock SJ, Lagerqvist B, Tijssen JGP, de Winter RJ. Long-term cardiovascular mortality after procedure-related or spontaneous myocardial infarction in patients with non-ST-segment elevation acute coronary syndrome: a collaborative analysis of individual patient data from the FRISC II, ICTUS, and RITA-3 tri. *Circulation* 2012;**125**:568–576.
23. Hagstrom E, Eriksson N, Johansson A, Bertilsson M, Axelsson T, Barratt BJ, Becker RC, Himmelmann A, James SK, Katus HA, Siegbahn A, Steg PG, Storey RF, Syvanen A-C, Varenhorst C, Akerblom A, Wallentin L. Are there any causal relations between growth differentiation factor 15 and outcomes in patients with acute coronary syndrome? – a report from the Plato Gwas Study. *Circulation* 2013;**128**:A17372.
24. Rossaint J, Vestweber D, Zarbock A. GDF-15 prevents platelet integrin activation and thrombus formation. *J Thromb Haemost* 2013;**11**:335–344.

Downloaded from https://academic.oup.com/eurheartj/article-abstract/37/16/1325/1748693
by guest
on 09 November 2017