

Growth Differentiation Factor 15 at 1 Month After an Acute Coronary Syndrome Is Associated With Increased Risk of Major Bleeding

Daniel Lindholm, MD, PhD; Emil Hagström, MD, PhD; Stefan K. James, MD, PhD; Richard C. Becker, MD; Christopher P. Cannon, MD; Anders Himmelmann, MD, PhD; Hugo A. Katus, MD; Gerald Maurer, MD; José Luis López-Sendón, MD, PhD; Philippe Gabriel Steg, MD; Robert F. Storey, MD, DM; Agneta Siegbahn, MD, PhD; Lars Wallentin, MD, PhD

Background—Growth differentiation factor-15 (GDF-15) is related to major bleeding when measured at initial presentation in patients with acute coronary syndromes (ACSs) treated with dual antiplatelet therapy. It is unknown whether follow-up measurements provide additional information. The objective of this study was to investigate whether GDF-15 measured 1 month after an ACS provides additional information beyond the baseline levels with regard to the risk of major bleeding.

Methods and Results—GDF-15 was measured at baseline and at 1 month after an ACS in 4049 patients included in the PLATelet inhibition and patient Outcomes (PLATO) trial. The association between 1-month GDF-15 level and non-coronary artery bypass grafting surgery-related major bleeding was assessed by a multivariable Cox model, adjusting for baseline GDF-15, age, anemia, impaired renal function, history of gastrointestinal bleeding, and sex. Elevated GDF-15 (>1800 ng/L) at 1 month was associated with an increased risk of non-coronary artery bypass grafting-related major bleeding (3.9% versus 1.2%; hazard ratio, 3.38; 95% CI, 1.89–6.06), independent of baseline GDF-15. Patients who had elevated GDF-15 levels at baseline and subsequent nonelevated GDF-15 at 1 month had a similar risk as patients who had nonelevated levels at both measurements.

Conclusions—GDF-15 at 1 month after an ACS is related to the risk of bleeding during DAPT and provides additional information on the bleeding risk beyond baseline GDF-15 levels. GDF-15 levels may therefore be useful as part of decision support concerning long-term antithrombotic treatment in patients post-ACS.

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Key Words: biomarker • bleeding • ischemic heart disease

Antithrombotic therapy is a cornerstone in the management of patients with acute coronary syndrome (ACS). Intense platelet inhibition in the acute phase and for 1 year after percutaneous coronary intervention (PCI) is associated with decreased rates of ischemic events, but at the cost of higher rates of bleeding.^{1–3} Recent studies have shown further reduction in ischemic events by dual antiplatelet

therapy (DAPT) beyond 1 year after an ACS and PCI.^{4–6} Patients with ACS are, however, heterogeneous regarding the risks for both ischemic and bleeding events. The most recent European Society of Cardiology non-ST-elevation ACS guidelines state that more-intense and prolonged P2Y₁₂ inhibition may be considered, taking into account the individual ischemic and bleeding risks.⁷ However, how these risks

From the Department of Medical Sciences, Cardiology and Uppsala Clinical Research Center (D.L., E.H., S.K.J., L.W.) and Department of Medical Sciences, Clinical Chemistry and Uppsala Clinical Research Center (A.S.), Uppsala University, Uppsala, Sweden; Division of Cardiovascular Health and Disease, Heart, Lung and Vascular Institute, University of Cincinnati College of Medicine, Cincinnati, OH (R.C.B.); Cardiovascular Division, Brigham and Women's Hospital, Boston, MA (C.P.C.); AstraZeneca Research and Development, Gothenburg, Sweden (A.H.); Medizinische Klinik, Universitätsklinikum Heidelberg, Heidelberg, Germany (H.A.K.); Medical University of Vienna, Austria (G.M.); Hospital Universitario La Paz, IdiPaz, Madrid, Spain (J.L.L.-S.); Département Hospitalo-Universitaire FIRE, AP-HP, Hôpital Bichat, Paris, France (P.G.S.); Paris Diderot University, Sorbonne Paris Cité, Paris, France (P.G.S.); NHLI Imperial College, ICMS, Royal Brompton Hospital, London, United Kingdom (P.G.S.); FACT (French Alliance for Cardiovascular Trials), an F-CRIN network, INSERM U1148, Paris, France (P.G.S.); Department of Infection, Immunity and Cardiovascular Disease, University of Sheffield, United Kingdom (R.F.S.).

Accompanying Table S1 and Figures S1, S2 are available at <http://jaha.ahajournals.org/content/6/4/e005580/DC1/embed/inline-supplementary-material-1.pdf>

Correspondence to: Daniel Lindholm, MD, PhD, Uppsala Clinical Research Center, Dag Hammarskjölds väg 14B, 1 tr, SE-752 37 Uppsala, Sweden. E-mail: daniel.lindholm@ucr.uu.se

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should be assessed and balanced during DAPT has not been specified so far.

In order to facilitate the estimation of bleeding risk, we have been searching for circulating biomarkers associated with bleeding during antithrombotic treatment. Recently, we identified that plasma level of growth differentiation factor-15 (GDF-15), a member of the transforming growth factor-beta superfamily, was independently associated with, and contributed to, estimation of the risk of major bleeding events in ACS on DAPT when measured at the time of initial presentation.⁸ The association between the GDF-15 level and risk of major bleeding has also been verified in patients with atrial fibrillation receiving different types of oral anticoagulant therapy.⁹

In the present study, we hypothesized that a repeated measurement of GDF-15 level 1 month after an ACS might provide additional information with regard to risk of major bleeding beyond the GDF-15 level at the index event.

Methods

Study Population

The PLATElet inhibition and patient Outcomes (PLATO) trial was a randomized, double-blind trial comparing ticagrelor with clopidogrel on background aspirin treatment in patients with ACS, with 6 to 12 months of follow-up. It adhered to the Declaration of Helsinki and was approved by ethical review boards. All patients gave their written informed consent to participate. Ticagrelor treatment was associated with reductions in ischemic events, including mortality, as compared with clopidogrel. The design and overall results have been previously published.^{1,10} Eligibility criteria of the trial are found in Table S1.

A biomarker substudy was also part of the study program that aimed to include all patients at randomization (16 876 of the 18 624 randomized patients were included). In addition, repeated blood sampling in a subset of around 4000 patients also at 1 month after randomization was prespecified in the biomarker substudy. In the present study, we included patients who had samples available for GDF-15 both at randomization and at 1 month (n=4049). See Figure S1 for a flow chart of the patient selection. The first and last authors had full access to the data.

Growth Differentiation Factor-15

Samples were obtained by direct venipuncture at randomization and at 1 month. Plasma was frozen in aliquots and stored at -70°C until central analysis at the Uppsala Clinical Research Center laboratory. Levels of GDF-15 in plasma were determined with Elecsys electrochemiluminescence

immunoassay on a Cobas Immunoanalyzer system (Roche Diagnostics, Rotkreuz, Switzerland). The analytical details of the assay have been published previously.¹¹ Based on previous studies, elevated level of GDF-15 was defined as >1800 ng/L.^{11–13}

Outcome

For this study, the end point of interest was major bleeding not related to coronary artery bypass grafting (CABG) surgery. The PLATO definition of non-CABG-related major bleeding included both life-threatening bleeding (fatal, intracranial, or intrapericardial bleeding; hypovolemic shock or severe hypotension attributed to bleeding and requiring vasopressors or surgery; and decline in hemoglobin of >50 g/L) and other major bleeding (bleeding leading to significant disability; decline in hemoglobin of 30–50 g/L). As a secondary end point in this study, we also assessed the composite of cardiovascular death/myocardial infarction (MI)/stroke.

End points were assessed from the landmark set at the 1-month follow-up visit, that is, outcomes between randomization and 1 month were not considered.

All events were adjudicated by an independent central adjudication committee.

Statistical Analysis

Patient characteristics are presented as medians and interquartile range (IQR) for continuous variables and as percentage and number for categorical variables.

\log_2 -transformed levels of GDF-15 at baseline and 1 month are presented as box plots, and the individual relative change in GDF-15 from baseline to 1 month is presented as a waterfall plot (ie, an ordered bar plot, where each bar represents an individual patient's relative change in biomarker level from baseline to 1 month).

To explore the relationship between GDF-15 levels at baseline and at 1 month, we fitted Cox models with GDF-15 level entered as restricted cubic splines (unadjusted). The predicted event rates up until 330 days after baseline, and 300 days after the 1-month visit, are plotted in relation to GDF-15 concentration at each time point. The x-axis was truncated at the 1st percentile of baseline GDF-15 at the lower end and at the 99th percentile at the higher end.

GDF-15 levels (\log_2 transformed) at 1 month were modeled in an ordinary least squares model, including the following covariates: \log_2 -transformed baseline GDF-15, age, sex, smoking status, diabetes mellitus, ACS management strategy (invasive or noninvasive), hypertension, heart failure, baseline hemoglobin, baseline estimated glomerular filtration rate (eGFR), history of gastrointestinal bleeding, and history of

peripheral arterial disease, where continuous covariates were entered as restricted cubic splines to account for nonlinearities. Each covariate's contribution to the model was calculated as the partial Chi^2 statistic minus the covariate's degrees of freedom.

Kaplan–Meier estimated event rates for non-CABG-related major bleeding are plotted from a 1-month landmark up to 300 days according to GDF-15 elevation status at 1 month. Similar Kaplan–Meier curves from 1 month according to GDF-15 at 1 month stratified by baseline GDF-15 elevation status are presented as well.

Risk of non-CABG-related major bleeding in relation to GDF-15 at 1 month was also assessed in a Cox model adjusted for baseline GDF-15 elevation (>1800 ng/L), age (<75 years versus ≥ 75 years), impaired renal function (eGFR <50 mL/min per 1.73 m²), history of gastrointestinal bleeding, and sex, stratified on presence of anemia at baseline (defined as baseline hemoglobin <130 g/L in men, <120 g/L in women). Results are presented as estimated hazard ratio (HR) and 95% CIs. We assessed the model's discriminatory performance using Harrell's C index and compared it with the C index of a model where 1-month GDF-15 was excluded.

The statistical software R (version 3.3.2; R Foundation for Statistical Computing, Vienna, Austria) was used for all analyses.

Results

Clinical Characteristics

Clinical characteristics at randomization are presented in Table. A majority of patients were of male sex, and risk factors such as habitual smoking, hypertension, and diabetes mellitus were highly prevalent. Approximately one fifth had a history of MI and 12% a history of previous PCI. When adjusting these rates to include events occurring after randomization, including PCI during the initial hospitalization, 89% had suffered an MI and 74% had a previous PCI procedure.

Growth Differentiation Factor-15 Levels at Baseline and 1 Month

Median GDF-15 level at baseline was 1509 ng/L (IQR, 1127–2106) and at 1 month 1381 ng/L (IQR, 1036–1927). GDF-15 levels were similar in ticagrelor- and clopidogrel-treated patients both at baseline and at 1 month (data not shown). In Figure 1A, \log_2 -transformed GDF-15 levels at baseline and 1 month are presented, and, in Figure 1B, the individual relative change in GDF-15 is shown. Even though GDF-15 levels were marginally lower at 1 month at the population level, there were patients with substantial relative increases in GDF-15 and, likewise, patients with substantial relative decreases.

Table. Clinical Characteristics

Characteristic	Total Study Population		Patients With Non-CABG-Related Major Bleeding From Baseline During Follow-up		Patients Without Non-CABG-Related Major Bleeding From Baseline During Follow-up	
	(n=4049)		(n=149)		(n=3900)	
Age, median (IQR)	61	(54–70)	68	(60–74)	61	(53–70)
Male sex	70%	(2849)	59%	(88)	71%	(2761)
Randomized treatment: Ticagrelor	50%	(2018)	52%	(77)	50%	(1954)
Habitual smoker	37%	(1509)	32%	(48)	37%	(1461)
Hypertension	65%	(2650)	68%	(102)	65%	(2548)
Diabetes mellitus	22%	(883)	26%	(39)	22%	(844)
Previous heart failure	5%	(219)	7%	(11)	5%	(208)
Peripheral arterial disease	6%	(251)	9%	(13)	6%	(238)
Chronic kidney disease	8%	(312/3908)	17%	(25)	8%	(287)
MI before index event	20%	(794)	19%	(28)	20%	(766)
Previous MI/Index event=MI	89%	(3611)	87%	(129)	89%	(3482)
PCI before index event	12%	(472)	14%	(21)	12%	(451)
Previous PCI/in-hospital PCI	74%	(2981)	83%	(124)	73%	(2857)
Baseline hemoglobin (g/L), median (IQR)	142	(132–151)	136	(124–145)	142	(132–151)
Baseline eGFR (mL/min per 1.73 m ²), median (IQR)	84	(67–101)	73.7	(55.5–94.3)	84.4	(67.3–101.4)

CABG indicates coronary artery bypass graft surgery; eGFR, estimated glomerular filtration rate; IQR, interquartile range; MI, myocardial infarction; PCI, percutaneous coronary intervention.

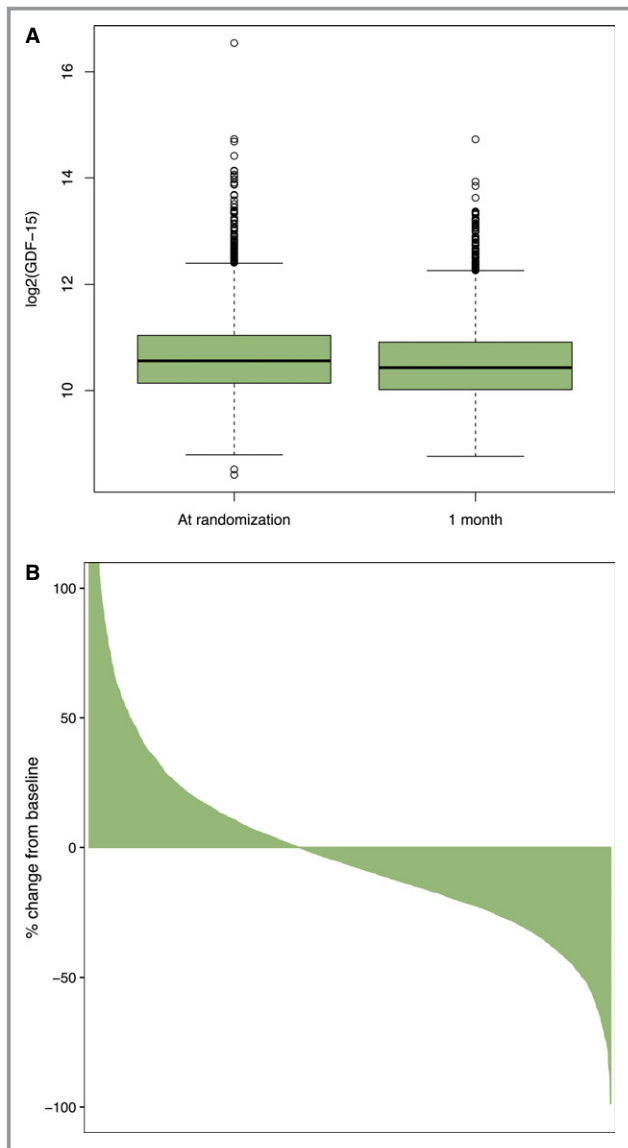


Figure 1. GDF-15 at baseline and at 1 month. A, Log_2 -transformed GDF-15 levels. B, Waterfall plot indicating relative change in GDF-15 levels between baseline and 1 month (please note that data are truncated at $\sim +100\%$; a few patients had $>100\%$ relative increase in GDF-15). The plot is an ordered bar plot, where each patient's % change in GDF-15 from baseline to 1 month is plotted. Although GDF-15 levels were only slightly lower on the population level (A), some patients had substantial relative changes in GDF-15 (B). GDF-15 indicates growth differentiation factor-15.

When modeling 1-month GDF-15 levels with an ordinary least squares model, the most important predictors were: baseline GDF-15 levels, age, diabetes mellitus, and eGFR (Figure S2), and this model explained 59% of the variance in 1-month GDF-15.

Major Bleeding in Relation to Growth Differentiation Factor-15 Levels

From 1 month onward, there were 71 events of non-CABG-related major bleeding. Increased GDF-15 was associated with

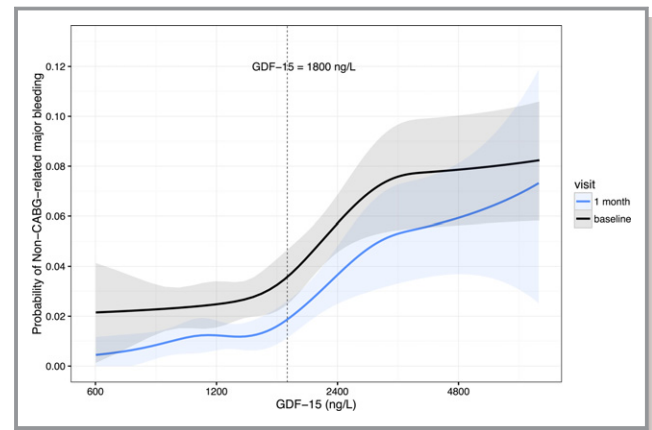


Figure 2. Probability of non-CABG-related major bleeding in relation to GDF-15 assessed from baseline (black) and from the 1-month follow-up visit (blue). The dashed line indicates a GDF-15 level of 1800 ng/L, which was used as a cutoff to define GDF-15 elevation in this study. CABG indicates coronary artery bypass grafting. CABG indicates coronary artery bypass grafting; GDF-15 indicates growth differentiation factor-15.

a higher probability of non-CABG-related major bleeding during follow-up, both when assessed from baseline and from the 1-month follow-up visit (Figure 2). The spline plot also indicated that the selected 1800 ng/L cutoff could separate patients at high risk of bleeding from those with low risk, given that the slope of the curves tended to increase at around that point.

In patients with GDF-15 levels above 1800 ng/L at 1 month, 3.9% experienced non-CABG-related major bleeding from 1 month up to 300 days thereafter, as compared with 1.2% in patients with nonelevated GDF-15 at 1 month (Figure 3). In the multivariable analysis, elevated GDF-15 at 1 month was associated with a 3-fold increased risk of non-CABG-related major bleeding (HR, 3.38; 95% CI, 1.89–6.06). The C index was 0.67, compared with 0.63 in a model excluding 1-month GDF-15. There was also an increased incidence of the composite end point cardiovascular death/MI/stroke in patients with elevated GDF-15 (9.2% in those with GDF-15 >1800 ng/L at 1 month, compared with 4.7% for patients with nonelevated GDF-15).

Patients who had nonelevated baseline GDF-15 levels and subsequent elevation of GDF-15 at 1 month were at increased risk for non-CABG-related major bleeding, compared with patients who had nonelevated values at both measurements. In those who had elevated baseline GDF-15 and subsequent nonelevated GDF-15, the risk of non-CABG-related major bleeding from 1 month onward was similar as in patients who had nonelevated levels at both measurements (Figure 4).

Discussion

The main finding in this study was that the GDF-15 level at 1 month post-ACS provided additional information regarding

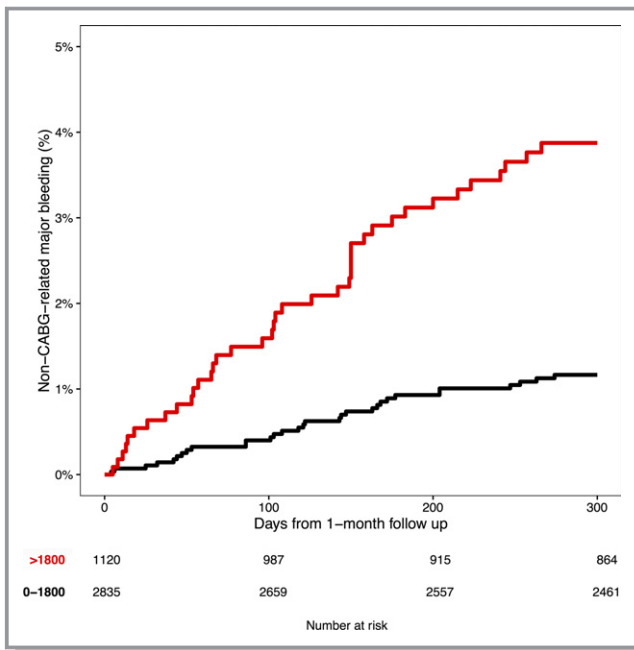


Figure 3. Kaplan-Meier estimates of non-CABG-related major bleeding from the 1-month follow-up visit according to 1-month GDF-15 levels (ng/L). CABG indicates coronary artery bypass grafting.

risk of non-CABG-related major bleeding beyond that of GDF-15 levels obtained in the acute phase. When adjusting for baseline characteristics, including baseline GDF-15 level, an elevated level of GDF-15 at 1 month was associated with a 3-fold increased risk of non-CABG-related major bleeding during DAPT after an ACS.

Previous studies have demonstrated an association between GDF-15 level and risk of bleeding both in patients with ACS treated with DAPT and in those with atrial fibrillation treated with oral anticoagulation, when measuring GDF-15 once at the initial presentation.^{8,9} The present findings validate the level of GDF-15 as a useful indicator of risk of bleeding beyond the acute setting, and indicate that GDF-15 might be a useful marker for monitoring bleeding risk in patients with coronary artery disease on antithrombotic treatment.

GDF-15 is, however, also a biomarker associated with other cardiovascular outcomes. It is independently related to the composite of recurrent ischemic events and mortality in ACS,^{8,12,14} as well as in stable coronary artery disease.¹⁵ GDF-15 is also associated with increased risk of the composite of MI and death in the general population.¹⁶⁻¹⁸ Furthermore, it is associated with both myocardial¹⁶ and renal dysfunction.¹⁹ Therefore, information on the GDF-15 level should preferably be used in multivariable clinical prediction models as one of several risk indicators when balancing the risk of bleeding and risk of ischemic events in patients on antithrombotic treatments.

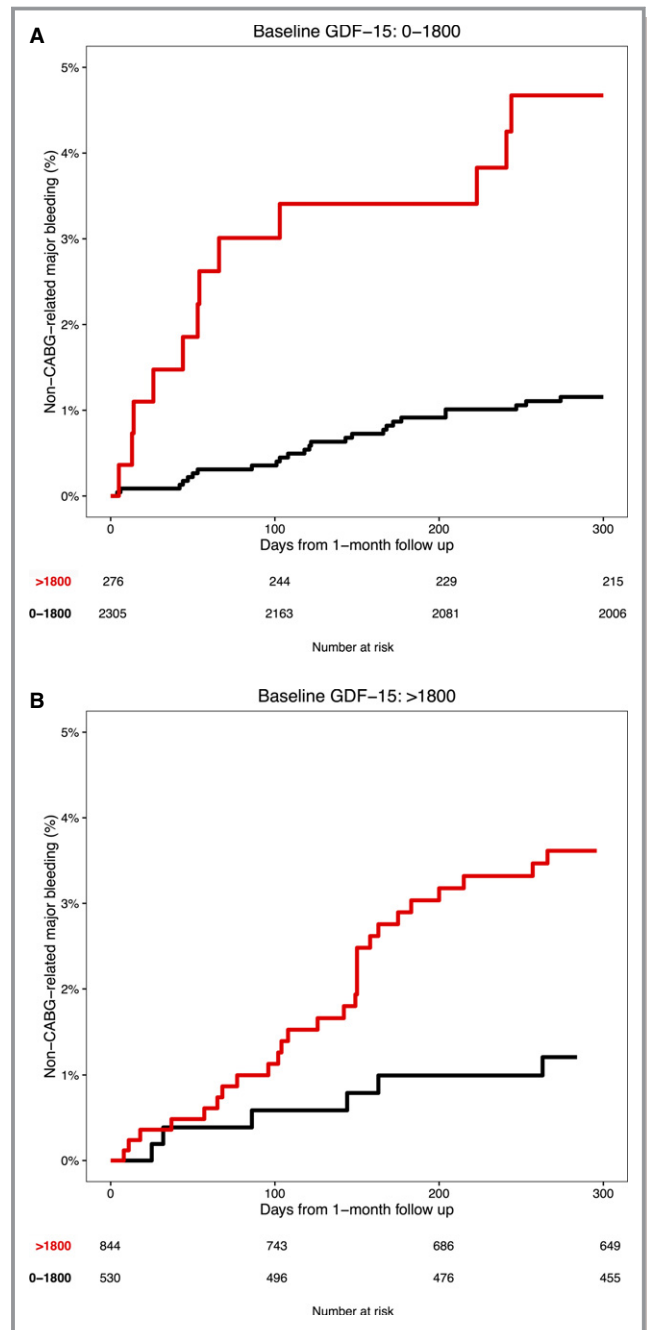


Figure 4. Kaplan-Meier estimates of non-CABG-related major bleeding from the 1-month follow-up visit according to 1-month GDF-15 levels in (A) patients with nonelevated (≤ 1800 ng/L) baseline GDF-15 levels and (B) patients with elevated (> 1800 ng/L) baseline GDF-15 levels. CABG indicates coronary artery bypass grafting.

The possible mechanisms underlying the independent association between GDF-15 and cardiovascular events have been proposed to be related to its association with inflammation,²⁰ oxidative stress,²¹ endothelial and myocardial dysfunction,¹⁶ atherosclerosis, and aging.²² In response to tissue injury like MI, there is a further elevation of GDF-

15 that might be related to further activation of inflammatory activity and myocardial stress.^{23,24} With regard to the more recently discovered bleeding association, the mechanism might still be related to frailty and cellular aging.²⁴ However, GDF-15 knockout mice show accelerated thrombus formation compared with wild type, and, *in vitro*, GDF-15 has been shown to inhibit platelet integrin activation,²⁵ which provides a possible pathophysiological link between the observed association between GDF-15 levels and bleeding in patients treated with antithrombotic and/or anticoagulant therapies.

Currently, there is intense discussion on the duration and intensity of DAPT and/or the combination of platelet inhibition with oral anticoagulation in patients with coronary artery disease. In this situation, there is often a delicate balance between ischemic risk and bleeding risk that has to be taken into account, as expressed in recent guidelines.⁷ In a substudy of the DAPT trial, a risk score to guide these decisions was proposed, although this used age as the only indicator of bleeding risk without providing any estimate of the discriminatory ability of the final model.²⁶ In the present study, the bleeding signal captured by GDF-15 levels provided incremental information beyond age and other clinical factors, both before start of treatment in the acute phase and when repeated during DAPT treatment in the chronic phase.

Limitations

There are several limitations to this work. First, patients with biomarkers at 1 month were a subset of all patients in the biomarker substudy of PLATO. Second, with a landmark analysis at 1 month, the risk of time-dependent confounding is acknowledged. Third, there were relatively few bleeding events, allowing for adjustment for few variables. Fourth, the exclusion criteria of the PLATO trial included known risk factors for bleeding (eg, oral anticoagulant therapy and thrombocytopenia), which precluded adjustment for these factors in the present study.

Conclusion

GDF-15 level measured at 1 month after an ACS is related to risk of bleeding during DAPT and provides additional information on bleeding risk beyond the level of GDF-15 at baseline and other clinical risk indicators of bleeding. GDF-15 level may therefore be useful to support decision making on the intensity and duration of long-term antithrombotic treatment in patients with coronary artery disease. Further prospective studies are, though, warranted to assess whether availability of GDF-15 levels at presentation and during follow-up after an ACS will improve clinical outcomes.

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SUPPLEMENTAL MATERIAL

Table S1. Eligibility criteria of the PLATO trial.

<i>Inclusion criteria</i>	<i>Exclusion criteria</i>
<ul style="list-style-type: none"> • STEMI (persistent ST-elevation ≥ 1 mm in ≥ 2 contiguous leads or new LBBB), with primary PCI planned <p style="text-align: center;"><i>or:</i></p> <ul style="list-style-type: none"> • NSTEMI-ACS with ≥ 2 of the following: <ul style="list-style-type: none"> ▪ ST-segment changes on ECG indicating ischemia (ST-depression or transient elevation ≥ 1 mm in two or more contiguous leads) ▪ Positive biomarker indicating myocardial necrosis (troponin or CK-MB above the upper limit of normal) ▪ ≥ 1 of the following: <ul style="list-style-type: none"> ○ ≥ 60 years of age ○ Previous MI or CABG ○ Coronary artery disease with $\geq 50\%$ stenosis in ≥ 2 vessels ○ Previous ischemic stroke/TIA, carotid stenosis ($\geq 50\%$), or cerebral revascularization ○ Diabetes mellitus ○ Peripheral artery disease ○ Chronic renal dysfunction 	<ul style="list-style-type: none"> • Contraindication to clopidogrel (e.g. hypersensitivity, moderate or severe liver disease, bleeding, major surgery within 30 days) • Oral anticoagulant therapy • Fibrinolytic therapy planned or within the previous 24 hours • Concomitant therapy with strong CYP3A inhibitors, CYP3A substrates with narrow therapeutic indices, or strong CYP3A inducers • Index event is an acute complication of PCI • PCI after index event and before first study dose • Increased risk of bradycardiac events • Dialysis required • Known clinically important thrombocytopenia • Known clinically important anemia • Any other condition that may put the patient at risk or influence study results in the investigators' opinion (e.g. cardiogenic shock, severe hemodynamic instability, active cancer) • Participation in another drug or device study within 30 days • Pregnancy or lactation

Figure S1. Selection of study population.

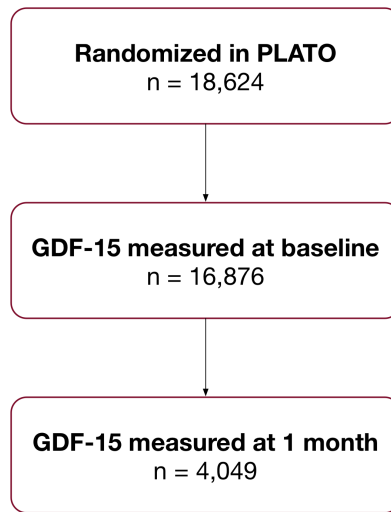
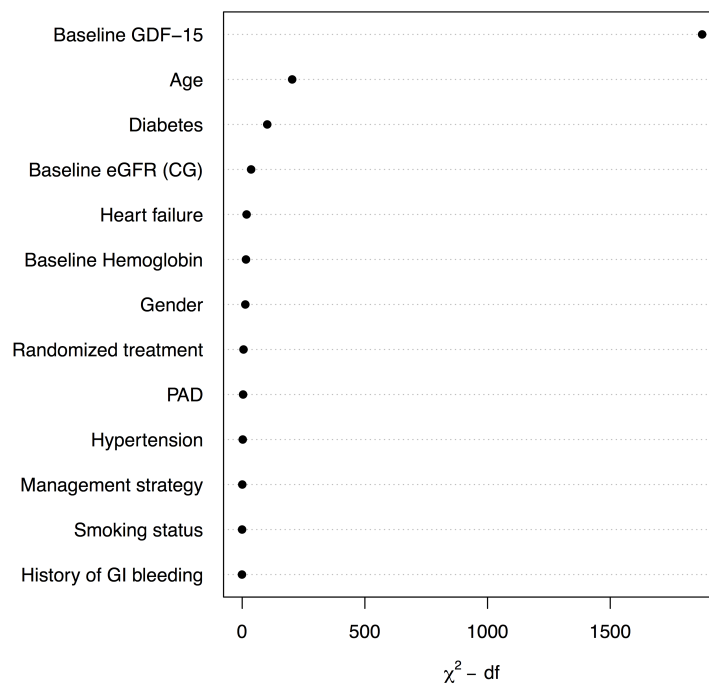


Figure S2. Importance of variables included in the ordinary least squares model for GDF-15 levels at 1 month, where importance is measured as $\chi^2 - df$.





Growth Differentiation Factor 15 at 1 Month After an Acute Coronary Syndrome Is Associated With Increased Risk of Major Bleeding

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