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) Trial

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Background—Growth differentiation factor 15 (GDF-15), high-sensitivity troponin, and N-terminal pro-brain natriuretic peptide levels are predictive of death and cardiovascular events in healthy elderly subjects, patients with acute coronary syndrome, and patients with heart failure. High-sensitivity troponin I and N-terminal pro-brain natriuretic peptide are also prognostic in patients with atrial fibrillation. We evaluated the prognostic value of GDF-15 alone and in addition to clinical characteristics and other biomarkers in patients with atrial fibrillation.

Methods and Results—The Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation (ARISTOTLE) trial randomized 18,201 patients with atrial fibrillation to apixaban or warfarin. Biomarkers were measured at randomization in 14,798 patients. Efficacy and safety outcomes during 1.9 years of follow-up were compared across quartiles of GDF-15 by use of Cox analyses adjusted for clinical characteristics, randomized treatment, and other biomarkers. The GDF-15 level showed a median of 1383 ng/L (interquartile range, 977–2052 ng/L). Annual rates of stroke or systemic embolism ranged from 0.9% to 2.03% (P<0.001); of major bleeding, from 1.22% to 4.53% (P<0.001); and of mortality, from 1.34% to 7.19% (P<0.001) in the lowest compared with the highest GDF-15 quartile. The prognostic information provided by GDF-15 was independent of clinical characteristics and clinical risk scores. Adjustment for the other cardiac biomarkers attenuated the prognostic value for stroke, whereas the prognostic value for mortality and major bleeding remained. Apixaban consistently reduced stroke, mortality, and bleeding, regardless of GDF-15 levels.

Conclusions—GDF-15 is a risk factor for major bleeding, mortality, and stroke in atrial fibrillation. The prognostic value for major bleeding and death remained even in the presence of N-terminal pro-brain natriuretic peptide and high-sensitivity troponin I.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT00412984.

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Key Words: atrial fibrillation • biological markers • cardiovascular physiological processes • growth differentiation factor 15 • natriuretic peptide • brain • risk assessment • troponin

Arrhythmia/Electrophysiology

Atrial fibrillation constitutes a major risk factor for stroke and death.1–3 The risk of stroke in atrial fibrillation is currently estimated by clinical risk factors, for example, the CHA2DS2-VASc score.4 The development of stroke can be prevented by oral anticoagulation. However, anticoagulant treatment is also associated with a risk of major bleeding, which can be predicted with the HAS-BLED clinical score.5

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The indication for stroke prevention by oral anticoagulation is based on balancing the reduction of stroke and the increase in bleeding in relation to the type of oral anticoagulation and patient characteristics. Unfortunately, the risk factors for stroke and major bleeding overlap to a large extent. Consequently, there is a need for new and independent risk indicators for stroke, bleeding, and mortality in patients with atrial fibrillation.

Recently, cardiac troponin, a marker of myocardial cell damage, was identified as an independent biomarker of the risk of stroke, mortality, and bleeding in patients with atrial fibrillation. N-terminal pro-brain natriuretic peptide (NT-proBNP), a marker of cardiac dysfunction, was also recently shown to contribute independent information on the risk of stroke and mortality in patients with atrial fibrillation. Markers of renal dysfunction, for example, creatinine clearance and cystatin-C, also provide additive information on cardiovascular outcomes and bleeding. In studies of healthy elderly subjects and in patients with congestive heart failure and chronic and acute coronary artery disease, provides independent prognostic information on cardiovascular events beyond cardiovascular risk factors and other biomarkers. In this predefined biomarker substudy within the Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation (ARISTOTLE) trial, we assessed the associations between GDF-15 levels at baseline and clinical outcomes, including adjustments for established cardiovascular risk factors and other biomarkers, in 14,798 patients with atrial fibrillation.

We also compared the prognostic information with that of the currently used CHA2DS2-VASc and HAS-BLED scores and evaluated the outcomes with apixaban compared with warfarin in relation to levels of GDF-15.

Methods

The ARISTOTLE Trial

The details of the ARISTOTLE trial have been published. The ARISTOTLE trial enrolled 18,201 patients with atrial fibrillation and at least 1 CHADS, risk factor for stroke. Patients were randomized in a double-blind manner to either dose-adjusted warfarin (n=9081) or apixaban (n=9120). The primary end point was stroke or systemic embolism. The median length of follow-up was 1.9 years for the participants in the biomarker cohort, consisting of 14,798 included patients with availability to plasma samples for determination of GDF-15 and other biomarkers. Approval by the appropriate ethics committees was obtained at all sites. All patients provided written informed consent.

End Points and Clinical Risk Classification

The end points in this study included stroke or systemic embolism; ischemic stroke and systemic embolism, hemorrhagic stroke, or myocardial infarction (MI); all-cause mortality; and cardiac death (excluding bleeding and other noncardiac causes). Bleeding was classified according to the International Society on Thrombosis and Haemostasis criteria, with International Society on Thrombosis and Haemostasis major bleeding as the primary safety end point. A blinded clinical events committee using prespecified criteria centrally evaluated all outcome events. Patients were classified by CHADS2 scores of 0 to 1, 2, or ≥3 and by CHA2DS2-VASc scores of 0 to 1, 2, 3, 4, and ≥5. Major bleeding outcomes were evaluated in relation to the HAS-BLED score classified by 0 to 1, 2, or ≥3.

Biochemical Methods

All patients were required to provide plasma samples at randomization that were frozen and stored in aliquots at −70°C until analyzed centrally. The GDF-15 level was determined by the Elecsys GDF-15 precommercial assay (Roche Diagnostics), which is composed of a monoclonal mouse antibody for capture and a monoclonal mouse antibody fragment, Fab (β’2), for detection and uses a sandwich assay format. Detection is based on an electrochemiluminescence immunoassay using a ruthenium(II) complex label. The precommercial assay correlates closely with a previously established immunoradiometric assay method (r=0.9801; regression Passing/Bablok: slope, 1.049; intercept, −136 ng/L). The assay is reported to have an interassay coefficient of variation of 2.3% at 100 ng/L and 1.8% at 17,200 ng/L; the intra-assay coefficient of variation was 0.8% at 1100 ng/L and 0.9% at 18,900 ng/L with a lower detection limit <10 ng/L. When intra-assay and interassay variabilities were combined, the coefficients of variation at our laboratory was 4.4% at 1500 ng/L and 4.5% at 5900 ng/L.

The other biomarker levels were analyzed with immunoassays: high-sensitivity cardiac troponin I (cTnI-hs) on the ARCHITECT i1000SR (Abbott Diagnostics), the NT-proBNP level on the Cobas Analytics e601 and c501 Immunoanalyzer (Roche Diagnostics), and cystatin-C with the Architect system ci8200 (Abbott Laboratories, Abbott Park, IL) using the particle-enhanced turbidimetric immunoassay from Gentian (Gentian, Moss, Norway). All central biochemical analyses were performed at the Uppsala Clinical Research Center laboratory (Uppsala, Sweden).

Statistical Analyses

Demographics and other baseline characteristics were summarized using frequencies for categorical variables and median and 25th and 75th percentiles for continuous variables. For tests of differences among groups, the χ2 test was used for categorical variables, and the Kruskal-Wallis test was used for continuous variables. Multivariable analysis of variance with natural logarithms of GDF-15 as response variables and categorized baseline characteristics as explanatory variables was used to investigate the independent effect of each variable. Geometric means, calculated by antilogs of the model-adjusted means, were compared. Correlations between biomarkers were analyzed by scatterplots and the Spearman rank correlation coefficient.

Efficacy analyses included all randomized patients and included all events from randomization until the efficacy cutoff date. Bleeding analyses were “on treatment,” including all randomized patients who received at least 1 dose of study drug.

The outcomes in relation to treatment and GDF-15 (continuous using restricted cubic splines and in quartile groups) were analyzed with a Cox proportional hazards model including treatment group, GDF-15, and treatment by GDF-15 interaction as covariates. Interactions with continuous GDF-15 were illustrated by plotting the estimated probability of 1-year events according to the continuous level of GDF-15, with separate curves for each treatment group.

The outcomes in relation to GDF-15 quartiles were evaluated in 3 multivariable Cox proportional hazards models. Model 1 adjusted for randomized treatment, previous warfarin/vitamin K antagonist treatment, and geographic region; model 2 also adjusted for established clinical risk factors; and model 3 adjusted for variables in model 2 and other biomarkers (cTnI, NT-proBNP, cystatin-C). Established risk factors included age (continuous); sex; body mass index; smoking status; systolic blood pressure; heart rate; atrial fibrillation type; diabetes mellitus; history of symptomatic congestive heart failure, previous stroke/systemic embolism/transient ischemic attack, hypertension, previous MI, or previous peripheral artery disease/coronary artery bypass graft/percutaneous coronary intervention; treatment at randomization with aspirin, angiotensin-converting...
enzyme inhibitor, or angiotensin receptor blocker; and use of amiodarone. For the major bleeding end point, history of anemia, history of spontaneous or clinical relevant bleeding, chronic liver disease, hematocrit, and treatment at randomization with nonsteroidal anti-inflammatory drugs were also included. Nonlinearity in the relationship between continuous variables and outcomes was handled by including restricted cubic spline terms in the Cox model, fitted with 4 knots located at the 5th, 35th, 65th, and 95th percentiles. The increased discriminative value of GDF-15, when added to a model including the variables in model 1 and the CHADSVASc or the HAS-BLED score was investigated by estimating the C index for survival data27 for models with and without GDF-15 and the continuous (category-free) net reclassification improvement index or survival data as described by Pencina et al.28 The net reclassification improvement index among events and among nonevents and the total net reclassification improvement index were analyzed. We performed likelihood ratio tests to evaluate whether the global model fit improved after the addition of GDF-15. Estimates of the cumulative incidence improvement index among events and among nonevents and the total net reclassification improvement index were calculated and plotted. All presented event rates were 2 tailed and performed at the 0.05 significance level. Because the analyses were exploratory, no adjustments for multiple comparisons were made. The Statistics Section at the Uppsala Clinical Research Center conducted the statistical analyses using SAS software, version 9.3 (SAS Institute Inc, Cary, NC).

**Results**

**Distribution of GDF-15**

The GDF-15 levels had a skewed distribution, with a median of 1383 ng/L (25th percentile, 977 ng/L; 75th percentile, 2052 ng/L) in the total population without any differences between the randomized treatment groups. Accordingly, 60.4% had GDF-15 levels >1200 ng/L, which is the 90% percentile of healthy subjects of similar age, and >32.3% had levels >1800 ng/L, considered to identify the highest-risk group among patients with coronary artery disease.

**Baseline Characteristics in Relation to GDF-15**

Almost all baseline characteristics, risk scores and medications were associated with GDF-15 (Table I). GDF-15 level was correlated to the levels of cystatin-C, NT-proBNP, and cTnI-hs (Figure I in the online-only Data Supplement). In multivariable analyses, the main independent drivers of higher GDF-15 levels were age, diabetes mellitus, cystatin-C (renal dysfunction), NT-proBNP and cTnI-hs (cardiac dysfunction), history of anemia, and current smoking, as shown in Table 2.

**Relation of GDF-15 to Stroke or Systemic Embolism in Atrial Fibrillation**

There were 396 events (1.40%/y) of stroke or systemic embolism during the median follow-up of 1.9 years. Annual rates of stroke increased significantly with rising GDF-15 levels. In unadjusted analysis, there was a doubling in the rate of stroke or systemic embolism, with an annual rate of 2.03% in patients with GDF-15 in the highest quartile (>2052 ng/L) compared with an annual rate of 0.90% in the lowest quartile (≤977 ng/L). These associations between GDF-15 levels at baseline and the risk for subsequent stroke remained stable over time (Figure 1A). The association remained significant after adjustment for baseline characteristics, including clinical risk factors and the CHA₂DS₂VASC score (Figure 2A). The GDF-15 level and the CHA₂DS₂VASC score provided statistically significant independent prognostic information on the risk of stroke; the c index was 0.667 for CHA₂DS₂VASC score alone and increased only slightly to 0.670 (P=0.0091) when GDF-15 was added. After adjustment also for other cardiac biomarkers, the association with stroke was no longer statistically significant (Figure 2A). Accordingly, the c index for stroke was unchanged when GDF-15 was added to a model including CHA₂DS₂VASC score and the other cardiac biomarkers. Continuous plasma GDF-15 level, modeled with restricted cubic regression spline, suggested that there is a consistent increase in hazard for the primary end point from 1000 to 3500 ng/L of GDF-15 (Figure 3A). There were no interactions between GDF-15 levels and the relative reduction in stroke and systemic embolism by apixaban compared with warfarin, which was similar throughout the range of GDF-15 (Figure 3A and Table I and Figure II in the online-only Data Supplement).

**GDF-15 in Relation to Mortality and Myocardial Infarction**

A total of 1061 all-cause deaths (3.69%/y), 537 cardiac deaths (1.88%/y), and 149 MIs (0.52%/y) were observed in this substudy cohort. Higher GDF-15 levels were strongly and significantly associated with total and cardiac mortality, with 4- to 5-times-higher rates in the highest compared with the lowest GDF-15 quartile in unadjusted analyses and a stable association between GDF-15 level and mortality over time (Figure 1B). After adjustment for baseline characteristics, risk factors, CHA₂DS₂VASC score, and other cardiac biomarkers, these associations remained significant for total mortality-adjusted hazard ratios of 2.10 (95% confidence interval, 1.62–2.73) in the highest compared with the lowest quartile of GDF-15 (Figure 2B and Table II in the online-only Data Supplement). The GDF-15 level and the CHA₂DS₂VASC score provided statistically significant independent prognostic information on mortality; the c index was 0.637 for CHA₂DS₂VASC score alone and increased substantially to 0.707 (P<0.0001) when GDF-15 was added. In unadjusted analysis, there was also a significant association between the GDF-15 level and subsequent MI, which vanished after adjustment for baseline characteristics, risk factors, and other biomarkers (Table I in the online-only Data Supplement). The analysis of continuous GDF-15 showed a consistent increase in hazard for total and cardiovascular death and MI throughout the range of GDF-15 levels (Figures II and III in the online-only Data Supplement).

The GDF-15 level, CHA₂DS₂VASC score, and other biomarkers provided statistically significant independent prognostic information on mortality; the c index was 0.744 without and increased to 0.755 (P<0.0001) with GDF-15. This corresponded to a net reclassification improvement of 26% with the addition of GDF-15. There were no interactions between GDF-15 levels and the relative reduction in mortality by apixaban compared with warfarin, which was similar throughout the range of GDF-15 levels (Table II and Figure II in the online-only Data Supplement).
GDF-15 in Relation to Major Bleeding

A total of 669 major bleedings (2.61%/y) were observed in this substudy cohort. Higher GDF-15 levels were significantly associated with a 3.5-times-higher rate of major bleeding in the highest compared with the lowest quartile in unadjusted analyses, and these associations remained stable over time (Figure 1C). These associations remained significant even after adjustment for all baseline characteristics, established risk factors, and cardiac biomarkers, with an adjusted hazard ratio of 2.00 (1.48–2.69) in the highest versus the lowest quartile of GDF-15 (Figure 2C).

The GDF-15 level and the HAS-BLED score provided significant independent prognostic information on major bleeding; the c index was 0.633 for HAS-BLED score alone and increased to 0.677 (P<0.0001) when GDF-15 was added. The analysis of continuous GDF-15 showed a consistent increase in the rate of major bleeding throughout the range of GDF-15 levels (Figure 3B).

Table 1. Demographics and Clinical Characteristics at Baseline According to GDF-15 Quartiles

<table>
<thead>
<tr>
<th>GDF-15 Level, ng/L</th>
<th>≤977</th>
<th>&gt;977–1383</th>
<th>&gt;1383–2052</th>
<th>&gt;2052</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>3705</td>
<td>3697</td>
<td>3699</td>
<td>3697</td>
<td></td>
</tr>
<tr>
<td>Age, y†</td>
<td>64.0 (57.0–70.0)</td>
<td>69.0 (63.0–75.0)</td>
<td>72.0 (66.0–78.0)</td>
<td>73.0 (68.0–79.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>2418 (65.3)</td>
<td>2303 (62.3)</td>
<td>2362 (63.9)</td>
<td>2441 (66.0)</td>
<td>0.0045</td>
</tr>
<tr>
<td>Smoker, n (%)</td>
<td>303 (8.2)</td>
<td>291 (7.9)</td>
<td>300 (8.1)</td>
<td>305 (8.2)</td>
<td>0.9374</td>
</tr>
<tr>
<td>Weight, kg†</td>
<td>85.0 (73.5–98.0)</td>
<td>82.1 (70.0–96.0)</td>
<td>81.0 (69.0–94.0)</td>
<td>80.0 (67.9–94.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>497 (13.4)</td>
<td>716 (19.4)</td>
<td>915 (24.7)</td>
<td>1535 (41.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>3271 (88.3)</td>
<td>3244 (87.7)</td>
<td>3198 (86.5)</td>
<td>3233 (87.4)</td>
<td>0.1123</td>
</tr>
<tr>
<td>CrCl, mL/min†</td>
<td>90.8 (73.3–113.0)</td>
<td>77.0 (62.0–96.0)</td>
<td>68.1 (53.8–86.3)</td>
<td>59.7 (44.7–78.4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Nonparoxysmal AF, n (%)</td>
<td>2983 (80.5)</td>
<td>3069 (83.7)</td>
<td>3206 (86.7)</td>
<td>3265 (88.3)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Previous stroke or TIA, n (%)</td>
<td>592 (16.0)</td>
<td>681 (18.4)</td>
<td>736 (19.9)</td>
<td>771 (20.9)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CHF within 3 mo, n (%)</td>
<td>1039 (28.0)</td>
<td>1042 (28.2)</td>
<td>1128 (30.5)</td>
<td>1383 (37.4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MI, n (%)</td>
<td>339 (9.1)</td>
<td>407 (11.0)</td>
<td>488 (13.2)</td>
<td>664 (18.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>PAD, n (%)</td>
<td>83 (2.2)</td>
<td>143 (3.9)</td>
<td>200 (5.4)</td>
<td>297 (8.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>History of anemia, n (%)</td>
<td>127 (3.4)</td>
<td>161 (4.4)</td>
<td>248 (6.7)</td>
<td>461 (12.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>History of clinical bleeding, n (%)</td>
<td>520 (14.0)</td>
<td>550 (14.9)</td>
<td>652 (17.6)</td>
<td>692 (18.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>PCI/CABG, n (%)</td>
<td>305 (8.2)</td>
<td>396 (10.7)</td>
<td>542 (14.7)</td>
<td>769 (20.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CHADS2/VASC≤1, n (%)</td>
<td>705 (19.0)</td>
<td>310 (8.4)</td>
<td>184 (5.0)</td>
<td>86 (2.3)</td>
<td>&lt;0.0001</td>
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<tr>
<td>CHADS2/VASC=2, n (%)</td>
<td>1100 (29.7)</td>
<td>914 (24.7)</td>
<td>618 (16.7)</td>
<td>448 (12.1)</td>
<td></td>
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<tr>
<td>CHADS2/VASC=3, n (%)</td>
<td>946 (25.5)</td>
<td>994 (26.9)</td>
<td>1030 (27.8)</td>
<td>865 (23.4)</td>
<td></td>
</tr>
<tr>
<td>CHADS2/VASC=4, n (%)</td>
<td>574 (15.5)</td>
<td>794 (21.5)</td>
<td>933 (25.2)</td>
<td>987 (26.7)</td>
<td></td>
</tr>
<tr>
<td>CHADS2/VASC≥5, n (%)</td>
<td>380 (10.3)</td>
<td>685 (18.5)</td>
<td>934 (25.3)</td>
<td>1311 (35.5)</td>
<td></td>
</tr>
<tr>
<td>On warfarin/VKA &lt;7 d, n (%)</td>
<td>1954 (52.7)</td>
<td>1996 (54.0)</td>
<td>2045 (55.3)</td>
<td>1962 (53.1)</td>
<td>0.1094</td>
</tr>
<tr>
<td>Aspirin, n (%)</td>
<td>1048 (28.3)</td>
<td>1074 (29.1)</td>
<td>1146 (31.0)</td>
<td>1305 (35.3)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ACE inhibitor or ARB, n (%)</td>
<td>2545 (68.7)</td>
<td>2618 (70.8)</td>
<td>2627 (71.0)</td>
<td>2685 (72.6)</td>
<td>0.0029</td>
</tr>
<tr>
<td>Calcium channel blocker, n (%)</td>
<td>989 (26.7)</td>
<td>1164 (31.5)</td>
<td>1159 (31.3)</td>
<td>1205 (32.6)</td>
<td>&lt;0.0001</td>
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<td>β-Blocker, n (%)</td>
<td>2351 (63.5)</td>
<td>2290 (61.9)</td>
<td>2337 (63.2)</td>
<td>2381 (64.4)</td>
<td>0.1784</td>
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<tr>
<td>Digoxin, n (%)</td>
<td>1040 (28.1)</td>
<td>1153 (31.2)</td>
<td>1265 (34.2)</td>
<td>1341 (36.3)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Amiodarone, n (%)</td>
<td>434 (11.7)</td>
<td>424 (11.5)</td>
<td>423 (11.4)</td>
<td>413 (11.2)</td>
<td>0.9100</td>
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<tr>
<td>Antiplatlet or NSAID, n (%)</td>
<td>2449 (66.1)</td>
<td>2369 (64.1)</td>
<td>2216 (59.9)</td>
<td>2056 (55.6)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Liver dysfunction, n (%)</td>
<td>116 (3.1)</td>
<td>110 (3.0)</td>
<td>98 (2.6)</td>
<td>98 (2.7)</td>
<td>0.5064</td>
</tr>
<tr>
<td>Hematocrit, %†</td>
<td>44.0 (41.0–46.0)</td>
<td>43.5 (41.0–46.0)</td>
<td>43.0 (40.0–46.0)</td>
<td>42.0 (38.0–45.0)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

ACE indicates angiotensin-converting enzyme; ARB, angiotensin receptor blocker; AF, atrial fibrillation; CABG, coronary artery bypass graft; CHF, congestive heart failure; CrCl, creatinine clearance; GDF-15, growth differentiation factor 15; MI, myocardial infarction; NSAID, nonsteroidal anti-inflammatory drug; PAD, pulmonary artery disease; PCI, percutaneous coronary intervention; TIA, transient ischemic attack; and VKA, vitamin K antagonist.

*The P value is for the comparison between groups and is based on the χ² test for categorical variables and Kruskal-Wallis test for continuous variables.
†Measured levels are median (Q1–Q3). Percentages are percentage of patients within GDF-15 quartile group with the specific baseline characteristic.
### Table 2. Impact of Baseline Characteristics, Including Biomarkers, on GDF-15 Level

<table>
<thead>
<tr>
<th>Variable</th>
<th>Variable Value</th>
<th>Model Including All Baseline Characteristics*</th>
<th>Model Also Including Biomarkers†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Geometric Mean</td>
<td>Ratio of Geometric Mean (95% CI)</td>
</tr>
<tr>
<td>Age category, y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;65</td>
<td>4454</td>
<td>1137.5</td>
<td>1.84 (1.81–1.87)</td>
</tr>
<tr>
<td>65–75</td>
<td>5804</td>
<td>1468.3</td>
<td>1.22 (1.19–1.24)</td>
</tr>
<tr>
<td>≥75</td>
<td>4540</td>
<td>1828.0</td>
<td>1.43 (1.40–1.46)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>5274</td>
<td>1390.1</td>
<td>1.00 (0.98–1.02)</td>
</tr>
<tr>
<td>Male</td>
<td>9524</td>
<td>1491.2</td>
<td>1.04 (1.02–1.06)</td>
</tr>
<tr>
<td>Current smoker</td>
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<tr>
<td>No</td>
<td>13585</td>
<td>1438.2</td>
<td>1.03 (0.97–1.09)</td>
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<tr>
<td>Yes</td>
<td>1199</td>
<td>1649.9</td>
<td>1.15 (1.11–1.18)</td>
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<tr>
<td>Diabetes mellitus</td>
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<tr>
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CABG indicates coronary artery bypass graft; CHF, congestive heart failure; CI, confidence interval; cTnI, cardiac troponin I; GDF-15, growth differentiation factor 15; MI, myocardial infarction; NSAID, nonsteroidal anti-inflammatory drug; NT-proBNP, N-terminal brain natriuretic peptide; PAD, pulmonary artery disease; PCI, percutaneous coronary intervention; and TIA, transient ischemic attack.

*The geometric means are calculated by the antilogs of the model-adjusted means of the natural log-transformed GDF-15. The geometric means by baseline characteristic category and ratios of geometric means are estimated in a model including only baseline characteristics. The geometric means for each baseline biomarker quartile group are estimated in models including baseline characteristics and the specific biomarker. Baseline characteristics also included in the model but not shown include body mass index, heart rate, bleeding history, region, baseline statin treatment, type of atrial fibrillation, and previous vitamin K antagonist use.

†The ratio of geometric means is estimated in a model including baseline characteristics and all 3 biomarkers.
with GDF-15. This corresponded to a net reclassification improvement of 33% with the addition of GDF-15. There were no interactions between GDF-15 levels and the relative reduction in major bleeding by apixaban compared with warfarin, which was similar throughout the range of GDF-15 levels (Figure 3B and Table II and Figure II in the online-only Data Supplement).

**Discussion**

The major findings of this study were that GDF-15 levels in patients with atrial fibrillation are higher than in healthy subjects\(^\text{18}\) but are fairly similar to those in elderly community dwellers.\(^\text{14}\) As in previous studies, the GDF-15 level was related to almost all cardiovascular risk factors and comorbidities, with age, diabetes mellitus, renal dysfunction (cystatin-C), myocardial dysfunction (NT-proBNP, cTnI-hs), history of anemia, and smoking being the main associations with a higher GDF-15 level.\(^\text{14,15,20,22,24,29,30}\) In unadjusted analyses, higher GDF-15 level was related to a higher risk of all cardiovascular outcomes, that is, stroke, total and cardiac mortality, MI, and major bleeding. After adjustment for all baseline characteristics, comorbidities, and biomarkers, the

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**Figure 1. A.** Cumulative hazard rate of stroke or systemic embolic events in relation to quartiles of growth differentiation factor 15 (GDF-15) at entry. **B.** Cumulative hazard rate of death in relation to quartiles of GDF-15 at entry. **C.** Cumulative hazard rate of major bleeding in relation to quartiles of GDF-15 at entry.
**Figure 2.** Outcomes by growth differentiation factor 15 (GDF-15) quartiles by Cox proportional hazards models. *Model 1 was adjusted for randomized treatment, previous warfarin/vitamin K antagonist treatment, and geographic region; **model 2 was also adjusted for established clinical risk factor; and ***model 3 was adjusted for both established clinical risk factors and other biomarkers (cardiac troponin I, N-terminal pro-brain natriuretic peptide, cystatin-C). A, Stroke and systemic embolic events (SEEs). B, Total death. C, Major bleeding. CI indicates confidence interval; and HR, hazard ratio.
GDF-15 level remained an independent risk indicator for major bleeding and death. Therefore, GDF-15 seems to be a biomarker improving risk stratification for bleeding and mortality beyond clinical risk factors in anticoagulated patients with atrial fibrillation.

So far, only clinical risk factors have been used for risk stratification of patients with atrial fibrillation in terms of their risk of stroke. The CHA2DS2-VASC score is currently the most commonly used risk score for stroke. Recently, we showed that the levels of the biomarkers NT-proBNP and cardiac troponin were able to improve the risk stratification for stroke compared with clinical risk factors alone. In addition, these biomarkers provided prognostication of mortality beyond clinical risk factors. In the present study, the measurement of GDF-15, like NT-proBNP and cardiac troponin, appeared to be a risk indicator for stroke and mortality beyond clinical characteristics and clinical risk scores. However, these 3 biomarkers partly reflect the same processes, that is, myocardial dysfunction and cardiovascular comorbidity. Therefore, in multivariable analysis, GDF-15 did not appear to be an independent risk indicator for stroke in the presence of both the other biomarkers and clinical characteristics. However, even in the presence of the other biomarkers and clinical characteristics, GDF-15 provided additional information on the risk of death, which might be useful if included in future risk stratification strategies.

Recommending anticoagulant treatment to patients with atrial fibrillation is based on balancing the risk of stroke and the risk of major bleeding during different kinds of anticoagulant treatment. The risk of bleeding during anticoagulant treatment might be predicted by the HAS-BLED score on the basis of the following factors: hypertension, abnormal renal or liver function, stroke history, bleeding history, labile international normalized ratio, advanced age, or drug use (antiplatelets, nonsteroidal anti-inflammatory drugs, or alcohol). Estimating the risk of bleeding is notoriously difficult, and the c index for the HAS-BLED score usually, as in this study, ends up <0.65. A c index rising from 0.63 to 0.68 with a 30% net reclassification improvement with the addition of GDF-15 to the HAS-BLED score indicates that GDF-15 might be a useful component of a biomarker-based score for the risk of bleeding. In the balance between risk of stroke and risk of major bleeding, it seems important to have biomarkers mainly indicating stroke, that is, NT-proBNP, and another mainly indicating bleeding, that is, GDF-15. In this context, it is important to emphasize that the reductions in major bleeding, including hemorrhagic stroke, with apixaban were similar throughout the range of GDF-15 levels and accordingly seen in patients with high and low risk of bleeding.

The identification of GDF-15 as a biomarker independently related to major bleeding might lead to both

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Figure 2. Continued.
improved risk stratification and a better understanding of the underlying causes of bleeding during anticoagulant treatment. GDF-15 is a divergent member of the transforming growth factor-β family that can be secreted from a broad range of cells, for example, cytokine secreted from adipocytes and myocytes in response to, and maybe protecting against, stress such as cellular ischemia and mechanical and oxidative stress. Plasma levels of GDF-15 are increased in response to inflammation and may be involved in maintaining the inflammatory activity. These experimental data and the results from this and other clinical studies suggest a link—protective or harmful—between GDF-15 and cellular stress as supported by the associations with age, diabetes mellitus, renal disease, smoking, congestive heart failure, and biomarkers of cardiac and renal dysfunction and inflammation. The understanding of the GDF-15 is limited because the GDF-15 receptor and the involved signaling pathways are unknown. Currently, the level of GDF-15 may to be interpreted mainly as an integrative signal of severity of disease in several different pathological conditions.

Figure 3. A. Estimated 12-month rate of stroke and systemic embolic events in relation to continuous plasma growth differentiation factor 15 (GDF-15) levels modeled with restricted cubic regression spline. Note the reference lines at the 25th, 50th (median), and 75th percentiles. B. Estimated 12-month rate of major bleeding in relation to continuous plasma GDF-15 levels modeled with restricted cubic regression spline. Note the reference lines at the 25th, 50th (median), and 75th percentiles. CI indicates confidence interval.
In the present study, the associations between the GDF-15 level and the clinical risk factors and biomarkers of cardiac and renal dysfunction seemed to explain the relations between the GDF-15 level and ischemic stroke. However, as in other clinical studies, an independent relation between the GDF-15 level and mortality remained, indicating the importance of an underlying cellular process, which currently might be detected only by the GDF-15 elevation. The independent association between GDF-15 and the risk of bleeding confirmed our recent publication of the same relationship in patients treated with dual antiplatelet treatment after acute coronary syndrome in the Platelet Inhibition and Patient Outcomes (PLATO) trial. The independent relation between the GDF-15 level and bleeding in the present trial was seen in relation to major and clinically relevant bleeding and trended in the same direction for hemorrhagic stroke. The cause of the association between GDF-15 and the risk of bleeding might be that GDF-15 is increased at cellular stress and cellular vulnerability, which might be associated with an increased risk of bleeding at different kinds of tissue damage. There is also a potential for a specific mechanism because GDF-15 has an inhibitory effect on platelet activation mediated via a mechanism similar to glycoprotein IIb/IIIa inhibition, resulting in a reduced ability to form thrombus.

Limitations
These data come from a population with atrial fibrillation and at least 1 risk factor for stroke who were included in a clinical trial and therefore may not be immediately generalized to the total atrial fibrillation population. The results concerning relations to clinical characteristics, other biomarkers, and outcome events are in accordance with those in other patient populations but should preferably also be verified in other cohorts with atrial fibrillation.

Conclusions
In patients with atrial fibrillation, GDF-15 is a risk factor for major bleeding, death, and stroke, in addition to and independently of clinical information. The additive prognostic value for major bleeding and death remains even in the presence of other biomarkers such as cTnI-hs and NT-proBNP. The addition of GDF-15 to clinical risk stratification models such as the CHA2DS2-VASc and HAS-BLED risk scores has the potential to improve risk assessment of bleeding and mortality. The benefits of apixaban compared with warfarin were consistent regardless of the GDF-15 levels.

Sources of Funding
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Disclosures
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References


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**CLINICAL PERSPECTIVE**

The risk of stroke and bleeding in atrial fibrillation is currently estimated by clinical risk factors, for example, the CHA₂DS₂-VASA and HAS-BLED scores. The clinical risk factors for stroke and major bleeding overlap to a large extent, therefore, provide limited guidance for tailoring of anticoagulant treatment. Recently, cardiac troponin and N-terminal pro-brain natriuretic peptide were shown to contribute independent information on the risk of stroke and mortality in patients with atrial fibrillation. In this biomarker substudy of 14,798 patients with atrial fibrillation randomized to oral anticoagulation with either apixaban or warfarin during a median time of 1.9 years, we evaluated the prognostic value of growth differentiation factor 15 (GDF-15), a marker of oxidative stress and inflammation, alone and in addition to clinical characteristics. The results showed that a higher level of GDF-15 was associated with a higher risk of all cardiovascular outcomes, that is, stroke, total and cardiac mortality, myocardial infarction, and major bleeding. After adjustment for all baseline characteristics, comorbidities, and other biomarkers, the GDF-15 level remained an independent risk indicator for major bleeding and death. Thus, GDF-15 seems to be a biomarker improving risk stratification for bleeding and mortality beyond clinical risk factors and other biomarkers in anticoagulated patients with atrial fibrillation. The benefits of apixaban compared with warfarin in terms of stroke, mortality, and major bleeding were consistent, regardless of the GDF-15 levels. The addition of GDF-15 to clinical risk stratification models has the potential to improve risk assessment of bleeding and mortality during anticoagulant treatment.
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) Trial

Lars Wallentin, Ziad Hijazi, Ulrika Andersson, John H. Alexander, Raffaele De Caterina, Michael Hanna, John D. Horowitz, Elaine M. Hylek, Renato D. Lopes, Signild Åsberg, Christopher B. Granger and Agneta Siegbahn

on behalf of the ARISTOTLE Investigators

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SUPPLEMENTAL MATERIAL

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) Trial

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Supplemental Table I. Outcomes by GDF-15 quartile group and effect of randomized treatment. Cox proportional hazards model with treatment, biomarker and interaction as covariates.

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<tr>
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<td>1839</td>
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<td>55 (1.47)</td>
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<tr>
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<tr>
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<td>1843</td>
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</tr>
<tr>
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<td>1871</td>
<td>1813</td>
<td>114 (3.85)</td>
<td>148 (5.25)</td>
<td>0.73 (0.58-0.94)</td>
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<tr>
<td>Major bleed/Clinical significant non-major bleed</td>
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<td></td>
</tr>
<tr>
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<td>1862</td>
<td>1836</td>
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<tr>
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<td>1880</td>
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</tr>
<tr>
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<tr>
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<td>1813</td>
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<td>235 (8.55)</td>
<td>0.74 (0.61-0.89)</td>
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GDF-15, growth differentiation factor 15; HR, hazard ratio; CI, confidence interval
### Supplemental Table II. Outcomes by GDF-15 quartiles by Cox proportional hazards models

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<tr>
<th>GDF-15 level (ng/L)</th>
<th>Events (%/yr)</th>
<th>Model 1† p-value</th>
<th>Model 2‡ p-value</th>
<th>Model 3§ p-value</th>
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<td>HR (95% CI)</td>
<td>HR (95% CI)</td>
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<td>Q1 as reference</td>
<td>Q1 as reference</td>
<td>Q1 as reference</td>
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<td><strong>Stroke/Systemic embolism</strong></td>
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<tr>
<td>≤977*</td>
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<td>0.0107</td>
<td>0.2763</td>
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<tr>
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<td>86 (1.21)</td>
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<td>1.13 (0.81-1.57)</td>
<td>1.03 (0.74-1.44)</td>
</tr>
<tr>
<td>&gt;1383-2052</td>
<td>109 (1.58)</td>
<td>1.70 (1.25-2.30)</td>
<td>1.37 (0.99-1.89)</td>
<td>1.22 (0.87-1.70)</td>
</tr>
<tr>
<td>&gt;2052</td>
<td>134 (2.03)</td>
<td>2.14 (1.60-2.88)</td>
<td>1.65 (1.19-2.30)</td>
<td>1.35 (0.94-1.94)</td>
</tr>
<tr>
<td><strong>Death</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤977</td>
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<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>&gt;977-1383</td>
<td>185 (2.54)</td>
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<td>1.73 (1.35-2.22)</td>
<td>1.51 (1.17-1.94)</td>
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<tr>
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<td>286 (4.04)</td>
<td>3.04 (2.43-3.82)</td>
<td>2.40 (1.89-3.05)</td>
<td>1.85 (1.44-2.37)</td>
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<tr>
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<td>5.40 (4.36-6.70)</td>
<td>3.55 (2.80-4.49)</td>
<td>2.10 (1.62-2.73)</td>
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<tr>
<td><strong>Cardiac death</strong></td>
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</tr>
<tr>
<td>≤977</td>
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<td>&lt;.0001</td>
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<tr>
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<td>105 (1.44)</td>
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<td>1.79 (1.29-2.49)</td>
<td>1.45 (1.04-2.03)</td>
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<tr>
<td>&gt;1383-2052</td>
<td>146 (2.06)</td>
<td>2.76 (2.04-3.75)</td>
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</tr>
<tr>
<td>&gt;2052</td>
<td>228 (3.36)</td>
<td>4.50 (3.36-6.01)</td>
<td>2.96 (2.14-4.08)</td>
<td>1.48 (1.04-2.11)</td>
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<tr>
<td><strong>Myocardial infarction</strong></td>
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<tr>
<td>≤977</td>
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<tr>
<td>&gt;977-1383</td>
<td>26 (0.36)</td>
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<td>1.07 (0.60-1.90)</td>
<td>0.91 (0.51-1.63)</td>
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<tr>
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<td>1.86 (1.10-3.15)</td>
<td>1.43 (0.82-2.49)</td>
<td>1.06 (0.60-1.87)</td>
</tr>
<tr>
<td>&gt;2052</td>
<td>62 (0.93)</td>
<td>3.04 (1.86-4.97)</td>
<td>1.93 (1.11-3.34)</td>
<td>1.12 (0.61-2.05)</td>
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<tr>
<td><strong>Major bleed</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤977</td>
<td>85 (1.22)</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
<tr>
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<td>132 (2.01)</td>
<td>1.60 (1.22-2.11)</td>
<td>1.35 (1.02-1.78)</td>
<td>1.28 (0.97-1.70)</td>
</tr>
<tr>
<td>&gt;1383-2052</td>
<td>190 (3.05)</td>
<td>2.40 (1.85-3.10)</td>
<td>1.84 (1.41-2.41)</td>
<td>1.65 (1.25-2.18)</td>
</tr>
<tr>
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<td>262 (4.53)</td>
<td>3.49 (2.73-4.47)</td>
<td>2.46 (1.87-3.22)</td>
<td>1.98 (1.48-2.67)</td>
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<tr>
<td><strong>Major bleed/Clinical significant non-major bleed</strong></td>
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<td></td>
<td></td>
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<tr>
<td>≤977</td>
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<td>292 (4.56)</td>
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<td>1.17 (0.98-1.41)</td>
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<tr>
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<td>340 (5.56)</td>
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<tr>
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<td>1.61 (1.34-1.94)</td>
<td>1.43 (1.17-1.76)</td>
</tr>
</tbody>
</table>

GDF-15, growth differentiation factor 15; HR, hazard ratio; CI, confidence interval

*GDF-15 median (interquartile range) 1383 (977, 2052) ng/L
†Model 1 adjusted for randomized treatment, prior Warfarin/vitamin K antagonist treatment and geographic region.
‡Model 2 adjusted for established risk factors: age (continuous); sex; body mass index; smoking status; systolic blood pressure; heart rate; atrial fibrillation type; diabetes; history of symptomatic congestive heart failure; previous stroke/systemic embolism/transient ischemic attack; hypertension; previous myocardial infarction; previous peripheral artery disease/coronary
artery bypass graft/percutaneous coronary intervention; treatment at randomization with aspirin, angiotensin-converting enzyme inhibitors or angiotensin receptor blocker; and use of amiodarone. For the major bleeding endpoint: history of anemia; chronic liver disease; hematocrit; use of nonsteroidal anti-inflammatory drug and history of spontaneous or clinical relevant bleeding were also included.

§Model 3 also adjusted for biomarker levels (cystatin-C, cTnI-hs and NT-proBNP).
Supplemental Figure I. Correlations between levels of GDF-15 and a) NT-pro-BNP b) Cardiac troponin-I-hs and c) Cystatin-C.
For results below the limit of detection a value of the detection limit divided by 2 was imputed. Results below the limit of detection: for GDF-15 77 results were reported as <400 ng/L, for NT-proBNP 22 results were reported as <5 ng/L, and for Troponin I 227 results were reported as <1.3 ng/L. For cystatin-C 44 patients had the minimum level of 0.30 mg/L. Spearman rank correlation coefficients were estimated for GDF-15 vs NT-proBNP $\rho=0.36$, for GDF-15 vs Troponin I $\rho=0.33$ and for GDF-15 vs cystatin-C $\rho=0.52$ (all significantly different from zero, $p<0.0001$).

Figure Ia. NT-proBNP

NT-proBNP, N-terminal brain natriuretic peptide; GDF-15, growth differentiation factor 15
Figure Ib. Cardiac troponin-I-hs

GDF-15, growth differentiation factor 15
Figure Ic. Cystatin-C

GDF-15, growth differentiation factor 15
Supplemental Figure II. Outcomes by GDF-15 quartile group and effect of randomized treatment. Cox proportional hazards model with treatment, biomarker and interaction as covariates.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>GDF-15 (ng/L)</th>
<th>Apixaban</th>
<th>No of patients</th>
<th>Events (%/year)</th>
<th>Warfarin</th>
<th>No of patients</th>
<th>Events (%/year)</th>
<th>HR (95% CI)</th>
<th>p-value Interaction</th>
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</thead>
<tbody>
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<td>Stroke/SEE</td>
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<td>1806</td>
<td>1839</td>
<td>33 (0.87)</td>
<td>34 (0.90)</td>
<td>0.94 (0.59-1.51)</td>
<td>0.8466</td>
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<td></td>
<td>&gt;977 - ≤1383</td>
<td>1814</td>
<td>1883</td>
<td>27 (1.06)</td>
<td>49 (1.38)</td>
<td>0.78 (0.51-1.19)</td>
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<td>1944</td>
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<td>&gt;2052</td>
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<td>1822</td>
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<td>1839</td>
<td>47 (1.21)</td>
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<td>0.94 (0.59-1.51)</td>
<td>0.8466</td>
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<tr>
<td></td>
<td>&gt;977 - ≤1383</td>
<td>1814</td>
<td>1883</td>
<td>25 (1.05)</td>
<td>113 (2.02)</td>
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<td>143 (3.30)</td>
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<td>1822</td>
<td>245 (7.28)</td>
<td>239 (7.09)</td>
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<td>1855</td>
<td>1944</td>
<td>77 (2.16)</td>
<td>69 (1.96)</td>
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<td>1822</td>
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<td>54 (1.67)</td>
<td>78 (2.34)</td>
<td>0.71 (0.50-1.01)</td>
<td>0.04 (0.38-0.98)</td>
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<td>1943</td>
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<td>&gt;2052</td>
<td>1871</td>
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<td>114 (3.85)</td>
<td>148 (5.25)</td>
<td>1.03 (0.86-1.23)</td>
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</table>

GDF-15, growth differentiation factor 15; SEE, systemic embolic events; HR, hazard ratio; CI, confidence interval
Supplemental Figure III. Rate of stroke and systemic embolism in relation to plasma GDF-15 levels modeled using restricted cubic regression spline.

GDF-15, growth differentiation factor 15