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# Cardiovascular Biomarker Score and Clinical Outcomes in Patients With Atrial Fibrillation
## A Subanalysis of the ENGAGE AF-TIMI 48 Randomized Clinical Trial

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**IMPORTANCE** Treatment decisions in atrial fibrillation (AF) are based on clinical assessment of risk. The $CHA_2DS_2$-VASc (cardiac failure or dysfunction, hypertension, age 65-74 [1 point] or ≥75 years [2 points], diabetes mellitus, and stroke, transient ischemic attack or thromboembolism [2 points]–vascular disease, and sex category [female]) risk score is pragmatic and widely used but has only moderate discrimination.

**OBJECTIVE** To develop and test a cardiovascular biomarker score for indication of risk in patients with AF.

**DESIGN, SETTING, AND PARTICIPANTS** The ENGAGE AF-TIMI 48 trial was a randomized, double-blind, double-dummy clinical trial comparing 2 once-daily edoxaban dose regimens with warfarin in 21 105 patients with AF at moderate to high risk of stroke. This prespecified subanalysis was performed in 4880 patients enrolled at randomization in the biomarker substudy. Cardiac troponin I, N-terminal pro-B-type natriuretic peptide, and D-dimer levels were measured at baseline. A multimarker risk score was developed to determine the probability of stroke, systemic embolic events, or death by assigning tiered points for higher concentrations of the biomarkers.

**MAIN OUTCOMES AND MEASURES** Risk score and clinical outcomes based on cardiac troponin I, N-terminal pro-B-type natriuretic peptide, and D-dimer levels at baseline.

**RESULTS** Of the 5002 patients enrolled in the biomarker substudy of the ENGAGE AF-TIMI 48 trial, 4880 patients (97.6%) had all 3 biomarkers available at randomization (1820 [37.3%] were women; median [interquartile range] age, 71 [64-77] years). After adjustment for the $CHA_2DS_2$-VASc score, each biomarker was associated with a 2.8-fold to 4.2-fold gradient of risk comparing the highest vs lowest concentrations across groups of increasing concentrations ($P < .001$ for trend for each). The multimarker risk score identified a more than 15-fold gradient of risk after adjustment for $CHA_2DS_2$-VASc score. When added to the $CHA_2DS_2$-VASc score, the biomarker score significantly enhanced prognostic accuracy by improving the C statistic from 0.586 (95% CI, 0.565-0.607) to 0.708 (95% CI, 0.688-0.728) ($P < .001$) and reclassification with a net reclassification improvement of 59.4% ($P < .001$).

**CONCLUSIONS AND RELEVANCE** A prototype multimarker risk score significantly enhanced risk assessment for stroke, systemic embolic events, or death compared with traditional clinical risk stratification. Incorporation of biomarkers into clinical decision making to define therapeutic management in AF warrants consideration.

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Atrial fibrillation (AF) predisposes patients to an increased risk of embolic stroke and is associated with higher rates of stroke and mortality compared with sinus rhythm.[1-3] Current risk stratification schemes rely on a combination of demographic and clinical characteristics to determine the probability of thromboembolism. The most commonly used and validated risk scores are $CHADS_2$ (congestive heart failure, hypertension, age ≥75 years, diabetes, and stroke or transient ischemic attack (TIA) [2 points]) and $CHA_2DS_2$-VASc (cardiac failure or dysfunction, hypertension, age 65-74 [1 point] or ≥75 years [2 points], diabetes mellitus, and stroke, TIA or thromboembolism [2 points]–vascular disease, and sex category [female]), which are based on clinical variables.[4,5] Although clinical risk scores, particularly $CHA_2DS_2$-VASc, are useful in identifying patients with low risk of thromboembolism who are unlikely to benefit from anticoagulation, the scores were not developed to determine the probability of mortality.[6] It is important to stratify risk for both thromboembolism and mortality because this expanded outcome is the most relevant to clinicians and, most important, patients. This end point is analogous to myocardial infarction and death or heart failure hospitalization and death—composite outcomes routinely assessed in other disease areas. In addition, mortality is reduced with anticoagulation and thus "modifiable" by therapy; therefore, it is important to incorporate mortality into risk prognostic schemes used to qualify whether patients may benefit from anticoagulation. There has been considerable interest in determining whether the addition of biomarkers to clinical risk scores enhances prognostic accuracy for thromboembolism and mortality.[7,8]

An emerging body of research has demonstrated that various markers of inflammation, coagulation activity, hemodynamic stress, myocardial injury, and renal dysfunction are associated with an increased risk of adverse events in patients with AF.[7] In several large, contemporary, phase 3 trials[9-13] investigating non-vitamin K antagonist oral anticoagulants vs warfarin in patients with AF, cardiac troponin, N-terminal pro-B-type natriuretic peptide (NT-proBNP), and D-dimer have been shown to be powerful predictors of stroke and mortality that significantly enhance prognostic ability when they were added individually to clinical risk scores. There is also evidence[9,14] that further improvement in risk stratification is achieved when biomarkers are used in combination.

Therefore, we developed a multimarker risk score using biomarkers collected in the ENGAGE AF-TIMI 48 trial[15,16] comparing 2 once-daily dose regimens of edoxaban with warfarin in patients with AF at moderate to high risk of stroke. We evaluated whether the performance of this multimarker risk score alone and in combination with the $CHA_2DS_2$-VASc score improved on current approaches to risk-stratify patients with AF.

### Key Points

**Question** Can a cardiovascular biomarker score improve risk prediction in patients with atrial fibrillation?

**Findings** In this prespecified subanalysis of 4880 patients enrolled in the biomarker study of the ENGAGE AF-TIMI 48 trial, a multimarker risk score determined by using troponin I, N-terminal pro-B-type natriuretic peptide, and D-dimer levels at baseline identified a more than 15-fold gradient of risk for stroke, systemic embolic events, or death after adjustment for the $CHA_2DS_2$-VASc score. Use of the multimarker risk score significantly enhanced prognostic accuracy and reclassification.

**Meaning** Incorporation of biomarkers into clinical decision making to define therapeutic management in atrial fibrillation warrants consideration.

## Methods

### Study Population

The ENGAGE AF-TIMI 48 trial[17] has been described previously.[15,16] A total of 21 105 patients with documented AF and a $CHADS_2$ risk score of 2 or higher were eligible for enrollment. Patients were randomly assigned (1:1:1) to receive warfarin (dose adjusted to an international normalized ratio of 2.0 to 3.0), higher-dose edoxaban (60 mg/d with dose reduction to 30 mg/d in selected patients), or lower-dose edoxaban (30 mg/d with dose reduction to 15 mg/d in selected patients). Individuals included in this analysis were enrolled in the biomarker substudy at randomization. The substudy population consisted of the first approximately 5000 patients at selected sites who elected to participate in the biomarker substudy, with a final population of 5002 patients. Written informed consent for participation in the trial, including the biomarker substudy, was approved by the governing institutional review board/ethics committee at each site and obtained from each participating patient. Brigham and Women's Hospital Human Research Committee reviewed and approved the protocol overseeing the TIMI Study Group as the Coordinating Center for the trial.

### Biomarker Testing

Blood samples for determination of cardiac troponin I, NT-proBNP, and D-dimer levels were collected at randomization and stored at −20°C or colder and then shipped frozen to the TIMI Clinical Trials Laboratory, where they were stored at −80°C or colder until thawed and analyzed by personnel blinded to treatment allocation and clinical outcomes. Cardiac troponin I was measured (EDTA plasma) using a commercially available sensitive assay (TnI-Ultra; Siemens Healthcare Diagnostics) with a lower limit of detection of 0.006 ng/mL and an established 99th percentile reference limit of 0.04 ng/mL, with a coefficient of variation of 10% at a concentration of 0.03 ng/mL.[18] The NT-proBNP levels were measured (EDTA plasma) using a sandwich immunoassay (proBNP II; Roche Diagnostics) with an analytical range of 5 to 35 000 pg/mL and a coefficient of variation of 4.6% at a concentration of 44 pg/mL.[19] D-dimer was measured (citrated plasma) using an immunoturbidimetric assay (STA-Liatest D-DI; Diagnostica Stago) with a reportable range 0.22 to 4.00 μg/mL and an intrarun coefficient of variation of 2.6% at a concentration of 2.29 μg/mL.[20]

### Outcomes

The primary outcome for this analysis was the composite of all-cause stroke (ischemic and hemorrhagic), systemic em-

bolic event (SEE), and all-cause mortality. We chose a composite outcome because, although most clinical risk scores were initially developed to indicate the probability of thromboembolic events, the scores also serve as indicators of mortality, and anticoagulation has been demonstrated to reduce mortality.[21] Moreover, the increased number of events was desirable to support statistical power for individual stratified analyses. As a sensitivity analysis, we also examined stroke and SEE separately from all-cause mortality. An independent clinical end point committee unaware of randomized treatment assignment or biomarker levels adjudicated all events for the entire trial.

### Statistical Analysis

The evaluation of the prognostic ability of cardiac biomarkers in the ENGAGE AF-TIMI 48 trial was prespecified. Descriptive statistics of baseline characteristics are given as numbers and percentages, medians with 25th and 75th percentiles, or means (SDs). All analyses were performed in the intention-to-treat population and were restricted to patients who had all 3 biomarkers available. Biomarker levels were stratified by quartiles with the exception of troponin because a substantial portion of patients (49.6%) had a troponin level below the limit of detection for the TnI-Ultra assay (<0.006 ng/mL), and there is a clinically established 99th percentile reference limit (≥0.04 ng/mL). Troponin levels were therefore stratified as follows: less than the limit of detection, tertiles above the limit of detection but less than the 99th percentile reference limit, and the 99th percentile limit or higher.

Event rates are expressed per 100 patient-years. Hazard ratios (HRs) for the individual biomarkers as well as the biomarker scores were determined using the Cox proportional hazards regression model with and without multivariable adjustment for the $CHA_2DS_2$-VASc risk score. Testing for trend of survivor functions was conducted. A multimarker score was constructed by assigning tiered integer values to each biomarker group by rounding each HR adjusted for the $CHA_2DS_2$-VASc score to the nearest whole number. Formal interaction terms were analyzed in a Cox proportional hazards regression model to test for a treatment interaction with the biomarker risk score. The prognostic accuracy of the biomarker and $CHA_2DS_2$-VASc scores were compared using the area under the curve derived from receiver operating characteristic curves (C statistic).[22] To further assess the discriminatory performance (C statistic) in our data set, bootstrapping methods with replacement for 100 replications were performed. The ability of the multimarker risk score to enhance discrimination and correctly reclassify patients was additionally tested with the integrated discrimination improvement (IDI) and the category-free net reclassification improvement (NRI).[23,24] The IDI measures enhancement in average sensitivity gained without forgoing specificity from the addition of the biomarker risk score to the $CHA_2DS_2$-VASc score. As an integral, the IDI grades the biomarker risk score's performance in improving overall sensitivity and specificity. The NRI is the probability that patients were appropriately assigned to a higher or lower risk and evaluates whether there is an additive benefit gained from reclassifying patients into different categories with the addition of the biomarker risk score. The NRI quantifies the degree to which adding the biomarker risk score to the $CHA_2DS_2$-VASc score drives correct movement between categories. Analyses were performed with SAS, version 9.3 (SAS Institute Inc) and Stata, version 13.1 (StataCorp LP).

## Results

### Biomarker Levels and Association with Outcomes

Of the 5002 patients enrolled in the biomarker substudy of the ENGAGE AF-TIMI 48 trial, 4880 patients (97.6%) had all 3 biomarkers available at randomization (1820 [37.3%] were women; median [interquartile range] age, 71 [64-77] years). Baseline characteristics between patients included in this analysis and all other patients in the ENGAGE AF-TIMI 48 trial were generally similar (eTable 1 in the Supplement).

The distribution of each biomarker and the association between biomarker levels and event rates are reported in **Table 1**. There was a graded association between increasing individual biomarker levels and the rate of stroke, SEE, or death ($P < .001$ for trend for each). The same pattern was observed for the individual components of the composite end point, including stroke and SEE (Table 1). After adjustment for the $CHA_2DS_2$-VASc score, there remained a 2.8-fold to 4.2-fold gradient of risk comparing the highest vs lowest concentrations across groups of increasing concentrations of individual biomarker levels for stroke, SEE, or death (**Figure 1**). There was a similar significant gradient for stroke and SEE alone (1.6-fold to 3.9-fold; $P < .03$ for each) comparing the highest vs lowest concentrations after adjustment for the $CHA_2DS_2$-VASc score.

### Multimarker Risk Score

A multimarker score was constructed by assigning tiered integer values to each biomarker group (eTable 2 in the Supplement). The biomarker risk score had a range of 0 to 11 and was calculated for each patient by summing the integer values assigned for the 3 biomarker levels for that patient. The distributions of $CHA_2DS_2$-VASc and biomarker scores are shown in eFigure 1A and B in the Supplement, respectively.

The rate of stroke, SEE, or death ranged from 1.2% per year in patients with a biomarker score of 0 to 21.1% per year in patients with a biomarker score of 10 to 11—a greater than 17-fold range. In contrast, using the $CHA_2DS_2$-VASc score, the rate of the same end point ranged from 2.2% in patients with a $CHA_2DS_2$-VASc score of 2 to 9.9% per year in patients with a $CHA_2DS_2$-VASc score of 8 to 9—a greater than 4-fold range (**Figure 2**). The biomarker score identified more than a 15-fold gradient of risk after adjustment for the $CHA_2DS_2$-VASc score (eFigure 2 in the Supplement). There was an approximately 7-fold gradient of risk for stroke and SEE alone after adjustment for the $CHA_2DS_2$-VASc score (6.5; 95%, CI 2.1-19.9) (comparing a biomarker risk score of ≥10 to a biomarker risk score of 0). Annual rates of stroke, SEE, or death according to biomarker and $CHA_2DS_2$-VASc scores are shown in **Figure 3**. Although event rates increased with higher levels of both scores, a greater relative increase in risk

Table 1. Association of Biomarker Levels With Outcomes

| Variable | Event Rates per 100 Patient-years, No. (%) | | | | |
|---|---|---|---|---|---|
| | Stroke, SEE, or Death | Stroke or SEE | Ischemic Stroke | Hemorrhagic Stroke | Death |
| D-dimer, μg/mL[a] | | | | | |
| Q1: 0-0.26 | 113 (2.85) | 52 (1.31) | 40 (1.01) | 9 (0.22) | 83 (2.06) |
| Q2: 0.27-0.39 | 118 (3.68) | 42 (1.31) | 34 (1.06) | 7 (0.22) | 100 (3.07) |
| Q3: 0.40-0.67 | 172 (5.66) | 56 (1.85) | 45 (1.48) | 10 (0.33) | 140 (4.53) |
| Q4: 0.68-16.00 | 266 (8.89) | 69 (2.32) | 50 (1.67) | 17 (0.56) | 230 (7.49) |
| *P* value for trend | <.001 | <.001 | .006 | .01 | <.001 |
| TnI-Ultra, ng/mL[b] | | | | | |
| <LOD: <0.006 | 204 (3.04) | 93 (1.39) | 69 (1.03) | 23 (0.34) | 156 (2.29) |
| T1: 0.006-0.008 | 85 (4.45) | 35 (1.84) | 31 (1.62) | 2 (0.10) | 63 (3.23) |
| T2: 0.009-0.014 | 136 (6.50) | 36 (1.72) | 28 (1.34) | 24 (0.38) | 117 (5.50) |
| T3: 0.015-0.039 | 168 (8.85) | 38 (2.01) | 27 (1.42) | 8 (0.42) | 149 (7.69) |
| ≥99th percentile: ≥0.04 | 76 (12.92) | 17 (2.91) | 14 (2.40) | 2 (0.33) | 68 (11.21) |
| *P* value for trend | <.001 | .003 | .008 | .64 | <.001 |
| NT-proBNP, pg/mL[c] | | | | | |
| Q1: 0-370 | 80 (2.31) | 24 (0.69) | 16 (0.46) | 6 (0.17) | 65 (1.85) |
| Q2: 371-776 | 113 (3.34) | 43 (1.27) | 35 (1.03) | 8 (0.23) | 88 (2.56) |
| Q3: 777-1360 | 157 (4.75) | 61 (1.85) | 48 (1.45) | 10 (0.30) | 122 (3.61) |
| Q4: 1361-18993 | 319 (10.45) | 91 (2.99) | 70 (2.29) | 19 (0.62) | 278 (8.90) |
| *P* value for trend | <.001 | <.001 | <.001 | .002 | <.001 |

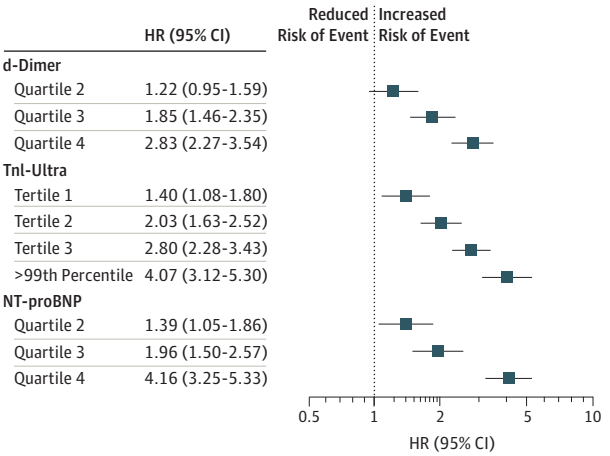
Abbreviations: LOD, level of detection; NT-proBNP, N-terminal pro-B-type natriuretic peptide; Q, quartile; SEE, systemic embolic event; T, tertile.

[a] For Q1, data were available from 1413 patients; Q2, 1167; Q3, 1124; and Q4, 1176.

[b] TnI-Ultra is a commercially available sensitive assay manufactured by Siemens Healthcare Diagnostics. For less than the LOD, data were available from 2421 patients; T1, 697; T2, 782; T3, 739; and greater than the 99th percentile, 241.

[c] For Q1, data were available from 1227 patients; Q2, 1218; Q3, 1217; and Q4, 1218.

Figure 1. Risk of Stroke, Systemic Embolic Event (SEE), and Death Associated With Individual Biomarker Levels




Referent quartile 1 for D-dimer and N-terminal pro-B-type natriuretic peptide (NT-proBNP) and less than the limit of detection for the TnI-Ultra assay (Siemens Healthcare Diagnostics). Hazard ratios (HRs) are adjusted for the $CHA_2DS_2$-VASc (cardiac failure or dysfunction, hypertension, age 65-74 [1 point] or ≥75 years [2 points], diabetes mellitus, and stroke, transient ischemic attack or thromboembolism [2 points]–vascular disease, and sex category [female]) score.

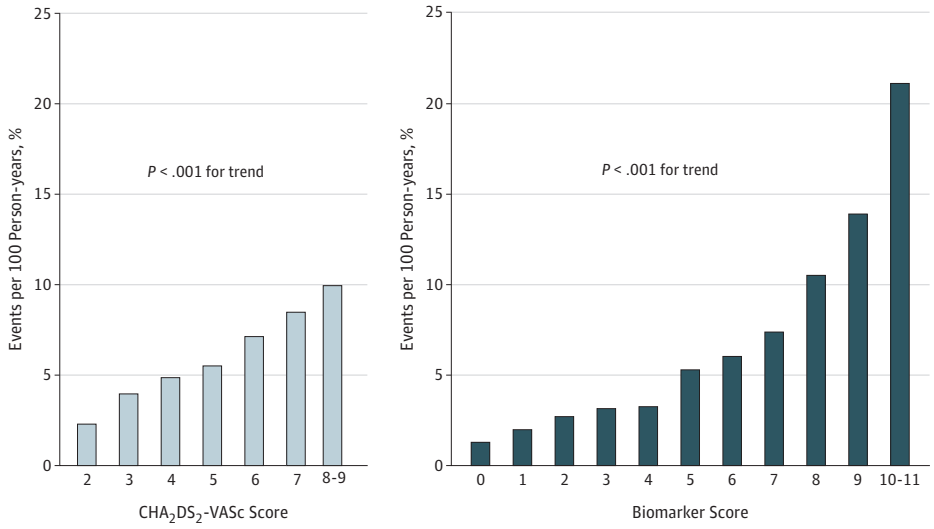
was observed with the biomarker score. There was no interaction between vitamin K antagonist-naive vs -experienced status at randomization or study treatment (edoxaban vs warfarin) and the predictive performance of the biomarker or $CHA_2DS_2$-VASc score.

## Prognostic Discrimination and Reclassification

Biomarkers significantly improved discrimination for stroke, SEE, or death when added individually to the $CHA_2DS_2$-VASc score (**Table 2**). The biomarker risk score alone had significantly greater prognostic accuracy than the $CHA_2DS_2$-VASc score, with a C statistic of 0.700 (95% CI, 0.679-0.720) compared with 0.586 (95% CI, 0.565-0.607) ($P < .001$) (Table 2). Bootstrapping methods resulted in similar C statistics, with a biomarker score of 0.700 (95% CI, 0.681-0.718) and a $CHA_2DS_2$-VASc score of 0.586 (95% CI, 0.568-0.605). Adding the biomarker score to the $CHA_2DS_2$-VASc score incrementally increased the C statistic to 0.708 (95% CI, 0.688-0.728) (bootstrapping C statistic, 0.708; 95% CI, 0.687-0.729) compared with the biomarker score alone ($P = .01$), with a relative IDI improvement of 590% ($P < .001$). Combining the biomarker and $CHA_2DS_2$-VASc scores also significantly enhanced reclassification compared with the $CHA_2DS_2$-VASc score alone; the NRI was 59.4% ($P < .001$), with events contributing 32.4% and nonevents contributing 27%.

In a sensitivity analysis, the biomarker score was independently associated with stroke or SEE (without including all-cause mortality) after adjustment for the $CHA_2DS_2$-VASc score. The C statistic for the $CHA_2DS_2$-VASc score was 0.584 (95% CI, 0.547-0.620), and the addition of the biomarker score provided significant improvement in discrimination (C statistic, 0.661; 95% CI, 0.627-0.696; $P < .001$) and reclassification (NRI, 0.318; $P < .001$) (eTable 3 in the Supplement). Again, bootstrapping methods resulted in similar C statistics: biomarker score, 0.635 (95% CI, 0.600-0.670); $CHA_2DS_2$-VASc score, 0.584 (95% CI, 0.557-0.610); and $CHA_2DS_2$-VASc plus biomarker score, 0.661 (95% CI, 0.627-0.696).

Figure 2. Rate of Stroke, Systemic Embolic Events, and Death Stratified by $CHA_2DS_2$-VASc (Cardiac Failure or Dysfunction, Hypertension, Age 65-74 [1 Point] or ≥75 Years [2 Points], Diabetes Mellitus, and Stroke, Transient Ischemic Attack or Thromboembolism [2 Points]–Vascular Disease, and Sex Category [Female]) and Biomarker Scores



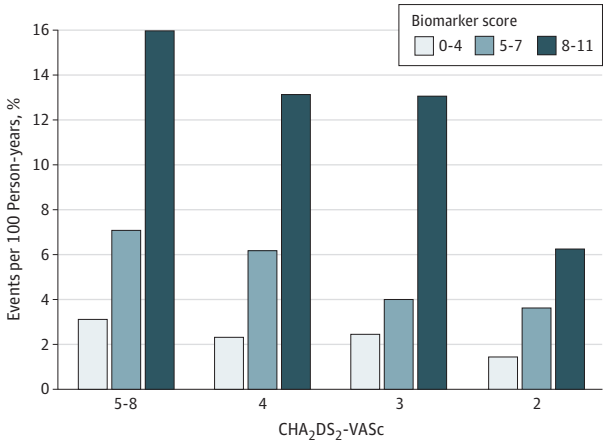

The unadjusted rate of stroke, systemic embolism, or death increased in a significant graded fashion with both the established risk score and the biomarker score.

## Discussion

Clinical practice guidelines[1,2] endorse risk stratification as central to the management of AF since risk stratification provides the framework to guide physicians and patients with respect to the most important decision they must make: whether an individual patient's risk of thromboembolism is high enough to warrant indefinite anticoagulation therapy and the obligate associated bleeding risk. The $CHA_2DS_2$-VASc score is commonly used for this purpose owing to its ability to more accurately identify low-risk patients who are unlikely to benefit from anticoagulant therapy compared with the $CHADS_2$ score.[25] The $CHA_2DS_2$-VASc score, like others that rely solely on clinical factors, has only modest prognostic ability. In addition, $CHA_2DS_2$-VASc and other clinical risk scores were developed to determine the risk of thromboembolism rather than its composite with mortality. We believe the composite is an important outcome for risk stratification since both thromboembolism and mortality are reduced with effective anticoagulation and, from a patient's perspective, they want to be stroke free and alive.[21] However, clinical risk scores are simple to use, which has made them attractive to clinicians. Our analyses sought to address 2 important questions. Can risk stratification be improved by the incorporation of a biomarker score? Would such an improvement be clinically meaningful to warrant the additional complexity and expense of measuring biomarkers?

Prior work[9-13] has established that individual biomarkers are associated with a gradient of risk for stroke and mortality even after adjusting for $CHA_2DS_2$-VASc risk factors. The recently developed ABC stroke risk score[26] demonstrated that 2 biomarkers—troponin and NT-proBNP—added to age and history of stroke or TIA performed significantly better than $CHA_2DS_2$-VASc in determining the probability of stroke or systemic embolism. This finding supports the premise that there is important independent information regarding underlying risk captured in this manner. We have extended these findings and provided proof of principle that a multimarker score developed by assigning tiered points for higher concentrations of troponin, NT-proBNP, and D-dimer significantly improved risk prediction compared with the addition of individual biomarkers alone. Although each biomarker was

Figure 3. Rate of Stroke, Systemic Embolic Event (SEE), or Death Stratified by $CHA_2DS_2$-VASc (Cardiac Failure or Dysfunction, Hypertension, Age 65-74 [1 Point] or ≥75 Years [2 Points], Diabetes Mellitus, and Stroke, Transient Ischemic Attack or Thromboembolism [2 Points]–Vascular Disease, and Sex Category [Female]) and Biomarker Scores




Simultaneous categorization of risk using the established risk and biomarker scores demonstrates that an elevated biomarker score identified patients with a $CHA_2DS_2$-VASc score of 2 who were at higher risk than patients with a $CHA_2DS_2$-VASc score of 5 to 8 with a low biomarker score.

Table 2. Discrimination and Reclassification for Stroke, SEE, or Death

| Variable | C Statistic (95% CI) | NRI (95% CI) | IDI |
|---|---|---|---|
| $CHA_2DS_2$-VASc score[a] | 0.586 (0.565-0.607) | 1 [Reference] | 1 [Reference] |
| With D-dimer | 0.647 (0.627-0.668)<br>*P* < .001 | NA | NA |
| With TnI-Ultra[b] | 0.662 (0.641-0.683)<br>*P* < .001 | NA | NA |
| With NT-proBNP | 0.674 (0.654-0.694)<br>*P* < .001 | NA | NA |
| Biomarker score[c] | 0.700 (0.679-0.720)<br>*P* < .001 | NA | NA |
| $CHA_2DS_2$-VASc with biomarker score | 0.708 (0.688-0.728)<br>*P* < .001[c]<br>*P* = .01[d] | 0.594 (0.513-0.676)<br>Event: 0.324 (0.249-0.400)<br>Nonevent: 0.270 (0.240-0.300)<br>*P* < .001[e] | 0.098 (0.086-0.111)<br>Relative IDI, 590%<br>*P* < .001 |

Abbreviations: $CHA_2DS_2$-VASc, congestive heart failure (signs or symptoms of heart failure confirmed with objective evidence of cardiac dysfunction), hypertension, age 65-74 (1 point) or ≥75 years (2 points), diabetes mellitus, and stroke, transient ischemic attack or thromboembolism (2 points)–vascular disease, and sex category (female); IDI, integrated discrimination improvement; NA, not applicable; NRI, net reclassification improvement; NT-proBNP, N-terminal pro-B-type natriuretic peptide; SEE, systemic embolic event.

[a] Each biomarker score compared with $CHA_2DS_2$-VASc score alone.

[b] TnI-Ultra is a commercially available sensitive assay manufactured by Siemens Healthcare Diagnostics.

[c] Compared with $CHA_2DS_2$-VASc score alone.

[d] Compared with biomarker score alone.

[e] Overall NRI for the $CHA_2DS_2$-VASc plus biomarker score compared with $CHA_2DS_2$-VASc alone.

associated with an approximately 3-fold to 4-fold gradient of risk across groups of increasing concentration after adjustment for the $CHA_2DS_2$-VASc score, the multimarker score identified a greater than 15-fold gradient. When added individually to the $CHA_2DS_2$-VASc score, each biomarker enhanced prognostic accuracy by improving the C statistic, but no single biomarker captured the scope of the spectrum of risk that was achieved by the multimarker score. Our results reinforce the concept that multiple biomarkers that capture orthogonal information may be useful to assess the risk that results from the complex interplay among prothrombotic and vascular injury and stress pathways more effectively than binary questions that simply assess, for example, whether a history of vascular disease or heart failure is present or information captured from a single biomarker that may offer only partial information from a single biological pathway.

## Potential Role of Biomarkers in AF

Although biomarkers significantly enhance risk indication in patients with AF, it is important to ask how this refinement might be used in clinical practice. It has been argued[27] that the primary values of clinical risk scores such as $CHA_2DS_2$-VASc are their easy use and ability to identify low-risk patients (eg, $CHA_2DS_2$-VASc score, 0) who do not require anticoagulation. Acknowledgment that the assumptions and simplifications (equal weighting of most risk factors) decrease prognostic precision is implicit. However, in many cases, it may not be important to know a patient's precise risk. For example, if all patients above a certain threshold of risk of 2% per year benefit from anticoagulation, is it necessary to know whether a patient's risk is 12% instead of 6% if that difference does not change therapeutic management? Improving prognostication in patients with AF by incorporating a multimarker score would be helpful to clinicians if the improvement has the potential to change clinical decision making.

Our data support the concept that biomarkers may contribute significant additional information regarding risk that could affect management. Figure 3 demonstrates that, across each $CHA_2DS_2$-VASc stratum, the multimarker score consistently identified a 4-fold to 6-fold gradient of risk regardless of whether patients had a $CHA_2DS_2$-VASc score of 2 or 8. Based on trial entry criteria, patients with a $CHA_2DS_2$-VASc score of 0 or 1 were not included in the ENGAGE AF-TIMI 48 trial; therefore, we do not have data regarding the performance of the multimarker score in patients with AF at the lowest risk of stroke as defined by the $CHA_2DS_2$-VASc risk score. However, our data suggest the potential of the multimarker score to identify patients categorized into this lower risk category who have a significantly elevated risk that may warrant anticoagulation. The converse may also be true: patients with clinical risk factors that qualify them for anticoagulation may be at lower risk based on their biomarker profile. This difference has clinical importance because the decision to initiate anticoagulation therapy in a patient carries an inherent risk of potential serious bleeding for an indefinite period since treatment is often continued for the lifetime of the patient. Incorporating biomarkers into clinical decision making in patients with AF may bring us closer to our goal of precision medicine in which more accurate risk assessment allows us to use anticoagulant therapy in patients who will achieve benefit and avoid exposing other patients to unnecessary risk. Both of these hypotheses need to be tested in studies that include patients across the full spectrum of $CHA_2DS_2$-VASc risk scores.

A more accurate assessment of risk with the incorporation of biomarkers may also affect clinical decision making outside of determining eligibility for anticoagulation. One area of potential applicability is catheter ablation. Restoring sinus rhythm and decreasing the burden of AF makes intuitive sense considering the impaired prognosis associated with AF, but there has been no established benefit of rhythm vs rate control strategy in reducing stroke or mortality.[28] Part of the reason for this lack of benefit may be that current assessments of the extent of atrial structural and functional changes and patient comorbidities are incomplete. A more individualized strat-

egy that incorporates biomarkers may allow identification of patients at an early stage of remodeling when the potential long-term benefits of a rhythm control strategy may be realized. An appeal of the use of biomarkers is that, unlike clinical characteristics that are static over time, biomarkers are dynamic and change with the course of disease. This concept can be extended more broadly to risk factor management in patients with AF. It has been demonstrated[29-31] that intensive risk factor management of obesity and obstructive sleep apnea reduces AF symptom burden and severity with associated beneficial effects in cardiac remodeling. Biomarkers may allow for a more precise phenotypic profile of patients to enable early identification of patients for intensive risk factor management before irreversible structural damage has occurred.

## Limitations

Several limitations of this analysis should be acknowledged. Clinical risk stratification scores were developed in patients who were not receiving anticoagulants, and all patients in the ENGAGE AF-TIMI 48 trial received anticoagulation; however, the performance of clinical risk scores has been demonstrated to be similar in patients receiving[9-13] vs those not receiving[4,5] anticoagulants. Moreover, when evaluated head to head in this population, the addition of biomarkers improved prognostic discrimination. Clinical risk scores, such as $CHA_2DS_2$-VASc, were also developed to predict thromboembolic events in particular, while we included mortality in the composite end point. However, clinical risk scores also indicate the probability of mortality, and the results were consistent when restricted to stroke or SEE alone. In addition, as mentioned above, our trial did not enroll patients with a $CHADS_2$ score and, thus, $CHA_2DS_2$-VASc scores of 0 or 1. Therefore, performance of the biomarker score in those patients is unknown.

In addition, the incremental discriminatory performance of any new prognostic instrument has the potential to be overstated when developed in a new data set and compared with an established risk score in that development set. Reestimation of the regression coefficients for the established risk predictors can be performed in the development set to optimize performance of the established risk score; however, such rederivation is not practical from a clinical viewpoint because the revised score would not reflect what is being used in practice. Alternatively, one can test both the established and new prognostic instruments (eg, our multimarker score) in the same external validation set. Therefore, although it provides an important proof of principle with this articulation of a multimarker score in AF with bootstraping that supports reproducible discrimination, our biomarker-based score requires validation and refinement in additional studies that should include cohorts not receiving anticoagulants outside of the highly selected clinical trial population. To facilitate clinical utility, optimal cut points for the biomarkers should be developed and externally validated. The multimarker score developed in this analysis is a prototype that will no doubt undergo further refinement with assessment in other populations.

## Conclusions

A prototype multimarker risk score was developed that significantly enhanced risk assessment for stroke, SEE, or death compared with traditional clinical risk stratification. Incorporation of biomarkers into clinical decision making to define therapeutic management in AF warrants consideration.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*Study concept and design:* Ruff, Giugliano, Braunwald, Mercuri, Morrow.
*Acquisition, analysis, or interpretation of data:* All authors.
*Drafting of the manuscript:* Ruff.
*Critical revision of the manuscript for important intellectual content:* All authors.
*Statistical analysis:* Murphy.
*Administrative, technical, or material support:* Ruff, Jarolim, Mercuri, Antman.
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