

# Repeated Measurements of Cardiac Biomarkers in Atrial Fibrillation and Validation of the ABC Stroke Score Over Time

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**Background**—Cardiac biomarkers are independent risk markers in atrial fibrillation, and the novel biomarker-based ABC stroke score (age, biomarkers, and clinical history of prior stroke) was recently shown to improve the prediction of stroke risk in patients with atrial fibrillation. Our aim was to investigate the short-term variability of the cardiac biomarkers and evaluate whether the ABC stroke risk score provides a stable short-term risk estimate.

**Methods and Results**—According to the study protocol, samples were obtained at entry and also at 2 months in 4796 patients with atrial fibrillation followed for a median of 1.8 years in the ARISTOTLE (Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation) trial. Cardiac troponin I, cardiac troponin T, and N-terminal pro-B-type natriuretic peptide were measured with high-sensitivity immunoassays. Associations with outcomes were evaluated by Cox regression. C indices and calibration plots were used to evaluate the ABC stroke score at 2 months. The average changes in biomarker levels during 2 months were small (median change cardiac troponin T +2.8%, troponin I +2.0%, and N-terminal pro-B-type natriuretic peptide +13.5%) and within-subject correlation was high (all  $\geq 0.82$ ). Repeated measurement of cardiac biomarkers provided some incremental prognostic value for mortality but not for stroke when combined with clinical risk factors and baseline levels of the biomarkers. Based on 8702 person-years of follow-up and 96 stroke/systemic embolic events, the ABC stroke score at 2 months achieved a similar C index of 0.70 (95% CI, 0.65–0.76) as compared with 0.70 (95% CI, 0.65–0.75) at baseline. The ABC stroke score remained well calibrated using predefined risk classes.

**Conclusions**—In patients with stable atrial fibrillation, the variability of the cardiac biomarkers and the biomarker-based ABC stroke score during 2 months are small. The prognostic information by the ABC stroke score remains consistent and well calibrated with similar good predictive performance if patients are retested after 2 months.

**Clinical Trial Registration** —URL: <http://www.clinicaltrials.gov>. Unique identifier: NCT00412984. (*J Am Heart Assoc.* 2017;6:e004851. DOI: 10.1161/JAHA.116.004851.)

**Key Words:** atrial fibrillation • cardiac biomarkers • natriuretic peptide • risk score • stroke • troponin

Atrial fibrillation (AF) is the most common sustained arrhythmia and constitutes a major risk factor for stroke and mortality.<sup>1,2</sup> Cardiac biomarkers, troponin and natriuretic peptides, have repeatedly been shown to provide independent prognostic information for the risk of stroke, vascular events, and mortality in patients with AF treated with

anticoagulation.<sup>3–6</sup> Recently, a biomarker-based risk model consisting of the 4 strongest predictors for stroke in patients with AF was developed, the ABC stroke score (age, biomarkers [troponin and NT-proBNP (N-terminal pro-B-type natriuretic peptide)], and clinical history of prior stroke).<sup>7</sup> The ABC stroke score was derived in 14 701 patients, validated in 2

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external cohorts of 1400 and 8356 patients achieving C indices between 0.65 and 0.68, and showed superiority over contemporary risk scores.<sup>7,8</sup> However, so far, there is no information on the variability over time for these biomarkers and the associations between changed levels and clinical events in patients with stable AF. Therefore, the utility of repeated measurements for reevaluation of the ABC stroke score within different time frames needs to be evaluated because changes of the biomarker levels and the ABC score may be potentially useful for monitoring disease progress and the effects of different treatments (eg, for hypertension, heart failure, and rate control) both in clinical trials and in the routine management of patients with AF.

In the present prespecified serial biomarkers substudy within the ARISTOTLE (Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation) trial, we investigated the changes in the concentrations of the cardiac biomarkers, cardiac troponin (cTn) I (cTnI), cTn T (cTnT), and NT-proBNP, at 2 months and whether the added information improved the prognostication of outcomes in patients with AF treated with anticoagulation. The availability of repeated measurements also provided an opportunity to determine whether the novel biomarker-based ABC stroke risk score provided a stable estimate over a period of time.

## Methods

### The ARISTOTLE Trial

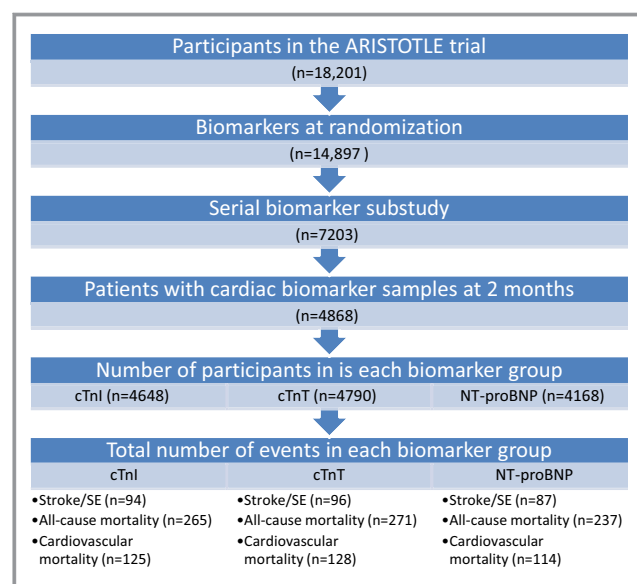
The details of the ARISTOTLE trial have been previously published.<sup>9,10</sup> Briefly, ARISTOTLE was a double blind, double-dummy, randomized clinical trial that enrolled 18 201 patients with AF and at least one CHADS<sub>2</sub> risk factor (congestive heart failure, hypertension, age  $\geq 75$  years, diabetes mellitus, prior stroke) for stroke or systemic embolism. Patients were randomized to warfarin (n=9081) or apixaban (n=9120). The primary end point was stroke or systemic embolism. The median duration of follow-up was 1.8 years.

The biomarker serial substudy aimed to include 5000 patients to provide blood samples for later biomarker measurements at randomization and at the preplanned outpatient visit after 2 months. Details are provided in Figure 1.

Approval by the appropriate institutional review committees was obtained at all sites. All patients provided written informed consent.

### End Points

The end points in this substudy were new events after 2 months and included the primary efficacy outcome in the ARISTOTLE trial (stroke or systemic embolism) and the secondary efficacy outcomes (all-cause mortality and



**Figure 1.** CONSORT flow diagram of the number of participants included in the serial biomarker substudy and total number of patients with events after 2 months stratified for each biomarker. ARISTOTLE indicates Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation; cTnI, cardiac troponin I; cTnT, cardiac troponin T; NT-proBNP, N-terminal pro-B-type natriuretic peptide; SE, systemic embolism.

cardiovascular death [excluding bleeding and other noncardiac causes]). A blinded clinical events committee using prespecified criteria adjudicated all end points.<sup>10</sup>

### Biochemical Methods

At randomization and at 2 months, blood samples were collected in vacutainer tubes containing EDTA and were centrifuged immediately. Plasma was frozen in aliquots and stored at  $-70^{\circ}\text{C}$  until analyzed centrally at the Uppsala Clinical Research Center. The cTnI concentration was determined with high-sensitivity sandwich immunoassays on the ARCHITECT i1000SR (Abbott Diagnostics). The dynamic range is 0.7 to 50 000 ng/L, limit of detection in the Uppsala Clinical Research Center laboratory 1.3 ng/L, and the 99th percentile upper reference limit for healthy persons 23 ng/L.<sup>11</sup> The coefficient of variation (CV) for this cTnI assay is 10% at 3.3 with a local CV of 7% at 21 ng/L. The cTnT concentration was determined with high-sensitivity sandwich immunoassays on the Cobas Analytics e601 Immunoanalyzers (Roche Diagnostics). The measuring range is 3 to 10 000 ng/L, limit of detection 5 ng/L, and the 99th percentile upper reference limit for healthy persons 14 ng/L.<sup>12</sup> The CV is 10% at 13 ng/L with a local CV of 3% at 27 ng/L. NT-proBNP concentration was determined on the Cobas Analytics e601 Immunoanalyzers (Roche Diagnostics). The limit of detection is 5 ng/L. The analytical range is 20 to

**Table 1.** Demographics and Clinical Characteristics at Baseline

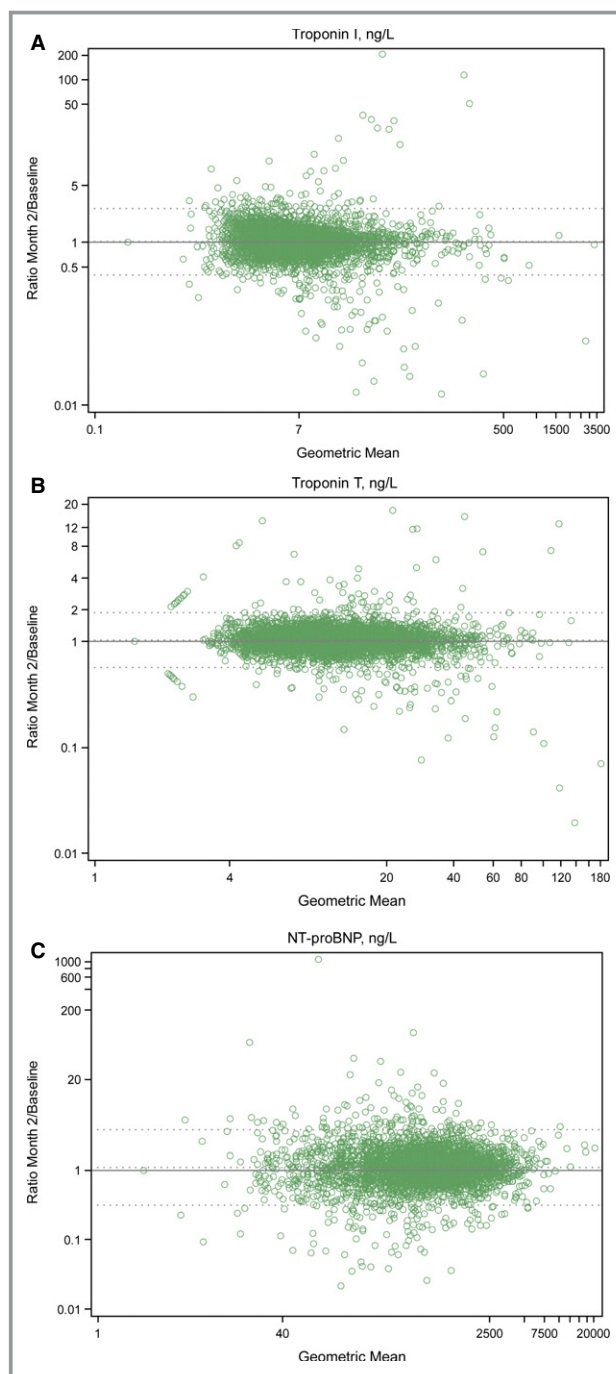
No.	N=4796
Age, median (Q1–Q3), y	70.0 (63.0–76.0)
Male sex	3156 (65.8%)
Region, No. (%)	
Asia/Pacific	684 (14.3)
Europe	2353 (49.1)
Latin America	419 (8.7)
North America	1340 (27.9)
Weight, median (Q1–Q3), kg	83.5 (71.3–96.6)
Renal function, median (Q1–Q3), mL/min (n=4779)	75.5 (58.5–96.7)
Systolic blood pressure, median (Q1–Q3) (n=4788)	130.0 (120–140)
Baseline heart rate, median (Q1–Q3), beats per min (n=4785)	75.0 (65–85)
Smoker, No. (%)	424 (8.8)
Myocardial infarction, No. (%)	650 (13.6)
Prior stroke or TIA, No. (%)	853 (17.8)
Heart failure, No. (%)	1721 (35.9)
Diabetes mellitus, No. (%)	1194 (24.9)
Hypertension, No. (%)	4187 (87.3)
Permanent/persistent atrial fibrillation, No. (%)	4022 (83.9)
Prior VKA status, naive, No. (%)	1802 (37.6)
ACEI or ARB, No. (%)	3397 (70.8)
Aspirin, No. (%)	1401 (29.2)
hs Troponin I, median (Q1–Q3), ng/L (n=4744)	5.1 (3–9)
hs Troponin T, median (Q1–Q3), ng/L (n=4791)	10.8 (8–16)
GDF-15, median (Q1–Q3), ng/L (n=4764)	1363.5 (978–2038)
NT-proBNP, median (Q1–Q3), ng/L (n=4791)	713.0 (366–1231)

ACEI indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; GDF, growth differentiation factor; hs, high-sensitivity; NT-proBNP, N-terminal pro-B-type natriuretic peptide; TIA, transient ischemic attack; VKA, vitamin K antagonist.

35 000 ng/L. The upper reference limit is 269 in men and 391 ng/L in women.<sup>13</sup> The CV is <10% at 30 ng/L with a local CV of 3% at 27 ng/L.<sup>14</sup>

## Statistical Analyses

The analyses included randomized patients with biomarkers available at randomization and at 2 months follow-up. The biomarkers were natural log transformed before analysis. Associations between baseline characteristics and cTn or NT-proBNP at 2 months were examined in linear regression models where the biomarker at 2 months was a response



**Figure 2.** Bland-Altman plot of the ratio between the 2-month level and the baseline level against the geometric mean of the 2 observations for cardiac troponin I (cTnI) (A), cardiac troponin T (cTnT) (B), and N-terminal pro-B-type natriuretic peptide (NT-proBNP) (C). The dotted lines represent mean and 95% CI. A, Association between continuous cTnI levels at baseline (x axis) and cardiovascular mortality by group according to changes of cTnI at 2 months (lines). B, Association between continuous cTnT levels at baseline (x axis) and cardiovascular mortality by group according to changes of cTnT at 2 months (lines). C, Association between continuous NT-proBNP levels at baseline (x axis) and cardiovascular mortality by group according to changes of NT-proBNP at 2 months (lines).

**Table 2.** Baseline Characteristic With Significant Associations With the Continuous cTnI (ng/L) at Month 2 Adjusted for Baseline cTnI Level and Randomized Treatment

Variable	Variable Value at Baseline	No.	Geometric Mean	Ratio of Geometric Means (95% CI)	P Value*
Diabetes mellitus	No	3495	6.0		<0.0001
	Yes	1153	6.3	1.06 (1.03–1.09)	
Prior myocardial infarction	No	4013	6.0		0.0010
	Yes	635	6.4	1.06 (1.03–1.10)	
Prior stroke or systemic embolism	No	3784	6.0		0.0120
	Yes	864	6.3	1.04 (1.01–1.08)	
Prior warfarin	No	1740	6.0		0.0325
	Yes	2908	6.1	1.03 (1.00–1.06)	
Vascular disease	No	3454	6.0		0.0008
	Yes	1194	6.30	1.05 (1.02–1.08)	
Renal function Cystatin C (quartiles), mg/L	≤0.82	1179	6.0		0.0106
	0.83–0.99	1123	5.9	0.99 (0.95–1.04)	
	1.0–1.2	1180	6.1	1.02 (0.98–1.07)	
	>1.2	1160	6.3	1.05 (1.01–1.10)	
NT-proBNP (quartiles), ng/L	≤363	1158	4.6		0.5973
	364–713	1174	5.4	1.00 (0.96–1.04)	
	714–1250	1183	6.2	1.02 (0.97–1.06)	
	>1250	1128	8.9	1.02 (0.97–1.07)	
Troponin T (quartiles), ng/L	≤7.5	1188	5.8		0.0007
	7.6–11.0	1205	6.0	1.02 (0.98–1.06)	
	11.1–16.7	1180	6.2	1.06 (1.01–1.10)	
	>16.7	1072	6.4	1.09 (1.04–1.15)	

cTnI indicates cardiac troponin I; NT-proBNP indicates N-terminal pro-B-type natriuretic peptide.

\*P value from a linear regression model with the natural logarithm of the month 2 biomarker as the outcome variable and log baseline biomarker level and specific baseline characteristic as the independent variables.

variable and the baseline characteristic, randomized treatment, and biomarker at baseline were explanatory variables. Geometric means, calculated by antilogarithms of the model-adjusted means, were compared. Correlations between biomarker changes were evaluated using the Spearman rank correlation coefficient. The intraindividual CV was estimated by pooling individual CVs.

Analysis of clinical outcomes included all events from the month 2 measurement until the efficacy cutoff date. For patients with events between randomization and month 2, the first event occurring after month 2 was counted as the first event.

The associations between cTn or NT-proBNP and events after 2 months were investigated using Cox proportional hazards regression adjusted for study treatment and baseline levels of the respective biomarker. A relative change value of 50% was used in order to capture clinically significant

changes.<sup>15–17</sup> The increased discriminative value of month 2 measurement of each biomarker was estimated by comparing Harrell's C index before and after adding the month 2 value to a model with the baseline value (both in continuous form), randomized treatment, other biomarkers (cystatin C and either cTnI or NT-proBNP), and established risk factors (for stroke or systemic embolism outcomes collected at baseline (age, sex, hypertension, diabetes mellitus, heart failure, previous stroke/systemic embolism/transient ischemic attack, and history of vascular disease). For all-cause mortality and cardiovascular death, systolic blood pressure and smoking status were also included. Restricted cubic splines were used to allow for nonlinearities in the association between continuous variables and outcomes. Likelihood ratio tests were performed to evaluate whether the global model fit improved after the addition of the month 2 measurements of each biomarker.

**Table 3.** Baseline Characteristic With Significant Associations With the Continuous cTnT (ng/L) at Month 2 Adjusted for Baseline cTnT Level and Randomized Treatment

Variable	Variable Value at Baseline	No.	Geometric Mean	Ratio of Geometric Means (95% CI)	P Value*
Age category, y	<65	1464	11.1		<0.0001
	65–74	1892	11.7	1.05 (1.03–1.08)	
	≥75	1434	12.3	1.11 (1.08–1.13)	
Atrial fibrillation	Paroxysmal	773	11.4		0.0225
	Persistent/permanent	4016	11.7	1.03 (1.00–1.05)	
Current smoker	No	4366	11.7		0.0397
	Yes	423	11.4	0.97 (0.94–1.00)	
Diabetes mellitus	No	3598	11.5		<0.0001
	Yes	1192	12.1	1.05 (1.03–1.07)	
Prior warfarin use	No	1796	11.4		<0.0001
	Yes	2994	11.9	1.04 (1.02–1.06)	
Sex	Female	1638	11.4		0.0001
	Male	3152	11.8	1.03 (1.02–1.05)	
Vascular disease	No	3565	11.6		0.0030
	Yes	1225	11.9	1.03 (1.01–1.05)	
Renal function Cystatin C (quartiles), mg/L	≤0.82	1208	11.9		0.0274
	0.83–0.99	1159	11.5	0.97 (0.94–0.99)	
	1.0–1.2	1224	11.6	0.98 (0.95–1.01)	
	>1.2	1196	11.8	0.99 (0.96–1.02)	
NT-proBNP (quartiles), ng/L	≤363	1188	11.6		0.7181
	364–713	1208	11.7	1.00 (0.98–1.03)	
	714–1250	1224	11.7	1.01 (0.98–1.04)	
	>1250	1168	11.8	1.01 (0.98–1.04)	
Troponin I (quartiles), ng/L	≤3.3	1302	11.2		<0.0001
	3.4–5.4	1222	11.6	1.03 (1.00–1.06)	
	5.5–10.1	1173	12.1	1.07 (1.04–1.10)	
	>10.1	1043	12.0	1.06 (1.03–1.10)	

cTnT indicates cardiac troponin T; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

\*P value from a linear regression model with the natural logarithm of the month 2 biomarker as outcome variable and log baseline biomarker level and the specific baseline characteristic as independent variables.

The validation of the ABC stroke score at 2 months was performed by applying the prediction model using the measurements at 2 months. Discrimination was assessed by Harrell's C index<sup>18</sup> and by comparing Kaplan–Meier curves and hazard ratios between the predefined risk categories.<sup>7,19</sup> Clinical usefulness and net benefit were estimated with decision curve analysis.<sup>20</sup> Calibration was assessed by comparing observed 1-year event rates with predictions from the final model.

A P value <0.05 from 2-sided tests was considered statistically significant. Since the analyses were exploratory, the P values were not adjusted for multiple comparisons. All analyses were performed at Uppsala Clinical Research Center

using SAS version 9.4 for Windows (SAS Institute Inc) and R version 3.3 (The R Foundation).

## Results

### Baseline Characteristics

Baseline characteristics, medications, and concentration of the cardiac biomarkers for the serial biomarker cohort are shown in Table 1. At consent, the median age of patients was 70 years (25th percentile–75th percentile, 63–76), 3156 (65.8%) were men, and 3693 (77%) were from Europe/North America.

**Table 4.** Baseline Characteristic With Significant Associations With the Continuous NT-proBNP (ng/L) at Month 2 Adjusted for Baseline NT-proBNP Level and Randomized Treatment

Variable	Variable Value at Baseline	No.	Geometric Mean	Ratio of Geometric Means (95% CI)	P Value*
Age category, y	<65	1277	619.9		<0.0001
	65–74	1635	667.9	1.08 (1.02–1.13)	
	≥75	1256	747.8	1.21 (1.14–1.27)	
Atrial fibrillation	Paroxysmal	700	545.3		<0.0001
	Persistent/permanent	3467	705.3	1.29 (1.22–1.37)	
Heart failure	No	2891	667.9		0.0778
	Yes	1277	692.7	1.04 (1.00–1.08)	
Sex	Female	1460	696.8		0.0150
	Male	2708	664.2	0.95 (0.92–0.99)	
Renal function Cystatin C (quartiles), mg/L	≤0.82	1081	713.3		<0.0001
	0.83–0.99	1007	643.7	0.90 (0.85–0.96)	
	1.0–1.2	1057	635.2	0.89 (0.84–0.95)	
	>1.2	1022	712.6	1.00 (0.93–1.07)	
Troponin I (quartiles), ng/L	≤3.3	1131	618.5		<0.0001
	3.4–5.4	1060	676.7	1.09 (1.03–1.16)	
	5.5–10.1	1034	701.5	1.13 (1.06–1.21)	
	>10.1	907	717.0	1.16 (1.08–1.24)	
Troponin T (quartiles), ng/L	≤7.5	1078	629.5		<0.0001
	>7.5–11.0	1088	657.4	1.04 (0.98–1.11)	
	>11.0–16.7	1041	687.6	1.09 (1.02–1.16)	
	>16.7	959	738.1	1.17 (1.10–1.25)	

NT-proBNP indicates N-terminal pro-B-type natriuretic peptide.

### Changes of cTn and NT-proBNP Levels Over Time in AF

The median cTnI level at entry was 5.1 ng/L (25th–75th percentile, 3.2–9.1 ng/L). At 2 months' follow-up, median levels were slightly higher at 5.2 ng/L (3.4–8.9 ng/L), geometric mean change was 1.02 (95% CI, 1.00–1.03), CV was 7.6%, and within-subject correlation was 0.87. For cTnT, the median level at entry was 10.8 ng/L (7.5–16.1 ng/L) and after 2 months was 11.1 ng/L (7.9–16.9 ng/L), geometric mean change was 1.03 (95% CI, 1.02–1.04), CV was 3.6%, and within-subject correlation was 0.88. Median NT-proBNP level at entry was 702 ng/L (362–1215 ng/L) and 797 ng/L (392–1327 ng/L) after 2 months, geometric mean change was 1.10 (95% CI, 1.08–1.12), CV was 14.4%, and within-subject correlation was 0.82. The agreements between the biomarker concentrations at baseline and after 2 months are shown in Figure 2A through 2C.

There was a positive correlation between changes in cTnI and cTnT concentrations, Spearman  $\rho=0.45$ . The correlation

between changes in NT-proBNP and changes in cTn was lower (cTnI  $\rho=0.15$  and cTnT  $\rho=0.17$ ).

The randomized treatment group (apixaban or warfarin) had no statistically significant relationship with the change in biomarker concentration at 2 months' follow-up (all  $P\geq 0.1272$ ).

### Determinants of Increased Levels of cTn and NT-proBNP at Follow-Up

The effect of baseline characteristics on cTn and NT-proBNP levels at 2 months' follow-up was assessed with linear regression adjusted for baseline levels of each respective biomarker (Table 2–4). For both cTnI and cTnT, presence of coronary artery disease and diabetes mellitus as well as baseline troponin were associated with higher levels at 2 months' follow-up. Furthermore, prior stroke and prior myocardial infarction were associated with higher cTnI values in particular at follow-up, while higher age, male sex, and lower hematocrit level were associated with higher cTnT levels at follow-up. Higher NT-proBNP levels at 2 months were

**Table 5.** Relation to Cardiovascular Outcomes Per 50% Increase in cTnT and NT-proBNP Month 2 Levels

Biomarker/Outcome	No.	Events (%/y)	HR (95% CI)	P Value
<b>NT-proBNP</b>				
Stroke or systemic embolism	4168	87 (1.15)	1.24 (1.06–1.45)	0.0076
Death	4168	237 (3.09)	1.30 (1.18–1.43)	<0.0001
Cardiovascular death	4168	114 (1.49)	1.36 (1.18–1.57)	<0.0001
<b>cTnI</b>				
Stroke or systemic embolism	4648	94 (1.11)	1.12 (0.98–1.29)	0.1079
Death	4648	265 (3.10)	1.18 (1.10–1.28)	<0.0001
Cardiovascular death	4648	125 (1.46)	1.24 (1.13–1.37)	<0.0001
<b>cTnT</b>				
Stroke or systemic embolism	4790	96 (1.11)	1.21 (0.98–1.49)	0.0745
Death	4790	271 (3.08)	1.40 (1.26–1.56)	<0.0001
Cardiovascular death	4790	128 (1.46)	1.50 (1.30–1.74)	<0.0001

Hazard ratios (HRs) per 50% increase in biomarker level at month 2 with 95% CIs and *P* values from Cox proportional hazards model adjusted for randomized treatment and baseline biomarker (continuous, log-transformed). cTnI indicates cardiac troponin I; cTnT, cardiac troponin T; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

mainly affected by age, type of AF (persistent or permanent), and baseline cTn levels.

### Changes of Cardiac Biomarker Levels Over Time and Association With Cardiovascular Risk

In Cox regression analyses adjusted for baseline concentrations of NT-proBNP and randomized treatment, there was a positive association between risk of stroke or systemic embolism and NT-proBNP at month 2 (hazard ratio per 50% increase 1.24; 95% CI, 1.05–1.45 [*P*=0.0093]). For cTnI and cTnT, the association was attenuated (hazard ratio, 1.12 [95% CI, 0.98–1.29] and hazard ratio, 1.21 [95% CI, 0.98–1.49]), respectively. However, the association was more pronounced for cardiovascular mortality for NT-proBNP as well as the cTn biomarkers (Table 5). The association between cardiovascular mortality and changes of cTn and NT-proBNP levels at 2 months according to continuous level of the biomarker at baseline is illustrated in Figure 3A through 3C.

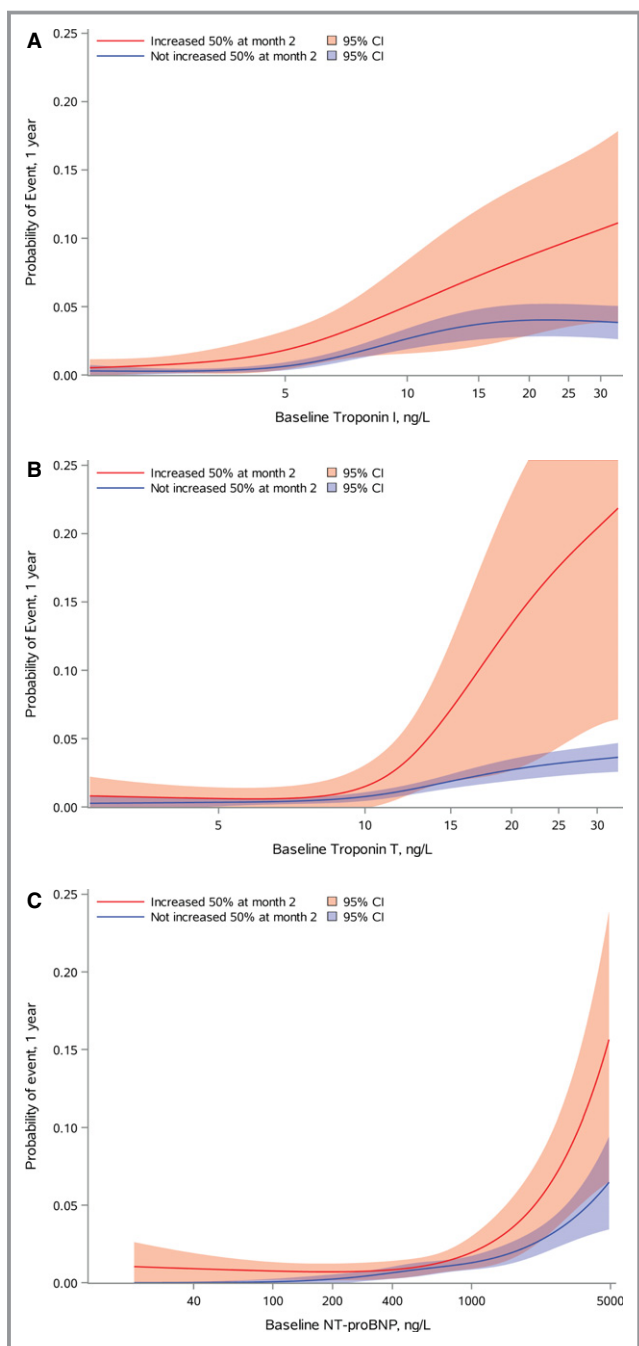
### Prognostic Discrimination Using Repeated Measurement of Cardiac Biomarkers

According to C indices, all 3 biomarkers showed similar discriminatory properties at baseline and after 2 months for the risks of stroke and cardiovascular mortality during follow-up after the 2-month visit. Addition of information from the repeated cTn or NT-proBNP measurement to baseline level on top of established risk factors and other baseline biomarkers (NT-proBNP and cystatin C for cTn; cTnI and cystatin C for the NT-proBNP-15 analyses) did not

improve the prognostication sufficiently for stroke or systemic embolism (no improvement in C index and/or *P*≥0.1031). For cardiovascular death, repeated measurement of the cardiac biomarkers at 2 months improved the C index slightly (cTnI 0.78 to 0.80 and cTnT 0.77 to 0.78; NT-proBNP 0.78 to 0.79 with statistically significant model improvement [*P*≤0.0006 for all]).

### Discrimination and Calibration of the ABC Stroke Score at 2-Month Follow-Up

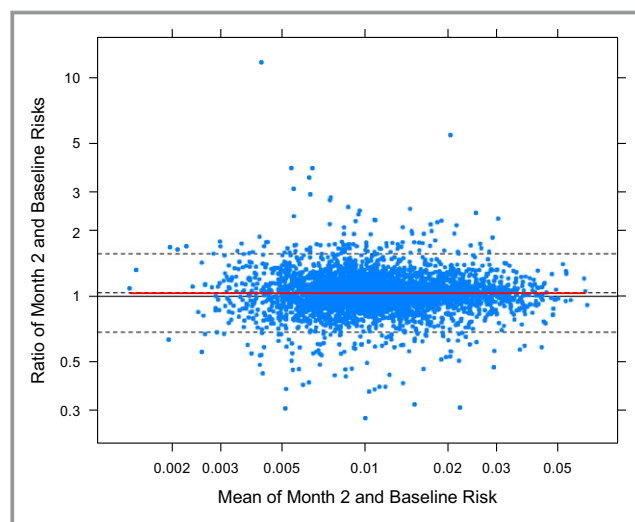
The newly developed biomarker-based stroke risk model, the ABC stroke score, was assessed in this cohort with a total of 8702 person-years of follow-up and a total of 96 adjudicated stroke or systemic embolic events after the blood sampling at 2 months. Using information from the cardiac biomarker levels at 2 months yielded a C index for the ABC stroke score of 0.72 (95% CI, 0.66–0.77) including cTnI and 0.70 (95% CI, 0.65–0.76) including cTnT. The C index for the ABC stroke score using data at baseline was almost identical, with cTnI 0.71 (95% CI, 0.66–0.76) and cTnT 0.70 (95% CI, 0.65–0.75). The similarity between the predicted risks of ABC stroke score at baseline and after 2 months is illustrated in Figure 4. Most predictions, 94.7%, differed less than a factor of 1.5 between the 2 measurement periods (Figure 4). The ABC stroke score at 2 months showed good calibration according to predefined risk classes (Figure 5). Similarly, decision curve analysis showed consistent net benefit for clinical usefulness when reassessing the ABC stroke score at 2 months compared with the ABC stroke score at baseline (Figure 6).



**Figure 3.** Association between continuous biomarker levels at baseline (x-axis) and cardiovascular mortality by group according to increase of each biomarker at 2 months (red lines) for (A) cTnI, (B) cTnT, and (C) NT-proBNP. NT-proBNP indicates N-terminal pro-B-type natriuretic peptide.

## Discussion

The main findings in this study were that the changes of cardiac biomarker levels in patients with stable AF during 2 months were small and that there was low intraindividual



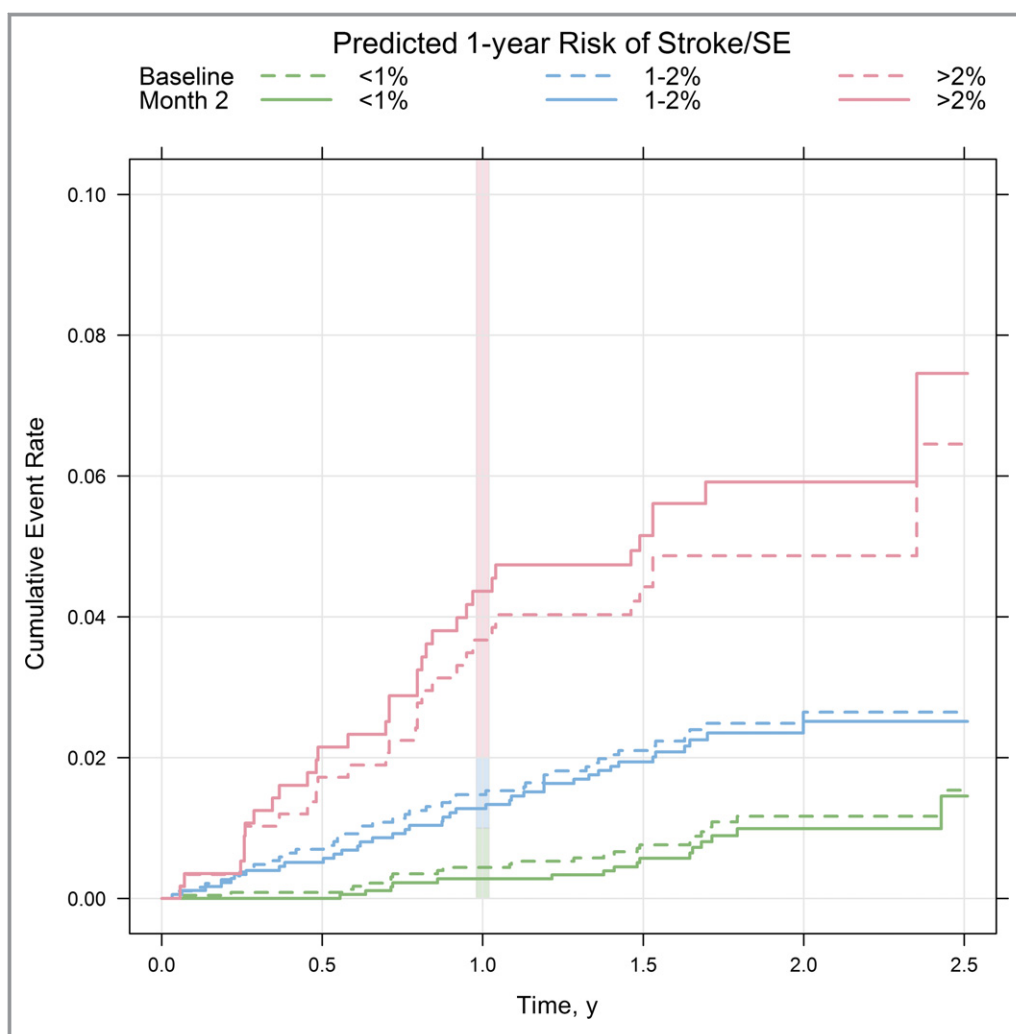
**Figure 4.** Bland-Altman plot of the log ratio (difference in log-predicted ABC [age, biomarkers, and clinical history of prior stroke] risk) between the 2-month and baseline estimates of the 1-year risk of stroke or systemic embolism against the geometric mean of the 2 estimates. The dashed lines indicate the mean and the 2-SDs distance from the mean. The red line indicates locally weighted scatterplot smoothing. Cardiac troponin T was used in the ABC stroke score.

variability. Increasing cTn levels were associated with higher baseline cTn levels, renal dysfunction, and cardiovascular comorbidities. Increasing levels of NT-proBNP were mainly associated with higher baseline cTn levels, older age, and persistent or permanent AF. In patients with AF in stable condition, repeated measurement of the cardiac biomarkers cTn and NT-proBNP provided some incremental prognostic value for mortality but not for stroke when combined with other prognostic biomarkers and established clinical risk factors. The biomarker-based ABC stroke risk score showed a reliable and stable predictive performance and remained well calibrated when reevaluated after 2 months.

## Repeated Measurements of cTn and Cardiovascular Risk

It has been demonstrated in patients with AF that cTn measured with high-sensitivity assays is independently associated with the risk of stroke, mortality, and major bleeding events.<sup>4,5</sup> We recently reported that a detectable level of cTnI during follow-up as measured with a conventional assay (AccuTnI assay, Beckman Coulter) was associated with an increased risk of stroke and cardiovascular events.<sup>21</sup> By using high-sensitivity cTn assays in the present study, in which more than 93% have detectable levels,<sup>4,5</sup> a more detailed analysis was possible concerning changes of troponin during a short period in a clinical setting. In both the RE-LY (Randomized



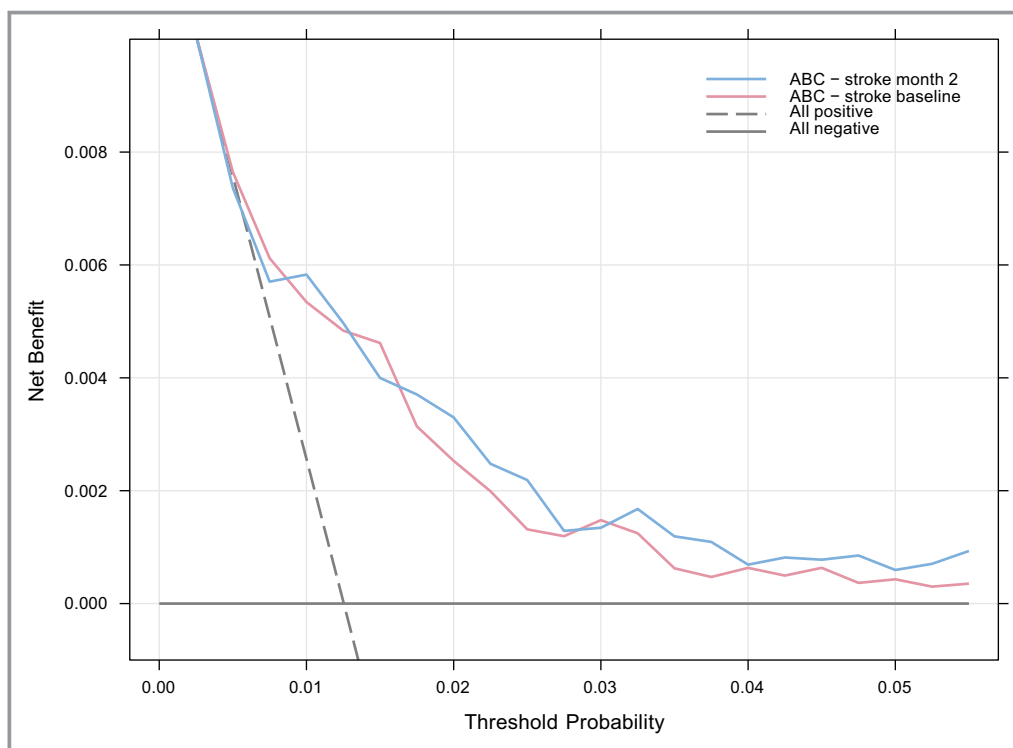


**Figure 5.** Cumulative event rate of stroke or systemic embolism by predicted 1-year ABC (age, biomarkers, and clinical history of prior stroke) risk (cardiac troponin T) group (green=0–1%, blue=1–2%, and red >2%) for the baseline (dashed lines) and temporal validation (solid lines) data. The figure shows the cumulative event rate within risk classes for the ABC stroke model evaluated independently at the 2 time points. It illustrates that the model performs equally well when it is applied using biomarker values at baseline as when it is applied using biomarker values at 2 months. SE indicates systemic embolism.

Evaluation of Long-Term Anticoagulation Therapy) and the present study, repeated measurement of cTnI improved risk stratification in patients with AF for cardiovascular mortality. However, there was seemingly a discrepancy between these studies concerning the findings for stroke or systemic embolism. In the RE-LY study, but not in the present serial markers substudy, a significant association between cTn and stroke or systemic embolism was seen. The differences are reasonably attributable to the vastly improved risk prediction gained by the initial cTnI measurement at baseline by using high-sensitivity assays compared with the conventional troponin assays. This was apparent as the C index for stroke or systemic embolism improved from 0.59 to 0.63 in the RE-LY study using the conventional cTnI assay, compared with

a C index from 0.69 to 0.70 using high-sensitivity assays in the present study.

The present findings demonstrating additive prognostic information of repeat measurement for the risk of cardiovascular mortality in patients with AF are in accordance with findings in previous studies with other patient populations, eg, those with coronary artery disease and heart failure and in apparently healthy adults, in which serial measurements of cTn have been shown to be powerful markers of mortality and morbidity.<sup>15,22–24</sup> Our findings in the present study therefore extend these observations to a novel population and validate previous results by displaying the prognostic importance of a repeated measurement of cTn in patients with AF related to mortality risk.



**Figure 6.** Decision curve analysis for the ABC stroke score (age, biomarkers, and clinical history of prior stroke) (cardiac troponin T) applied at baseline (red) and at 2 months (blue).

The additive value of repeated measurement was overall similar for cTnI and cTnT, with some potential differences of risks at different concentrations. There are some structural differences between cTnI and cTnT as well as differences in the assay properties that may contribute to these dissimilarities and provide insights into the moderate correlation between the 2 cTn subtypes.<sup>25</sup> The specific mechanism of elevated cTn in patients with AF is not known. It has been hypothesized that it might be related to changes in microvascular blood flow and myocyte ischemia and dysfunction, and possibly linked to underlying processes of atrial inflammation and fibrosis in AF.<sup>26,27</sup> Even though the average change during 2 months for cTn was small, there were some differences between the determinants for increasing levels of cTnI and cTnT. Recently, similar patterns between cTnI and cTnT have also been described in AF, although these findings are restricted by analyses based on single measurements.<sup>28</sup>

### Repeated Measurement of NT-proBNP and Cardiovascular Risk

NT-proBNP has demonstrated a strong independent association with risk of stroke and mortality in patients with AF.<sup>3,6</sup> Recently, we also reported that an elevated level during follow-up was associated with an increased risk of stroke

and cardiovascular events.<sup>21</sup> In the present study, using repeated measurement, we demonstrated that the variability of NT-proBNP over time is low, and that risk stratification improves for stroke and mortality in patients with AF during treatment with anticoagulation. In this study, as compared with the RE-LY biomarker substudy, we assessed the actual change in NT-proBNP levels, accounting for analytical imprecision and, importantly, also adjusting the analysis for the levels of NT-proBNP at baseline. The differences in the predictive importance most likely depends on the use of a larger number of clinical risk indicators (demographic variables and comorbidities) as well as the inclusion of biomarkers with high-sensitivity assays in the baseline model before assessing the added value of repeated NT-proBNP measurements.

### The ABC Stroke Score Over Time

This study presents the first temporal validation of the novel biomarker-based ABC stroke score for estimating the risk of stroke in patients with AF. Although cardiac biomarkers are powerful predictors of risk of stroke in patients with AF, analytical and temporal variation might influence the clinical usefulness of a score composed of biomarkers. However, there is an abundance of data demonstrating the stability of these analytes and the high precision in the used assays.<sup>25,28–30</sup> We

showed that the levels of cardiac biomarkers overall remain stable with a relatively low CV and a high within-subject correlation in patients with stable AF during 2 months of follow-up. The biomarker-based ABC stroke score remained well calibrated over time and provided similar clinical usefulness. The majority of patients had similar predicted risk of stroke using the ABC stroke score at baseline and at 2 months. Therefore, from a clinical perspective, short-term routine reassessment does not seem necessary in patients with stable AF. The results also support that reliable and similar prognostic information is obtained by biomarker determinations at different time points during the course of the disease.

### Limitations

Our results were derived from a clinical trial population of consenting patients with AF and at least 1 risk factor for stroke and therefore may not apply to a general unselected population. The change of the biomarker concentrations over time may be influenced by the analytical imprecision. Similarly, parts of the observed differences between cTnI and cTnT may be attributable to analytical differences between the assays rather than biological differences. However, both troponins were analyzed using high-sensitivity assays with high precision in the same plasma samples. Within the ARISTOTLE trial, repeated sampling of biomarkers was prespecified at 2 months; therefore, the importance of long-term (12 months or longer) changes of cardiac biomarkers in patients with AF may thus differ and requires further investigation. Nonetheless, the present results provide valuable information from a clinical perspective on the short-term stability of these biomarkers in patients with AF and on the consistent risk prediction with the ABC stroke score.

### Conclusions

In patients with stable AF, the variability of the levels of the cardiac biomarkers cTn and NT-proBNP levels during 2 months is small. Repeated measurements of these cardiac biomarkers do not seem useful for monitoring the risk of stroke, although it might be useful for improved information on the risk of cardiovascular mortality. The ABC stroke risk score provides reliable and stable prognostic information at different time points, and reassessment of stroke risk by repeating the ABC stroke score is overall not routinely required in patients with stable AF within a short time frame such as 2 months. Whether biomarker retesting at a later time point or after a change in clinical status would add additional prognostic information requires further investigation.

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## References

- Benjamin EJ, Wolf PA, D'Agostino RB, Silbershatz H, Kannel WB, Levy D. Impact of atrial fibrillation on the risk of death: the Framingham Heart Study. *Circulation*. 1998;98:946–952.
- Wolf PA, Abbott RD, Kannel WB. Atrial fibrillation as an independent risk factor for stroke: the Framingham Study. *Stroke*. 1991;22:983–988.
- Hijazi Z, Oldgren J, Andersson U, Connolly SJ, Ezekowitz MD, Hohnloser SH, Reilly PA, Vinereanu D, Siegbahn A, Yusuf S, Wallentin L. Cardiac biomarkers are associated with an increased risk of stroke and death in patients with atrial fibrillation: a Randomized Evaluation of Long-term Anticoagulation Therapy (RE-LY) substudy. *Circulation*. 2012;125:1605–1616.
- Hijazi Z, Siegbahn A, Andersson U, Granger CB, Alexander JH, Atar D, Gersh BJ, Hanna M, Harjola VP, Horowitz J, Husted S, Hylek EM, Lopes RD, McMurray JJ, Wallentin L, Investigators A. High-sensitivity troponin I 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. *Circulation*. 2014;129:625–634.
- Hijazi Z, Wallentin L, Siegbahn A, Andersson U, Alexander JH, Atar D, Gersh BJ, Hanna M, Harjola VP, Horowitz J, Husted S, Hylek EM, Lopes RD, McMurray JJ, Granger CB, Investigators A. High-sensitivity troponin T and risk stratification in patients with atrial fibrillation during treatment with apixaban or warfarin. *J Am Coll Cardiol*. 2014;63:52–61.
- Hijazi Z, Wallentin L, Siegbahn A, Andersson U, Christersson C, Ezekowitz J, Gersh BJ, Hanna M, Hohnloser S, Horowitz J, Huber K, Hylek EM, Lopes RD, McMurray JJ, Granger CB. N-terminal pro-B-type natriuretic peptide for risk assessment in patients with atrial fibrillation: insights from the ARISTOTLE Trial (Apixaban for the Prevention of Stroke in Subjects With Atrial Fibrillation). *J Am Coll Cardiol*. 2013;61:2274–2284.
- Hijazi Z, Lindback J, Alexander JH, Hanna M, Held C, Hylek EM, Lopes RD, Oldgren J, Siegbahn A, Stewart RA, White HD, Granger CB, Wallentin L, Aristotle and Investigators S. The ABC (age, biomarkers, clinical history) stroke risk score: a biomarker-based risk score for predicting stroke in atrial fibrillation. *Eur Heart J*. 2016;37:1582–1590.
- Oldgren J, Hijazi Z, Lindback J, Alexander JH, Connolly SJ, Eikelboom JW, Ezekowitz MD, Granger CB, Hylek EM, Lopes RD, Siegbahn A, Salim Y, Wallentin L. External validation of the biomarker-based ABC-stroke risk score for atrial fibrillation. *J Am Coll Cardiol*. 2016;67:879–879.
- Granger CB, Alexander JH, McMurray JJ, Lopes RD, Hylek EM, Hanna M, Al-Khalidi HR, Ansell J, Atar D, Avezum A, Bahit MC, Diaz R, Easton JD, Ezekowitz JA, Flaker G, Garcia D, Ghalibaf M, Gersh BJ, Golitsyn S, Goto S, Hermosillo AG, Hohnloser SH, Horowitz J, Mohan P, Jansky P, Lewis BS, Lopez-Sendon JL, Pais P, Parkhomenko A, Verheugt FW, Zhu J, Wallentin L. Apixaban versus warfarin in patients with atrial fibrillation. *N Engl J Med*. 2011;365:981–992.
- Lopes RD, Alexander JH, Al-Khatib SM, Ansell J, Diaz R, Easton JD, Gersh BJ, Granger CB, Hanna M, Horowitz J, Hylek EM, McMurray JJ, Verheugt FW, Wallentin L. Apixaban for reduction in stroke and other Thromboembolic events in atrial fibrillation (ARISTOTLE) trial: design and rationale. *Am Heart J*. 2010;159:331–339.
- Apple FS, Ler R, Murakami MM. Determination of 19 cardiac troponin I and T assay 99th percentile values from a common presumably healthy population. *Clin Chem*. 2012;58:1574–1581.
- Giannitsis E, Kurz K, Hallermayer K, Jarausch J, Jaffe AS, Katus HA. Analytical validation of a high-sensitivity cardiac troponin T assay. *Clin Chem*. 2010;56:254–261.
- Johnston N, Jernberg T, Lindahl B, Lindback J, Stridsberg M, Larsson A, Venge P, Wallentin L. Biochemical indicators of cardiac and renal function in a healthy elderly population. *Clin Biochem*. 2004;37:210–216.
- Yeo KT, Wu AH, Apple FS, Kroll MH, Christenson RH, Lewandowski KB, Sedor FA, Butch AW. Multicenter evaluation of the Roche NT-proBNP assay and comparison to the Biosite Triage BNP assay. *Clin Chim Acta*. 2003;338:107–115.
- de Filippi CR, de Lemos JA, Christenson RH, Gottdiener JS, Kop WJ, Zhan M, Seliger SL. Association of serial measures of cardiac troponin T using a sensitive assay with incident heart failure and cardiovascular mortality in older adults. *JAMA*. 2010;304:2494–2502.
- Nordenskjold AM, Ahlstrom H, Eggers KM, Frobert O, Jaffe AS, Venge P, Lindahl B. Short- and long-term individual variation in cardiac troponin in patients with stable coronary artery disease. *Clin Chem*. 2013;59:401–409.
- Nordenskjold AM, Ahlstrom H, Eggers KM, Frobert O, Venge P, Lindahl B. Short- and long-term individual variation in NT-proBNP levels in patients with stable coronary artery disease. *Clin Chim Acta*. 2013;422:15–20.
- Harrell FE. *Regression Modeling Strategies: With Applications to Linear Models, Logistic and Ordinal Regression, and Survival Analysis*. New York, NY: Springer; ISBN 978-3-319-19424-0; 2015.
- Kirchhof P, Benussi S, Kotecha D, Ahlsson A, Atar D, Casadei B, Castella M, Diener HC, Heidbuchel H, Hendriks J, Hindricks G, Manolis AS, Oldgren J, Popescu BA, Schotten U, Van Putte B, Vardas P, Agewall S, Camm J, Baron Esquivias G, Budts W, Carerj S, Casselman F, Coca A, De Caterina R, Deftereos S, Dobrev D, Ferro JM, Filippatos G, Fitzsimons D, Gorennek B, Guenoun M, Hohnloser SH, Koh P, Lip GY, Manolis A, McMurray J, Ponikowski P, Rosenhek R, Ruschitzka F, Savelieva I, Sharma S, Suwalski P, Tamargo JL, Taylor CJ, Van Gelder IC, Voors AA, Windecker S, Zamorano JL, Zeppenfeld K. 2016 ESC Guidelines for the management of atrial fibrillation developed in collaboration with EACTS. *Eur Heart J*. 2016;37:2893–2962.
- Vickers AJ, Cronin AM, Elkin EB, Gonen M. Extensions to decision curve analysis, a novel method for evaluating diagnostic tests, prediction models and molecular markers. *BMC Med Inform Decis Mak*. 2008;8:53.
- Hijazi Z, Oldgren J, Andersson U, Connolly SJ, Ezekowitz MD, Hohnloser SH, Reilly PA, Siegbahn A, Yusuf S, Wallentin L. Importance of persistent elevation of cardiac biomarkers in atrial fibrillation: a RE-LY substudy. *Heart*. 2014;100:1193–1200.
- Eggers KM, Lagerqvist B, Venge P, Wallentin L, Lindahl B. Persistent cardiac troponin I elevation in stabilized patients after an episode of acute coronary syndrome predicts long-term mortality. *Circulation*. 2007;116:1907–1914.
- Miller WL, Hartman KA, Burritt MF, Grill DE, Rodeheffer RJ, Burnett JC Jr, Jaffe AS. Serial biomarker measurements in ambulatory patients with chronic heart failure: the importance of change over time. *Circulation*. 2007;116:249–257.
- Xue Y, Clopton P, Peacock WF, Maisel AS. Serial changes in high-sensitive troponin I predict outcome in patients with decompensated heart failure. *Eur J Heart Fail*. 2011;13:37–42.
- Apple FS, Collinson PO, IFCC Task Force on Clinical Applications of Cardiac Biomarkers. Analytical characteristics of high-sensitivity cardiac troponin assays. *Clin Chem*. 2012;58:54–61.
- Hijazi Z, Oldgren J, Siegbahn A, Granger CB, Wallentin L. Biomarkers in atrial fibrillation: a clinical review. *Eur Heart J*. 2013;34:1475–1480.
- Jeremias A, Gibson CM. Narrative review: alternative causes for elevated cardiac troponin levels when acute coronary syndromes are excluded. *Ann Intern Med*. 2005;142:786–791.
- Hijazi Z, Siegbahn A, Andersson U, Lindahl B, Granger CB, Alexander JH, Atar D, Gersh BJ, Hanna M, Harjola VP, Horowitz J, Husted S, Hylek EM, Lopes RD, McMurray JJ, Wallentin L. Comparison of cardiac troponins I and T measured with high-sensitivity methods for evaluation of prognosis in atrial fibrillation: an ARISTOTLE substudy. *Clin Chem*. 2015;61:368–378.
- Lindahl B, Eggers KM, Venge P, James S. Evaluation of four sensitive troponin assays for risk assessment in acute coronary syndromes using a new clinically oriented approach for comparison of assays. *Clin Chem Lab Med*. 2013;51:1859–1864.
- Nowatzke WL, Cole TG. Stability of N-terminal pro-brain natriuretic peptide after storage frozen for one year and after multiple freeze-thaw cycles. *Clin Chem*. 2003;49:1560–1562.



## Repeated Measurements of Cardiac Biomarkers in Atrial Fibrillation and Validation of the ABC Stroke Score Over Time

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