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FASTTRACK – CLINICAL RESEARCH
Clinical trials

Biomarker-based prediction of sinus rhythm in atrial fibrillation patients: the EAST-AFNET 4 biomolecule study

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Abstract

Background and Aims In patients with atrial fibrillation (AF), recurrent AF and sinus rhythm during follow-up are determined by interactions between cardiovascular disease processes and rhythm control therapy. Predictors of attaining sinus rhythm at follow-up are not well known.

Methods To quantify the interaction between cardiovascular disease processes and rhythm outcomes, 14 biomarkers reflecting AF-related cardiovascular disease processes in 1586 patients in the EAST-AFNET 4 biomolecule study (71 years old, 45% women) were quantified at baseline. Mixed logistic regression models including clinical features were constructed for each biomarker. Biomarkers were interrogated for interaction with early rhythm control. Outcome was sinus rhythm at 12 months. Results were validated at 24 months and in external datasets.

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Results Higher baseline concentrations of three biomarkers were independently associated with a lower chance of sinus rhythm at 12 months: angiopoietin 2 (ANGPT2) (odds ratio [OR] .76 [95% confidence interval .65–.89], $P < .001$), bone morphogenetic protein 10 (BMP10) (OR .83 [.71–.97], $P = .017$), and N-terminal pro-B-type natriuretic peptide (NT-proBNP) (OR .73 [.60–.88], $P < .001$). Analysis of rhythm at 24 months confirmed the results. Early rhythm control interacted with the predictive potential of NT-proBNP ($P_{interaction} = .033$). The predictive effect of NT-proBNP was reduced in patients randomized to early rhythm control (usual care: OR .64 [.51–.80], $P < .001$; early rhythm control: OR .90 [.69–1.18], $P = .453$). External validation confirmed that low concentrations of ANGPT2, BMP10, and NT-proBNP predict sinus rhythm during follow-up.

Conclusions Low concentrations of ANGPT2, BMP10, and NT-proBNP identify patients with AF who are likely to attain sinus rhythm during follow-up. The predictive ability of NT-proBNP is attenuated in patients receiving rhythm control.

Structured Graphical Abstract

Key Question

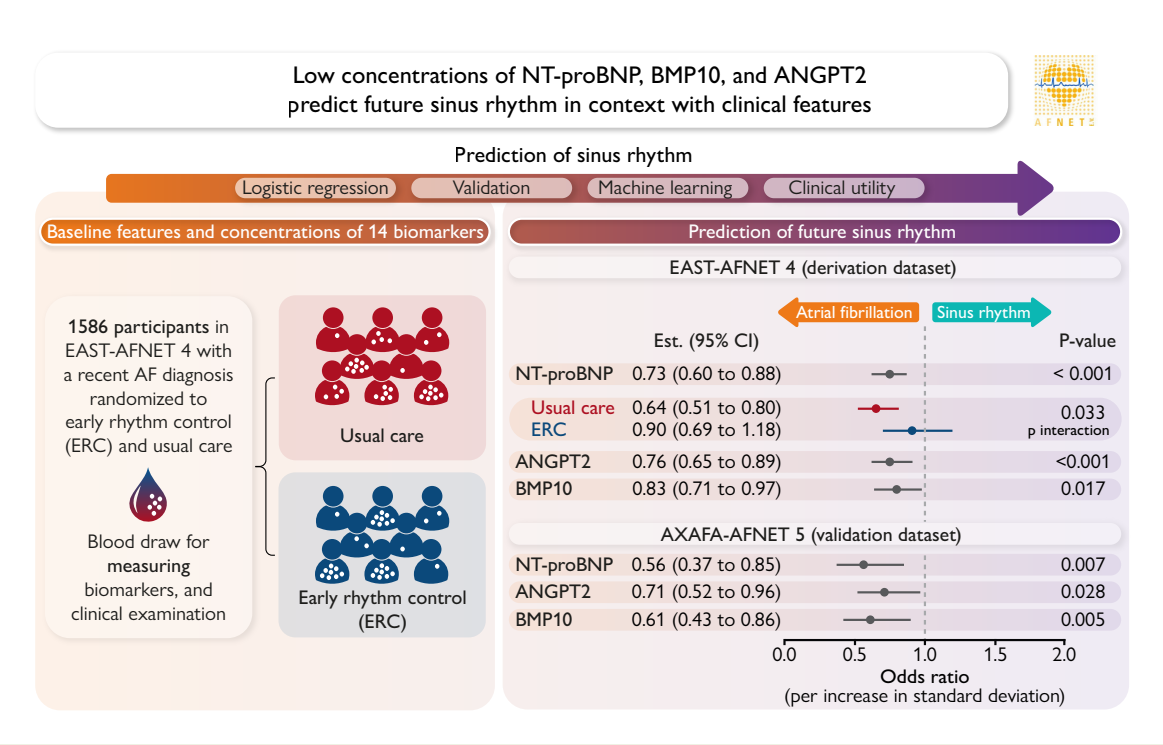
Can clinical features or circulating biomarkers measured at baseline predict sinus rhythm during mid-term follow-up in patients with atrial fibrillation (AF)? Which biomarkers interact with early rhythm control therapy?

Key Finding

Low baseline concentrations of NT-proBNP (<1000 pg/ml), ANGPT2 (<3.5 ng/ml) and BMP10 (<2 ng/ml) predicted sinus rhythm at follow-up in the EAST-AFNET 4 trial and in two external validation data sets. The three biomarkers improved sinus rhythm prediction compared to a clinical risk score.

Take Home Message

Biomarkers like NT-proBNP, ANGPT2 and BMP10 can improve the prediction of sinus rhythm at follow-up in patients with a history of AF. Further studies are needed to establish their role in clinical practice.



In patients diagnosed with atrial fibrillation, low concentrations of NT-proBNP, BMP10, and ANGPT2 at baseline predict sinus rhythm at 12-month follow-up in context with clinical features. This was validated in additional datasets, of which AXAFA-AFNET 5 is depicted here. A treatment interaction shows that NT-proBNP's predictive value is impacted by early rhythm control treatment. AF, atrial fibrillation; ANGPT2, angiopoietin 2; BMP10, bone morphogenetic protein 10; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

Keywords Atrial fibrillation • Blood biomarker • Sinus rhythm • Rhythm control • Natriuretic peptides • Bone morphogenetic protein 10 • Angiopoietin 2 • Risk prediction • Risk score

Introduction

In addition to improving atrial fibrillation (AF)-related symptoms,[1] rhythm control therapy[2] can prevent AF-related cardiovascular events such as stroke, heart failure hospitalizations, and cardiovascular death.[3] The cardiovascular complication-reducing effect of early rhythm control therapy shown in the EAST-AFNET 4 study is mainly mediated by attaining sinus rhythm at 12-month follow-up.[4] This potentially reflects a reduced AF burden[5] and lack of progression to non-paroxysmal patterns of AF.[6,7] Predicting sinus rhythm at 12 months could therefore help to identify patients requiring intensive rhythm control, e.g. with AF ablation.[3,8] Knowledge of treatable processes contributing to AF at 12-month follow-up can help to develop adjunct therapies aimed at maintaining sinus rhythm and preventing AF progression. Several chronic, interdependent disease processes[9,10] contribute to AF. Such processes can be aggravated by presence of AF, attenuated by rhythm control, or exist independent of AF.[1,11] Circulating biomarkers provide quantitative proxies for cardiomyocyte death or injury [troponin (TnT)]; atrial metabolic dysfunction and stress [bone morphogenetic protein 10 (BMP10), fatty acid binding protein 3 (FABP3), and insulin-like growth factor binding protein 7 (IGFBP7)][12,13]; thrombo-inflammation [D-dimer, C-reactive protein (CRP), interleukin-6 (IL-6)][14,15]; vascular and endothelial dysfunction [angiopoietin 2 (ANGPT2), endothelial specific molecule 1 (ESM1)][14,15]; frailty [growth differentiation factor 15 (GDF-15)]; and cardiac load [natriuretic peptides like N-terminal pro-B-type natriuretic peptide (NT-proBNP)].[16] Quantification of biomarkers selected to reflect these disease processes in a single blood draw identifies patient clusters with different risk of cardiovascular events.[17] Whether the disease processes reflected by these biomarkers modify future rhythm in patients with AF has not been investigated.

This analysis of the EAST-AFNET 4 biomolecule study embedded into the Early treatment of Atrial fibrillation for STroke prevention (EAST-AFNET 4) trial[2] quantified 14 biomarkers reflecting different disease processes in AF that were defined a priori.[9] The ability of each biomarker to predict sinus rhythm at 12-month follow-up in patients with and without early rhythm control therapy was evaluated (*Structured Graphical Abstract*).

Validation was performed internally at 24 months, by comparing biomarker-based clusters at baseline by association with sinus rhythm at 12- and 24-month follow-up and by machine learning integrating biomarkers and clinical parameters. Clinical utility was assessed by defining and testing threshold values and by comparison with a clinical score. External validation was performed in two independent datasets of patients with AF.

Methods

Details of the prespecified analysis plan of the EAST-AFNET 4 biomolecule study can be found in a separate Supplementary material file (Supplementary file Statistical analysis plan SAP). *Post hoc* exploratory analyses were added to gain more insight into the main findings.

Derivation dataset (EAST-AFNET 4)

EAST-AFNET 4 randomized patients with recently diagnosed AF and stroke risk factors to systematic early rhythm control or usual care including symptom-based rhythm control.[2] All patients were followed up for a median of 5.1 years. The EAST-AFNET 4 biomolecule study collected a baseline blood sample in 1586 patients enrolled in the EAST-AFNET 4 trial.[17,18] In brief, all consenting patients provided a blood sample at baseline. Samples were shipped to the core biostorage facility at UKE Hamburg, spun, shock-frozen, and stored at −80°C. EAST-AFNET 4 and its biomolecule study were approved at all participating study sites. Written informed consent was obtained from all patients.

Validation datasets

AXAFA-AFNET 5

The Anticoagulation using the direct factor Xa inhibitor apixaban during Atrial Fibrillation catheter Ablation: Comparison to vitamin K antagonist therapy (AXAFA-AFNET 5[19]) trial was a randomized, investigator-initiated trial comparing continuous vitamin K antagonist therapy to apixaban in 633 patients undergoing a first AF ablation in 49 European and US American study sites. The same 14 biomarkers quantified in the derivation dataset were quantified in the AXAFA-AFNET 5 blood samples using the same assays.[20] The outcome of interest was rhythm at the final follow-up visit, 120 days after enrolment.[19] All patients provided written informed consent.

BBC-AF atrial fibrillation snapshot

Details of the BBC-AF cohort have been described before.[21] In brief, consecutive patients eligible for recruitment had ECG-diagnosed AF or presented with at least two cardiovascular conditions (congestive heart failure, hypertension, diabetes, prior stroke, or vascular disease) to a large teaching hospital (Sandwell and West Birmingham NHS Trust). Patients who did not have a diagnosis of AF underwent 7-day ambulatory ECG monitoring to rule out undiagnosed ECG-documented AF. For this analysis, only patients with ECG-documented AF were included. Follow-up data were collected by assessing local hospital records corroborated against Hospital Episode Statistics data, general practitioner records, and mortality data from NHS Digital, up to 2.5 years after the final patient was recruited.[22] This study complied with the Declaration of Helsinki, was approved by the National Research Ethics Service Committee (IRAS ID 97753), and was sponsored by the University of Birmingham. All patients provided written informed consent.

TRUST snapshot

A snapshot of patients enrolled in the Long-term Outcome and Predictors for Recurrence after Medical and Interventional Treatment of Arrhythmias study (TRUST; NCT05521451), with biomarker concentrations and 12-month rhythm status was created. All patients provided written informed consent. A snapshot of all patients with biomarker concentrations and ECG follow-up at 12–18 months was obtained in June 2024 for validation.

Selection of biomarkers and their quantification

Circulating biomarkers were selected by scientists from the EU-funded CATCH-ME consortium based on relevant disease processes and available high-precision high-throughput assays.[9] Biomarkers were selected in four steps: (i) members of the consortium identified candidate biomarkers reflecting disease processes known to contribute to AF and its complications, (ii) deep literature and patent searches for candidate biomarkers and additional novel biomarkers were performed, (iii) expert discussion and Delphi-like votes by the consortium defined most promising candidates, and (iv) availability and feasibility checks to perform measurements of thousands of samples with high precision.

Fourteen biomarkers were selected (*Table 1* following clinical characteristics); ANGPT2, BMP10, cancer antigen 125 (CA125), CRP, D-dimer, ESM1, FABP3, fibroblast growth factor 23 (FGF23), GDF15, IGFBP7, IL-6, NT-proBNP, TnT, and serum creatinine (sCr).

Blood samples were collected at all participating sites and shipped to the core lab at University Heart and Vascular Center (UHZ) Hamburg by courier at ambient temperatures (24–48 h transport time). Upon arrival at UHZ, samples were spun, shock-frozen, and stored at −80°C for analysis. Biomarkers were centrally quantified using pre-commercial and commercial high-throughput, high-precision platforms (Roche, Penzberg, Germany) in EDTA plasma. The biomarker quantification was provided as an in-kind contribution of Roche to the CATCH ME consortium. Blood samples were shipped to, and quantifications were conducted at the Roche biomarker research facility in Penzberg, Germany.

Table 1 Baseline characteristics and biomarkers in the EAST-AFNET 4 biomolecule study

Treatment group		Early rhythm control	Usual care	*P*-value*
n		800	786	
Sex: female		355 (44%)	358 (46%)	.639
Age (years)		71 [66, 75]	71 [66, 76]	.711
BMI		28.7 [25.6, 32.1]	29.0 [25.6, 32.5]	.699
Blood pressure (systolic, mmHg)		135 [123, 150]	135 [125, 148]	.730
Blood pressure (diastolic, mmHg)		80 [74, 90]	80 [74, 90]	.716
LVEF (%)		60 [55, 65]	60 [55, 65]	.873
AF type (first episode)		290 (36%)	270 (34%)	
AF type (paroxysmal)		302 (38%)	288 (37%)	.839
AF type (persistent)		208 (26%)	228 (29%)	.202
Other clinical characteristics				
Diabetes		207 (26%)	189 (24%)	.400
Hypertension		494 (62%)	512 (65%)	.170
Chronic kidney disease		98 (12%)	97 (12%)	.956
Estimated glomerular filtration rate (mL/min 1.73 m^2)		75 [63–87]	76 [64–87]	.734
Previous stroke or transient ischaemic attack		114 (14%)	81 (10%)	.017
Chronic obstructive pulmonary disease		63 (8%)	61 (8%)	.991
Diastolic LA diameter (mm)		42 [38, 47]	43 [39, 47]	.730
NYHA class				
No heart failure		523 (65%)	509 (65%)	
I		82 (10%)	88 (11%)	.555
II		164 (21%)	160 (20%)	.985
III		31 (4%)	29 (4%)	.882
EHRA score				
I		232 (29%)	236 (30%)	
II		386 (48%)	374 (48%)	.679
III		122 (15%)	122 (15%)	.914
IV		8 (1%)	9 (1%)	.839
Missing		52 (7%)	45 (6%)	
Biomarker (unit)	Coefficient of variation			
NT-proBNP (pg/mL)	1.51	441 [175–966]	467 [187–1036]	.537
ANGPT2 (ng/mL)	.70	2.53 [1.87–3.65]	2.53 [1.87–3.75]	.456
BMP10 (ng/mL)	.24	2.10 [1.82–2.41]	2.11 [1.83–2.45]	.507
FGF23 (pg/mL)	1.27	155 [115–218]	153 [115–211]	.244
ESM1 (ng/mL)	.76	2.04 [1.64–2.59]	2.05 [1.63–2.63]	.818
GDF15 (pg/mL)	.80	1333 [990–2000]	1359 [971–2005]	.078
IGFBP7 (ng/mL)	.26	102 [90.7–117]	102 [90.1–117]	.457
IL-6 (pg/mL)	6.62	2.56 [1.64–4.04]	2.68 [1.67–4.18]	.479
FABP3 (ng/mL)	.50	32.0 [26.3–39.6]	31.9 [26.4–39.6]	.837
D-dimer (μg/mL)	1.74	.17 [.09–.34]	.16 [.08–.36]	.506

Continued

Table 1 *Continued*

Treatment group		Early rhythm control	Usual care	*P*-value*
TnT (ng/L)	2.26	11.1 [8.02–16.6]	11.4 [8.21–16.7]	.337
CRP (mg/L)	3.28	2.02 [.96–4.99]	2.38 [1.04–4.75]	.392
sCr (μmol/L)	.29	81.7 [70.7–95.5]	80.4 [70.0–94.5]	.771
CA125 (U/mL)	1.51	11.5 [8.08–15.9]	11.1 [7.93–16.1]	.433

Estimated glomerular filtration rate (eGFR) was calculated as CKD EPI, Chronic Kidney Disease Epidemiology Collaboration.
AF, atrial fibrillation; SR, sinus rhythm; ERC, early rhythm control; UC, usual care; BMI, body mass index; LVEF, left ventricular ejection fraction; LA, left atrium; NYHA, New York Heart Association Functional Classification of heart failure; EHRA, European Heart Rhythm Association score; ANGPT2, angiopoietin 2; BMP10, bone morphogenetic protein 10; CA125, cancer antigen 125; CRP, C-reactive protein; ESM1, endothelial specific molecule 1; FABP3, fatty acid binding protein 3; FGF23, fibroblast growth factor 23; GDF15, growth differentiation factor 15; IGFBP7, insulin-like growth factor binding protein 7; IL-6, interleukin-6; NT-proBNP, N-terminal pro-B-type natriuretic peptide; TnT, cardiac troponin; sCr, serum creatinine.
**P*-values were calculated on the unimputed dataset using mixed logistic regression model with site as random effect, for biomarkers additionally adjusted for sex, age, body mass index, diastolic blood pressure, left ventricular ejection fraction, and AF type. Distributions are shown as mean and SD for normally distributed values, as median and IQR for non-normally distributed values and biomarkers, and as frequency (percentage) for nominal features.

Statistical methods

As rhythm is a secondary outcome analysis of the EAST-AFNET 4 trial, all results are exploratory. Biomarker concentrations were 1% winsorized[23] from above and logarithmically transformed (log base e) to normalize skewed concentration ranges for all datasets. Concentrations below the detection limit for CA-125 and D-dimer were replaced with the lowest available value. For the initial testing of prespecified hypotheses, all 14 biomarkers were used. Validations were done with predictive biomarkers. This analysis does not take into account the probability of chance findings because of performance of multiple comparisons with 14 biomarkers. As a consequence, results should be interpreted as explorative/hypothesis generating and call for further validation.

Patients in AF at the time of blood sampling showed higher concentrations in most biomarkers (see Supplementary data online, *Table S1*). Rhythm at time of blood sampling was therefore included as a confounder in all subsequent analyses in addition to the features predicting rhythm at 12 months in the main EAST-AFNET 4 dataset.[4]

Mixed logistic regression models were used to assess the predictive value of the 14 biomarkers on rhythm at 12 months, with study centre as a random intercept. The lme4 R package[24] was used. Each biomarker was assessed in a separate model adjusted for sex, age, rhythm at baseline, body mass index, diastolic blood pressure, AF pattern (first episode, paroxysmal, persistent), left ventricular ejection fraction, rhythm at baseline, and randomized group (usual care or early rhythm control).[4] Nested models with additional interaction terms between treatment type and the biomarker of interest were constructed. To obtain *P*-values for the interaction, each nested model pair was compared by ANOVA for their goodness of fit. Odds ratios (ORs) and *P*-values for the biomarker effects under different treatment types were calculated by reference cell coding.[25] Missing values in heart rhythm and left ventricular ejection fraction were imputed in a 60-times multiple imputed dataset as described earlier,[2] following the recommendations of White, Royston, and Wood.[26,27] A sensitivity analysis constructed prediction models for recurrent AF at 12- and 24-month follow-up without imputation.

To further explore the effect of rhythm on the biomarkers, mixed regression models were repeated in subgroups split by baseline rhythm (sinus rhythm or AF) and by rhythm control therapy (early rhythm control or usual care) and ORs for the outcome sinus rhythm at 12 months were calculated using the methods described above.

As internal validation, analysis was repeated for sinus rhythm at 24-month follow-up. As sensitivity analysis, the analysis was repeated for recurrent AF up to 24 months.

As additional internal validation, patient clusters formed using all biomarker concentrations agnostic to clinical features[17] were tested for prediction of presence of sinus rhythm at 12- and 24-month follow-up. The lowest-risk cluster was used as a reference.

As another means of internal validation, we applied a random forest machine learning model (ML) and made use of a mixed effect random forest (MERF) wrapper to account for the centre as a random effect. The ML model was fitted with the features used for confounding the generalized linear model as well as of all 14 biomarkers at once. To assess the variable importance, we used the models' inherent Gini-based feature importance as well as the model agnostic SHapley Additive exPlanations (SHAP) values.

Clinical utility

Cut-off values for clinically useful probabilities of sinus rhythm at 12 months (80%) and for AF at 12 months (40%) were determined for all biomarkers that predicted the main outcome. A clinical risk score was developed based on a recent meta-analysis[28]: three accepted clinical features predicting recurrent AF, namely left atrial size, AF pattern, and age, were dichotomized with a point scored for persistent AF yes, anterior–posterior left atrial diameter > 50 mm, age > 75 years (see Supplementary data online, *Table S2*). As many patients with one of these three features attain sinus rhythm at 12 months, the score was considered as predictive of high risk of AF at 12 months if at least two of the three factors were present. Each of the biomarkers that were independently associated with sinus rhythm at 12 months was added to this clinical score separately, as well as in combination. If at least one biomarker was above the cut-off value, the patient was regarded as high risk of not attaining sinus rhythm. The confusion matrices for correctly and incorrectly classified patients at high-risk-classified of not attaining sinus rhythm were calculated for the reference clinical score alone and all additional, biomarker-enriched scores.

Biomarkers' predictive values were tested in the validation datasets using univariate and multivariate models restricted to the features that predicted sinus rhythm at 12 months in the derivation dataset.

Python version 3.8.13 was employed for data pre-processing and visualization, R version 4.2.2 for statistical computations.[29] Relevant code will be made publicly available (https://github.com/UCCSHH).

Results

Derivation analysis dataset

The 1586 patients with a recent history of AF and stroke risk factors (age 71 years, 45% women) with clinical features, biomarker concentrations, and cardiovascular outcomes were equally assigned to both randomized treatment groups (*Table 1*, Supplementary data online, *Figure S1*).

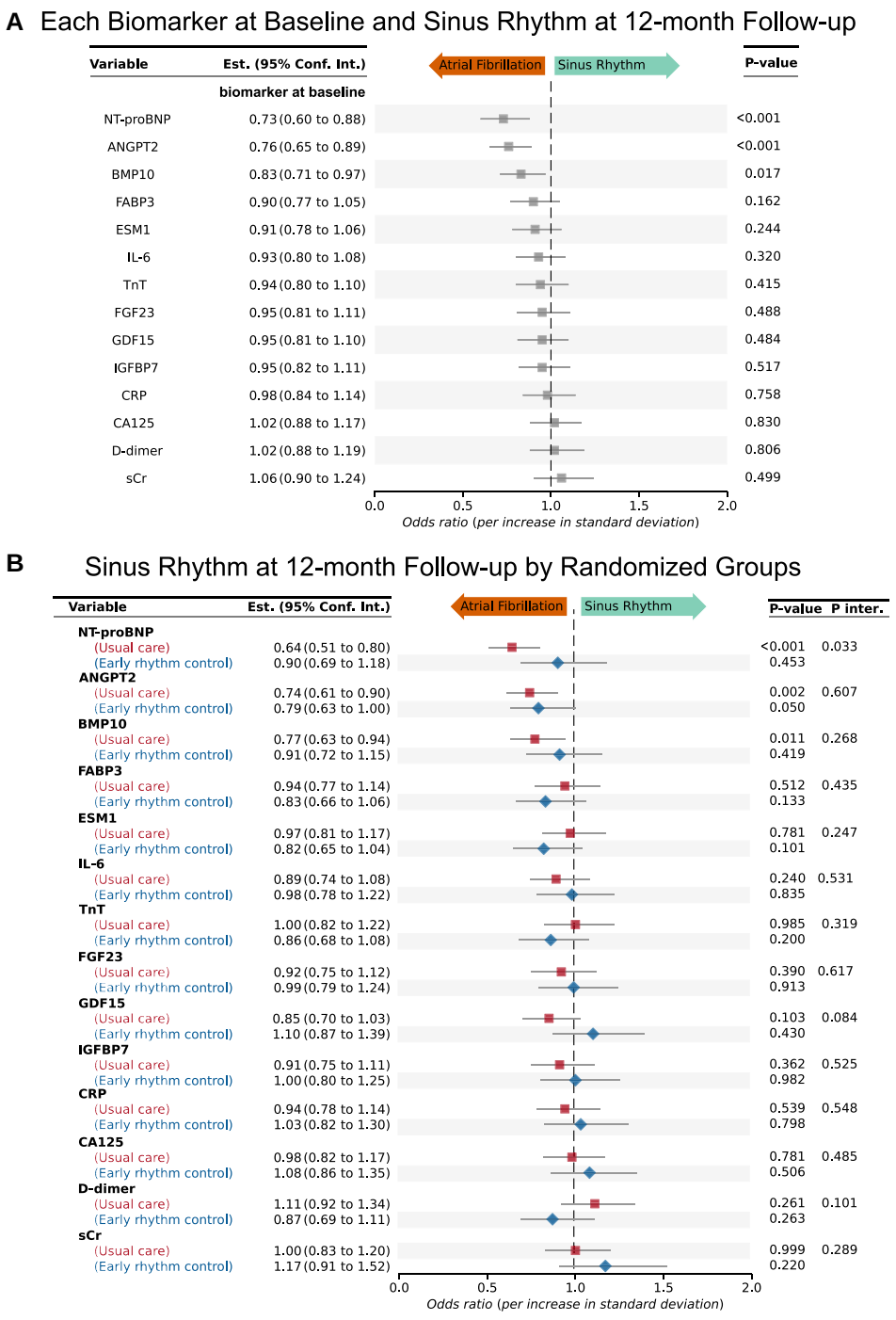


Figure 1 Low concentrations of the biomarkers NT-proBNP, angiopoietin 2, and bone morphogenetic protein 10 predict sinus rhythm at 12-month follow-up in the derivation dataset (EAST-AFNET 4). Odds ratios for sinus rhythm at 12-month follow-up (*A*) and odds ratios by randomized treatment group (*B*). Forest plot showing odds ratios for each biomarker for the outcome sinus rhythm at 12-month follow-up and 95% confidence intervals. The odds ratio for NT-proBNP shows an interaction between NT-proBNP concentrations and randomized treatment group (early rhythm control or usual care). All odds ratios are corrected for clinical features, age, sex, EAST study centre, rhythm at baseline, atrial fibrillation type, randomized treatment group, body mass index, diastolic blood pressure, and left ventricular ejection fraction. Even after multiple confounding, high biomarker concentrations indicate lower odds of sinus rhythm at 12-month follow-up. Low concentrations of NT-proBNP predict sinus rhythm at 12-month follow-up in patients with usual care (only symptomatic rhythm control). High concentrations of NT-proBNP do not necessarily predict lack of sinus rhythm at 12 months if patients receive early rhythm control. ANGPT2, angiopoietin 2; BMP10, bone morphogenetic protein 10; CA125, cancer antigen 125; CRP, C-reactive protein; D-dimer, ESM1, endothelial specific molecule 1; FABP3, fatty acid binding protein 3; FGF23, fibroblast growth factor 23; GDF15, growth differentiation factor 15; IGFBP7, insulin-like growth factor binding protein 7; IL-6, interleukin-6; NT-proBNP, N-terminal pro-B-type natriuretic peptide; TnT, cardiac troponin; sCr, serum creatinine

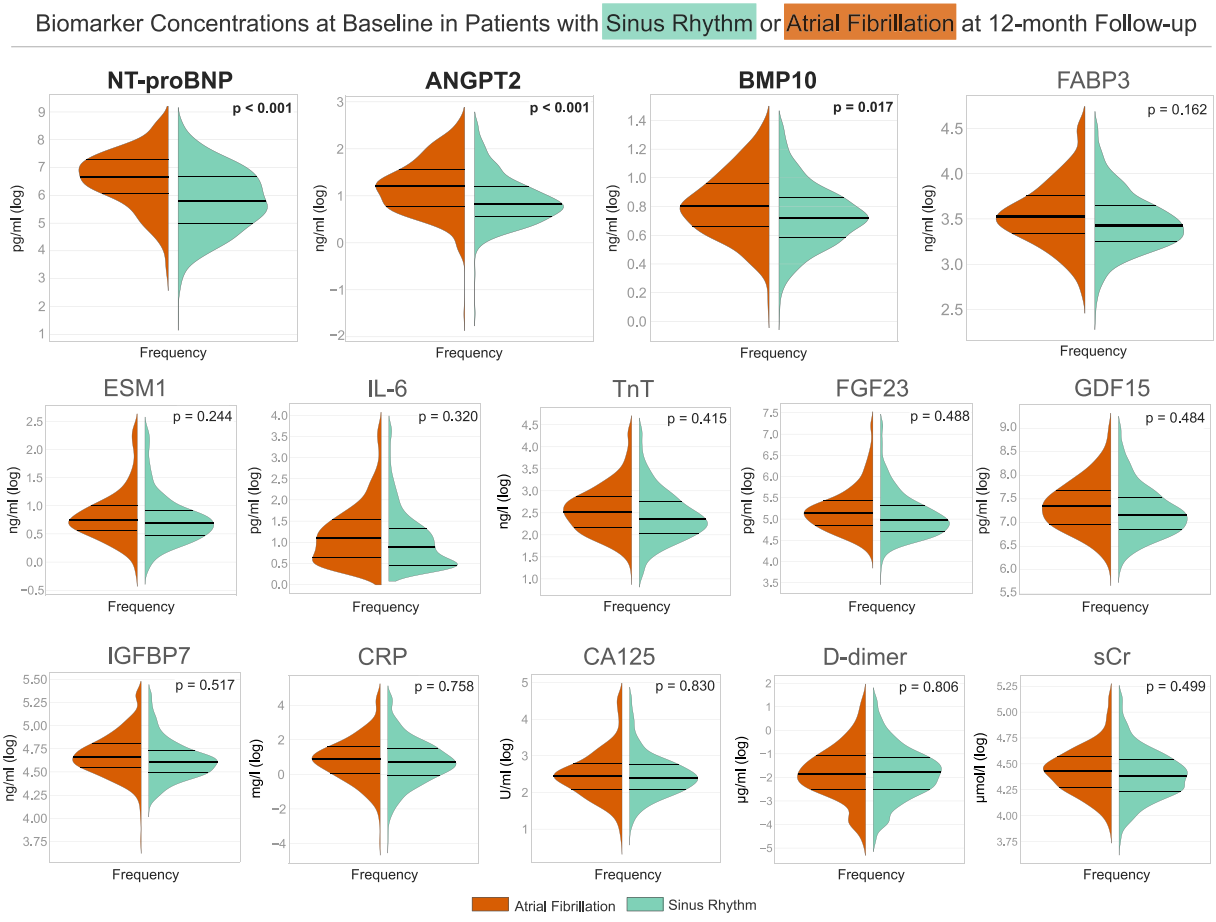


Figure 2 Biomarker concentration distributions at baseline in patients with sinus rhythm (teal right part of each plot) or atrial fibrillation (orange left part of each plot) at 12-month follow-up. Violin plot of the distribution of log-transformed biomarker concentrations for each of 14 biomarkers at baseline, split by the outcome of rhythm at 12-month follow-up. Log-transformed biomarker concentrations are shown on the *y*-axis and the kernel estimated frequency on the *x*-axis. Central thick horizontal lines are the median and the thinner lines represent interquartile range. N-terminal pro-B-type natriuretic peptide, angiopoietin 2, and bone morphogenetic protein 10 show an association with sinus rhythm at 12-month follow-up based on the acceptance of a Type 1 error of 5%. *P*-values were calculated using mixed logistic regression model with site as random effect, adjusted for age, sex, rhythm at baseline, randomized group (early rhythm control or usual care), body mass index, diastolic blood pressure, and left ventricular ejection fraction, those clinical features that were associated with outcomes including sinus rhythm in the main EAST-AFNET 4 trial. ANGPT2, angiopoietin 2; BMP10, bone morphogenetic protein 10; CA125, cancer antigen 125; CRP, C-reactive protein; D-dimer, ESM1, endothelial specific molecule 1; FABP3, fatty acid binding protein 3; FGF23, fibroblast growth factor 23; GDF15, growth differentiation factor 15; IGFBP7, insulin-like growth factor binding protein 7; IL-6, interleukin-6; NT-proBNP, N-terminal pro-B-type natriuretic peptide; TnT, cardiac troponin; sCr, serum creatinine

Association of biomarker concentrations with attaining sinus rhythm at 12 months

Three biomarkers (ANGPT2, BMP10, and NT-proBNP) showed lower concentrations at baseline in patients who were in sinus rhythm at the 12-month follow-up (*Figure 1A*). These three biomarkers were independently associated with sinus rhythm at the 12-month follow-up after multiple corrections for clinical features, early rhythm control, and baseline rhythm (*Figure 1A*). NT-proBNP interacted with early rhythm control therapy at 12-month follow-up ($P = .033$) and low NT-proBNP concentrations only predicted sinus rhythm at 12 months in patients randomized to usual care (*Figure 1B*). Early rhythm control impacted on the rhythm-predicting effect of NT-proBNP and dampened its predictive value in this group. There was no significant interaction detected between early rhythm control and any of the other 13 biomarkers in this dataset (*Figure 1B*).

Biomarker concentrations distributions depicted in violin plots after log transformation (*Figure 2*) show lower concentrations in sinus rhythm vs. AF at 12 months. Numbers of mean biomarker concentrations by rhythm at 12-month follow-up and by randomized treatment group are given in *Table 2*.

Baseline biomarker concentrations depending on baseline rhythm in the derivation dataset and clinical features are shown in *Table 3*, extended information shown in Supplementary data online, *Table S1*. *Post hoc* subgroup analyses by rhythm at the time of baseline assessment (sinus rhythm or AF) and by randomized group (early rhythm control or usual care) find NT-proBNP mainly associated with sinus rhythm at 12 months in patients under usual care. BMP10 and ANGPT2 retained their predictive ability shown in the joint group of all patients also if only the subgroup patients in AF at the time of blood sampling were analysed (*Figure 3*).

Table 2 Baseline biomarker concentrations are shown split by rhythm at 12-month follow-up (sinus rhythm or atrial fibrillation) and by randomized group (early rhythm control or usual care)

Randomization group	Early rhythm control		Usual care		SR vs. AF 12 months
Rhythm at 12-month follow-up	Sinus rhythm 12-month FU	Atrial fibrillation 12-month FU	Sinus rhythm 12-month FU	Atrial fibrillation 12-month FU	*P*-value*
NT-proBNP (pg/mL)	377 [164–859]	750 [376–1351]	294 [127–700]	782 [437–1454]	.001
ANGPT2 (ng/mL)	2.34 [1.78–3.41]	3.45 [2.43–5.62]	2.24 [1.7–3.09]	3.31 [2.15–4.62]	.001
BMP10 (ng/mL)	2.08 [1.8–2.39]	2.21 [1.96–2.58]	2.04 [1.79–2.34]	2.24 [1.94–2.66]	.010
FGF23 (pg/mL)	151 [112–209]	179 [125–238]	141 [108–197]	168 [129–226]	.429
ESM1 (ng/mL)	2.01 [1.61–2.52]	2.17 [1.75–2.88]	1.98 [1.57–2.52]	2.09 [1.73–2.66]	.218
GDF15 (pg/mL)	1304 [958–1934]	1441 [997–2008]	1254 [911–1782]	1589 [1071–2347]	.461
IGFBP7 (ng/mL)	100 [89–114]	108 [93–126]	98.8 [88.5–110]	104 [94.7–119]	.487
IL-6 (pg/mL)	2.47 [1.57–3.88]	2.6 [1.76–4.62]	2.37 [1.56–3.6]	3.02 [1.98–4.65]	.417
FABP3 (ng/mL)	31.4 [25.6–39]	35.3 [28.3–43.4]	30.4 [25.7–37.8]	33.5 [28.0–42.0]	.151
D-dimer (µg/mL)	.17 [.08–.33]	.19 [.1–.36]	.16 [.08–.32]	.16 [.08–.32]	.638
TnT (ng/L)	10.6 [7.81–15.7]	13.0 [9–17.6]	10.3 [7.53–15.5]	12.5 [8.68–17.7]	.415
CRP (mg/L)	2 [.95–4.65]	1.97 [.9–4.63]	2.07 [.93–4.37]	2.52 [1.12–4.87]	.910
sCr (µmol/L)	81.3 [70–95]	83 [72.7–94.8]	79.5 [68.0–91.9]	84.4 [72–97.2]	.541
CA125 (U/mL)	11.4 [8.0–15.8]	12.3 [8.3–17.1]	10.8 [7.8–15.7]	11.4 [7.96–15.9]	.779

AF, atrial fibrillation; SR, sinus rhythm; FU, follow-up; ANGPT2, angiopoietin 2; BMP10, bone morphogenetic protein 10; CA125, cancer antigen 125; CRP, C-reactive protein; D-dimer, ESM1, endothelial specific molecule 1; FABP3, fatty acid binding protein 3; FGF23, fibroblast growth factor 23; GDF15, growth differentiation factor 15; IGFBP7, insulin-like growth factor binding protein 7; IL-6, interleukin-6; NT-proBNP, N-terminal pro-B-type natriuretic peptide; TnT, cardiac troponin; sCr, serum creatinine.
**P*-values were calculated using mixed logistic regression model with site as random effect, adjusted for sex, age, body mass index, diastolic blood pressure, left ventricular ejection fraction. Values are shown as median [IQR].

Internal validations

As a first internal validation, the same analysis was performed for the 24-month follow-up. The same biomarkers, ANGPT2, BMP10, and NT-proBNP, were consistently associated with sinus rhythm at 24-month follow-up (*Figure 4*).

Repeating the analysis for recurrent AF up to 24 months showed similar results (see Supplementary data online, *Table S3*). As further internal validation analysis, unsupervised biomarker-based clustering of EAST patients previously performed was applied to sinus rhythm at 12-month follow-up. Clusters separated by risk of cardiovascular complications, with patients assigned to the high-risk cardiovascular outcome cluster showing a lower likelihood of sinus rhythm at 12 months, patients in the two intermediate cardiovascular risk biomarker clusters showing an intermediate likelihood of sinus rhythm, all tested against the low cardiovascular risk cluster, with the low-risk outcome patient cluster showing the highest likelihood of sinus rhythm at 12 months (*Figure 5A*). These findings were consistent for the biomarker-based clusters at 24-month follow-up (*Figure 5B*).

As further internal validation, a random forest classifier was trained on the EAST-AFNET 4 dataset. Its feature performance evaluation confirmed the importance of the three biomarkers alongside AF pattern, rhythm at baseline, and early rhythm control for the outcome of sinus rhythm (*Figure 6*).

Table 3 Baseline clinical characteristics used as confounders and biomarker concentrations in the derivation dataset (EAST-AFNET 4 biomolecule study) at baseline by randomized group and by baseline rhythm

Group	Early rhythm control		Usual care		*P*-value
Baseline rhythm	Sinus rhythm	Atrial fibrillation	Sinus rhythm	Atrial fibrillation	Rhythm*
N	452	348	438	348	
Women	220 (49%)	135 (39%)	221 (51%)	137 (39%)	<.001
Age, years	70 [65–75]	71 [67–76]	71 [66–75]	72 [66–76]	.035
BMI	28.7 [25.8–31.6]	28.4 [25.5–32.9]	28.4 [25.4–31.4]	29.4 [25.9–33.3]	.022
Blood pressure (diastolic) (mmHg)	80 [72, 87]	80 [76, 90]	80 [71, 89]	80 [76, 90]	<.001
LVEF (%)	60 [57, 65]	59 [50, 64]	60 [59, 65]	60 [51, 64]	<.001
AF type: first episode	172 (38%)	118 (34%)	155 (35%)	115 (33%)	
AF type: paroxysmal	235 (52%)	67 (19%)	223 (51%)	65 (19%)	<.001
AF type: persistent or long-standing persistent	45 (10%)	163 (47%)	60 (14%)	168 (48%)	<.001
Biomarker concentrations					
NT-proBNP (pg/mL)	228 [121–467]	890 [506–1496]	253 [124–504]	934 [529–1603]	<.001
ANGPT2 (ng/mL)	2.20 [1.65–2.76]	3.39 [2.29–5.14]	2.12 [1.63–3.00]	3.35 [2.31–4.81]	<.001
BMP10 (ng/mL)	2.03 [1.73–2.30]	2.22 [1.96–2.58]	2.01 [1.76–2.29]	2.25 [1.96–2.69]	<.001
FGF23 (pg/mL)	139 [106–194]	178 [128–247]	140 [110–192]	170 [130–243]	.003
ESM1 (ng/mL)	1.97 [1.58–2.44]	2.14 [1.74–2.84]	1.96 [1.57–2.56]	2.15 [1.74–2.78]	.002
GDF15 (pg/mL)	1251 [938–1847]	1478 [1058–2188]	1259 [914–1761]	1585 [1065–2272]	<.001
IGFBP7 (ng/mL)	99.0 [89.3–111.2]	106.8 [93.6–125]	99.2 [87.9–111]	105 [93.8–123]	<.001
IL-6 (pg/mL)	2.22 [1.50–3.58]	3.03 [1.99–4.88]	2.42 [1.58–3.89]	3.02 [1.95–4.59]	.041
FABP3 (ng/mL)	30.2 [25.1–38.1]	34.2 [28.2–42.1]	30.9 [25.6–37.9]	33.3 [27.1–42.6]	.020
D-dimer (μg/mL)	.17 [.08–.32]	.18 [.09–.36]	.15 [.08–.32]	.18 [.09–.4]	.267
TnT (ng/L)	10.1 [7.39–14.5]	12.7 [9–18.8]	10.7 [7.6–15.7]	12.5 [8.73–18.3]	.436
CRP (mg/L)	1.76 [.87–4.29]	2.48 [1.09–5.78]	2.08 [.93–4.52]	2.58 [1.26–5.03]	.130
sCr (μmol/L)	80.0 [69.0–93.7]	84.0 [71.0–97.0]	79.6 [68.1–92.0]	83.9 [71.0–97.2]	.296

Continued

Table 3 *Continued*

Group	Early rhythm control		Usual care		*P*-value
Baseline rhythm	Sinus rhythm	Atrial fibrillation	Sinus rhythm	Atrial fibrillation	Rhythm*
CA125 (U/mL)	11.1 [8.01–14.9]	12.3 [8.4–16.9]	10.8 [8.02–15.7]	11.4 [7.84–16.7]	.052

Rhythm at time of blood sampling was included as a fix factor in the analyses of outcome. Distributions are shown as mean and SD for normally distributed values, as median and IQR for non-normal distributed values and biomarkers, and as frequency (percentage) for nominal features. For biomarker concentrations, there were no differences between the randomized groups, but differences between sinus rhythm and AF during the baseline visit.

AF, atrial fibrillation; ERC, early rhythm control; UC, usual care; BMI, body mass index; AF, atrial fibrillation; LVEF, left ventricular ejection fraction; ANGPT2, angiopoietin 2; BMP10, bone morphogenetic protein 10; CA125, cancer antigen 125; CRP, C-reactive protein; D-dimer, ESM1, endothelial specific molecule 1; FABP3, fatty acid binding protein 3; FGF23, fibroblast growth factor 23; GDF15, growth differentiation factor 15; IGFBP7, insulin-like growth factor binding protein 7; IL-6, interleukin-6; NT-proBNP, N-terminal pro-B-type natriuretic peptide; TnT, cardiac troponin; sCr, serum creatinine.

**P*-values were calculated in the unimputed, pooled dataset (ERC and UC combined) using mixed logistic regression model with site as random effect, for the biomarkers additionally adjusted for sex, age, body mass index, diastolic blood pressure, left ventricular ejection fraction, and AF type, the clinical features that were associated with outcomes including sinus rhythm in the main EAST-AFNET 4 dataset.[4]

Clinical utility

Thresholds to predict a high probability of attaining sinus rhythm (>80%, low risk of AF) or a high probability of recurrent AF at follow-up (>40%, high risk of AF) were determined for each biomarker (*Table 4*, Supplementary data online, *Figures S2–S4*). To compare them to clinical features predicting sinus rhythm, a score combining clinical features predicting recurrent AF was created integrating age, left atrial size, and AF pattern[28] (see Supplementary data online, *Table S2*). Adding biomarkers using these thresholds improved identification of patients at risk of not attaining sinus rhythm at 12-month follow-up (*Table 5*, Supplementary data online, *Table S4*).

External validation

Several separate validation datasets (AXAFA-AFNET 5 trial, BBC-AF, and TRUST cohort snapshot Supplementary data online, *Tables S5–S7*) were used. The biomarkers NT-proBNP, BMP10, and ANGPT2 were confirmed as predictive of sinus rhythm in the final follow-up in AXAFA-AFNET 5 (*Figure 7*, *Structured Graphical Abstract*). The clinical utility of adding the biomarkers to clinical predictors was validated in both cohorts using the thresholds derived in EAST-AFNET 4 (see Supplementary data online, *Tables S8* and *S9*).

Discussion

Main findings

Three out of 14 candidate biomarkers, BMP10, ANGPT2, and NT-proBNP, are associated with sinus rhythm at 12-month and 24-month follow-up after correcting for clinical features. Low NT-proBNP, low ANGPT2, and low BMP10 concentrations independently predict sinus rhythm in patients at follow-up. NT-proBNP is less predictive of rhythm in patients receiving rhythm control therapy. Adding these biomarkers to a clinical score identifying patients with a low probability of sinus rhythm at 12 months (positive with two out of three features: left atrial size > 50 mm, persistent AF, or age > 75 years) refined risk prediction (*Structured Graphical Abstract*).

Relevance for clinical care and research

In view of the growing choice of medical,[2,30] interventional,[2,31] and surgical[32] treatment options for patients with AF, selecting the best strategy and the patients most benefitting from rhythm control therapy gains importance. Biomarker-based risk estimators have so far mainly been developed to refine anticoagulation decisions in patients with AF.[33–35] Actionable biomarkers to guide rhythm control therapy are lacking. Similar to stroke prevention estimators, rhythm estimators face the challenge of random factors determining a binary outcome (AF or sinus rhythm). The present results suggest that NT-proBNP, BMP10, and ANGPT2 can stratify patients at high and low risk of attaining sinus rhythm alone and in combination. These biomarkers reflect and identify diseases processes that promote future AF, pointing to potential therapeutic targets for adjunct therapy supporting rhythm control. While a simple clinical score combining enlarged left atrial size, persistent AF, and older age predicted future sinus rhythm reasonably well, adding biomarkers reclassifies a clinically relevant number of patients at high risk of not attaining sinus rhythm at the price of also classifying more patients in sinus rhythm as high risk.

Effect of baseline rhythm on biomarker concentrations

This study shows that ANGPT2 and BMP10 provide additional information on future sinus rhythm when combined with NT-proBNP, especially in patients who are in AF at the time of blood sampling. Most biomarkers studied were elevated when the blood sample was taken in AF. Furthermore, NT-proBNP lost its ability to predict sinus rhythm in patients on rhythm control therapy.[20,36] The effects of baseline rhythm on the concentrations and predictive ability of biomarkers should be further investigated in patients with AF undergoing rhythm control therapy.

Interpretation of NT-proBNP

NT-proBNP is released by atrial cardiomyocytes in response to stretch and strain, thereby acutely regulating fluid balance in the body, resulting in high concentrations during AF.[37] In heart failure, NT-proBNP is also released by ventricular cardiomyocytes, further enhancing its concentrations. Atrial stretch has proarrhythmic effects including shortening of the atrial effective refractory period[38] and conduction slowing,[39] partially explaining its prediction of sinus rhythm in this study. NT-proBNP reflects short- and mid-term cardiac load in patients with AF, probably explaining its interaction with rhythm control. The possibility that elevated NT-proBNP concentrations predict rhythm during follow-up have been reported before.[40–47] NT-proBNP is also associated with incident AF[48–51] and with cardiovascular events in patients with and

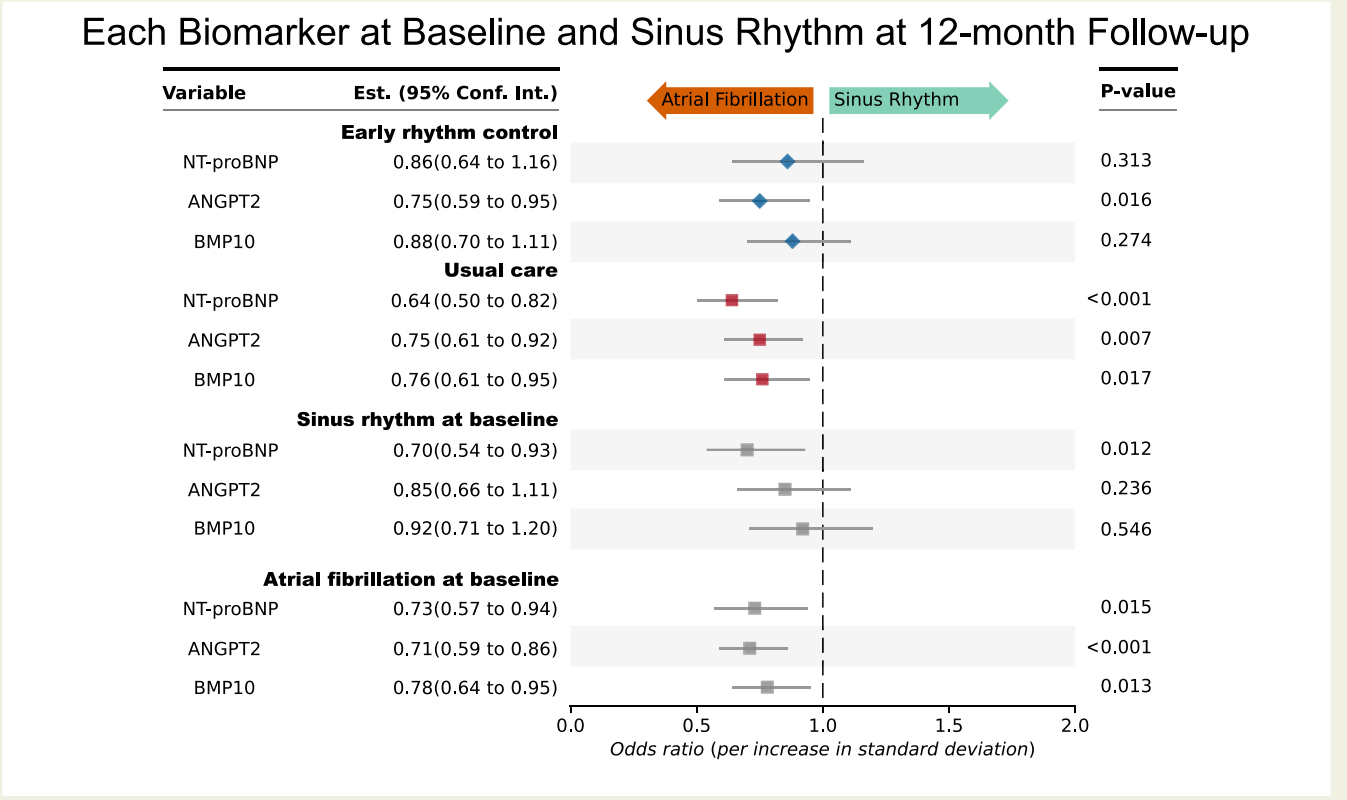


Figure 3 Biomarkers measured at baseline predicting sinus rhythm at 12-month follow-up in all participants of the biomarker study, separately analysed by rhythm at baseline (atrial fibrillation at baseline or sinus rhythm at baseline) and randomized treatment group (early rhythm control or usual care), respectively, in a *post hoc* analysis. Of the three biomarkers identified to be predictive of sinus rhythm in the whole cohort, NT-proBNP, ANGPT2, and BMP10, all three biomarkers retained their predictive value in the subgroup of patients randomized to usual care. All three biomarkers also retained their predictive value in the subgroup of patients in atrial fibrillation during blood draw at baseline. ANGPT2, angiopoietin 2; BMP10, bone morphogenetic protein 10; NT-proBNP, N-terminal pro-B-type natriuretic peptide

without AF and heart failure.[22] This analysis demonstrates that the rhythm-predicting ability of NT-proBNP is reduced in patients treated with rhythm control therapy.

The NT-proBNP thresholds associated with a high risk of AF at 12 months in this study (>1500 pg/mL) are comparable to the thresholds associated with cardiovascular events, but higher than currently used thresholds e.g. for AF screening[52] or for diagnosing heart failure with AF and heart failure with preserved ejection fraction.[53] Based on the present analysis, higher thresholds may have better clinical utility. This warrants further analysis.

Interpretation of BMP10 and ANGPT2

BMP10 and ANGPT2 are tightly regulated circulating biomarkers, illustrating their signalling roles in regulating disease processes contributing to AF.[3] Mechanistic studies of their role in AF are needed to define more precise clinical use cases for these biomarkers in patients with AF.

ANGPT2 is a vascular growth factor required for angiogenic remodelling.[54] Overexpression of ANGPT2 in murine models promotes perivascular cardiac inflammation and fibrosis.[55] Pro-inflammatory molecules such as thrombin increase ANGPT2 expression *in vitro*[56] and inhibition of thrombin in animals with persistent AF improves atrial cardiomyopathy.[15] Thus, ANGPT2 mediates the inflammatory communication between endothelial cells and myocardium in AF. Low ANGPT2 might reflect preserved vascular integrity, reducing the inflammatory burden in atrial vascular beds and thereby slowing AF progression.

ANGPT2 is associated with recurrent AF in patients after AF ablation[20] and with prevalent AF in unselected hospitalized patients.[57] ANGPT2 is elevated in patients with kidney disease,[58] acute lung injury,[59] and sepsis,[60] conditions associated with AF. ANGPT2 can also predict heart failure hospitalization in patients with AF,[61] similar to NT-proBNP.[22] This study is the first to suggest that ANGPT2 can predict sinus rhythm in patients with AF with and without rhythm control therapy. Further research into treatable atrial disease processes regulated by ANGPT2 is warranted.

BMP10 is selectively expressed in and released by atrial cardiomyocytes.[16,62] BMP10 is part of the TGFβ growth factor family and regulates vascular smooth muscle cell tone.[63] Its function in the atria is not well known. BMP10 concentrations are reduced in hereditary forms of pulmonary arterial hypertension,[64] possibly reflecting reduced atrial metabolism. Its inverse correlation and possible repression by PITX2 in atrial cardiomyocytes[16,65] may suggest that elevated BMP10 concentrations could identify a reversible atrial metabolic defect[13,17] that may be aggravated by the genomic basis of AF on chromosome 4q25.[13]

High concentrations of BMP10 are associated with recurrent AF,[57,66] and with cardiovascular events[17,67] and stroke in patients with AF. BMP10 may also be associated with atrial fibrosis.[68] Lower BMP10

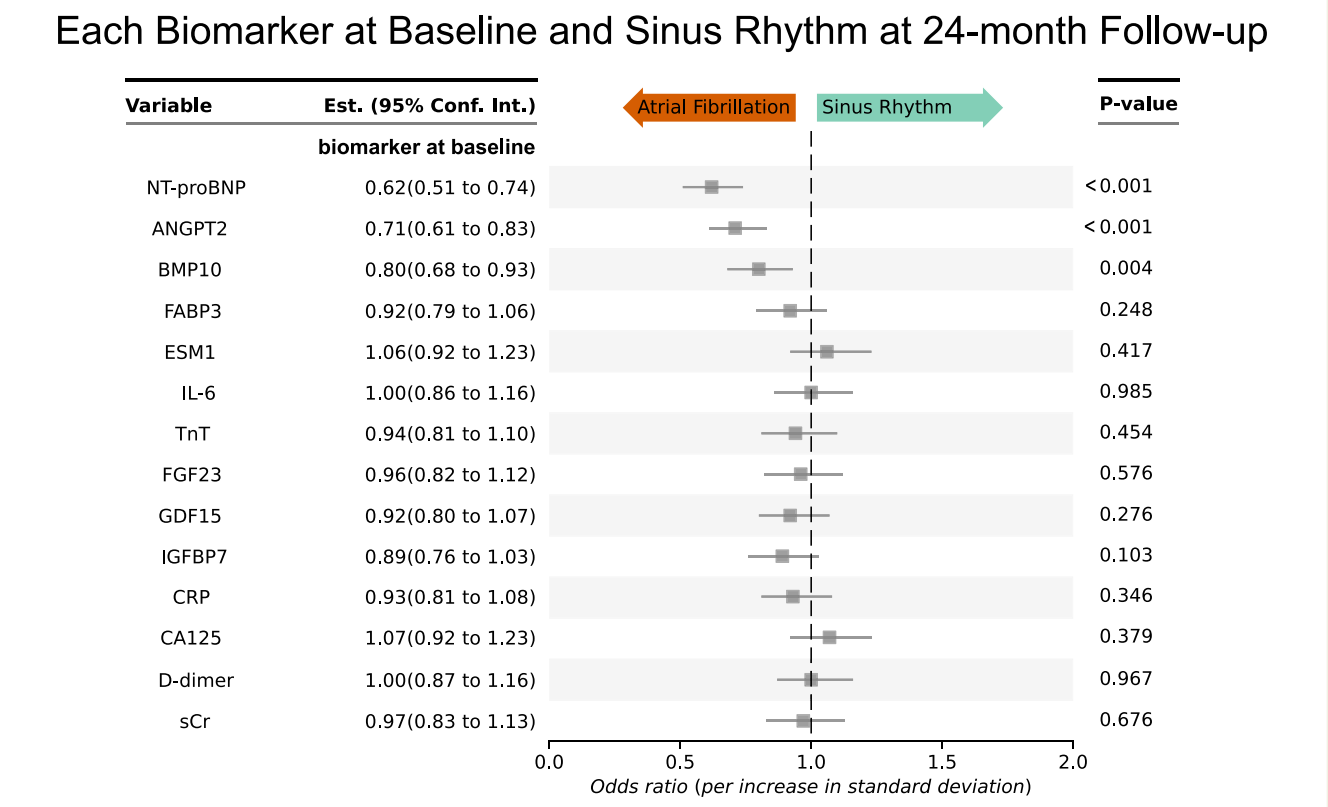


Figure 4 Internal validation: angiopoietin 2, bone morphogenetic protein 10, and NT-proBNP biomarkers at baseline predict sinus rhythm at 24-month follow-up even after correction for multiple confounders. Odds ratios are shown for sinus rhythm at 24-month follow-up. This analysis provides an internal validation of the biomarkers predicting sinus rhythm at 12-month follow-up (*Figure 1*). All odds ratios are corrected for clinical age, sex, study site, rhythm at baseline, randomized treatment group (early rhythm control or usual care), body mass index, diastolic blood pressure, and left ventricular ejection fraction, those clinical features that were associated with outcomes including sinus rhythm in the main EAST-AFNET 4 trial.[4] Low concentrations of NT-proBNP, ANGPT2, and BMP10 predict sinus rhythm at 24-month follow-up in patients. Accordingly, high concentrations predict lack of sinus rhythm at 24-month follow-up. ANGPT2, angiopoietin 2; BMP10, bone morphogenetic protein 10; CA125, cancer antigen 125; CRP, C-reactive protein; D-dimer, ESM1, endothelial specific molecule 1; FABP3, fatty acid binding protein 3; FGF23, fibroblast growth factor 23; GDF15, growth differentiation factor 15; IGFBP7, insulin-like growth factor binding protein 7; IL-6, interleukin-6; NT-proBNP, N-terminal pro-B-type natriuretic peptide; TnT, cardiac troponin; sCr, serum creatinine

concentrations in patients in sinus rhythm,[20] combined with its prediction of future sinus rhythm (*Figure 1*) suggest that a possible BMP10-mediated metabolic defect could partially be secondary to the metabolic demands of AF. Taken together, these results suggest that BMP10 is a potentially actionable biomarker indicative of atrial myopathy and atrial metabolic dysfunction. Further research into the atrial effects of BMP10 and its relation to AF burden[5] are warranted.

Biomolecule-based clustering of patients agnostic to clinical features previously identified four subgroups of patients with AF with a gradual increase in cardiovascular events.[17] The three biomarkers associated with sinus rhythm at 12 months in this study are among the six dominant biomarkers defining these patient clusters.[17] The biomarker-based clusters show a risk gradient for sinus rhythm at 12 months (*Figure 5*). At difference to the prior study that defined patient clusters based on biomarker concentrations agnostic to clinical information, this analysis shows that the three biomarkers NT-proBNP, ANGPT2, and BMP10 predict sinus rhythm in context with clinical parameters. Of note, a simple clinical score (see Supplementary data online, *Table S2*) was already quite useful in identifying patients who will attain sinus rhythm. This information can help clinicians to select different intensities of rhythm control therapy depending on the likelihood of attaining sinus rhythm. NT-proBNP, ANGPT2, and BMP10 can refine that selection (*Table 5*). The present result and the biomarker-clustering also identify potentially treatable drivers of recurrent AF and or cardiovascular events in patients with AF. Based on the known atrial effects of BMP10 and ANGPT2, antihypertensive therapy and metabolic interventions such as SGLT2 inhibitor therapy[12] could have beneficial effects in patients with elevated BMP10 and ANGPT2 concentrations.[16,67,69] The underlying disease processes suggest that the same biomarkers could also be useful to identify patients at risk of AF. The present analysis identifies potentially actionable biomarkers suitable to select the intensity of rhythm control therapy. Further research into the mechanistic links between these biomarkers with baseline and future rhythm, and further evaluations of their clinical utility in different scenarios are warranted.

Strengths and limitations

Central quantification of the biomarkers using high-precision assays combined with the rigorous, near-complete follow-up at 12 and 24 months in a controlled clinical trial is a strength of this analysis. The

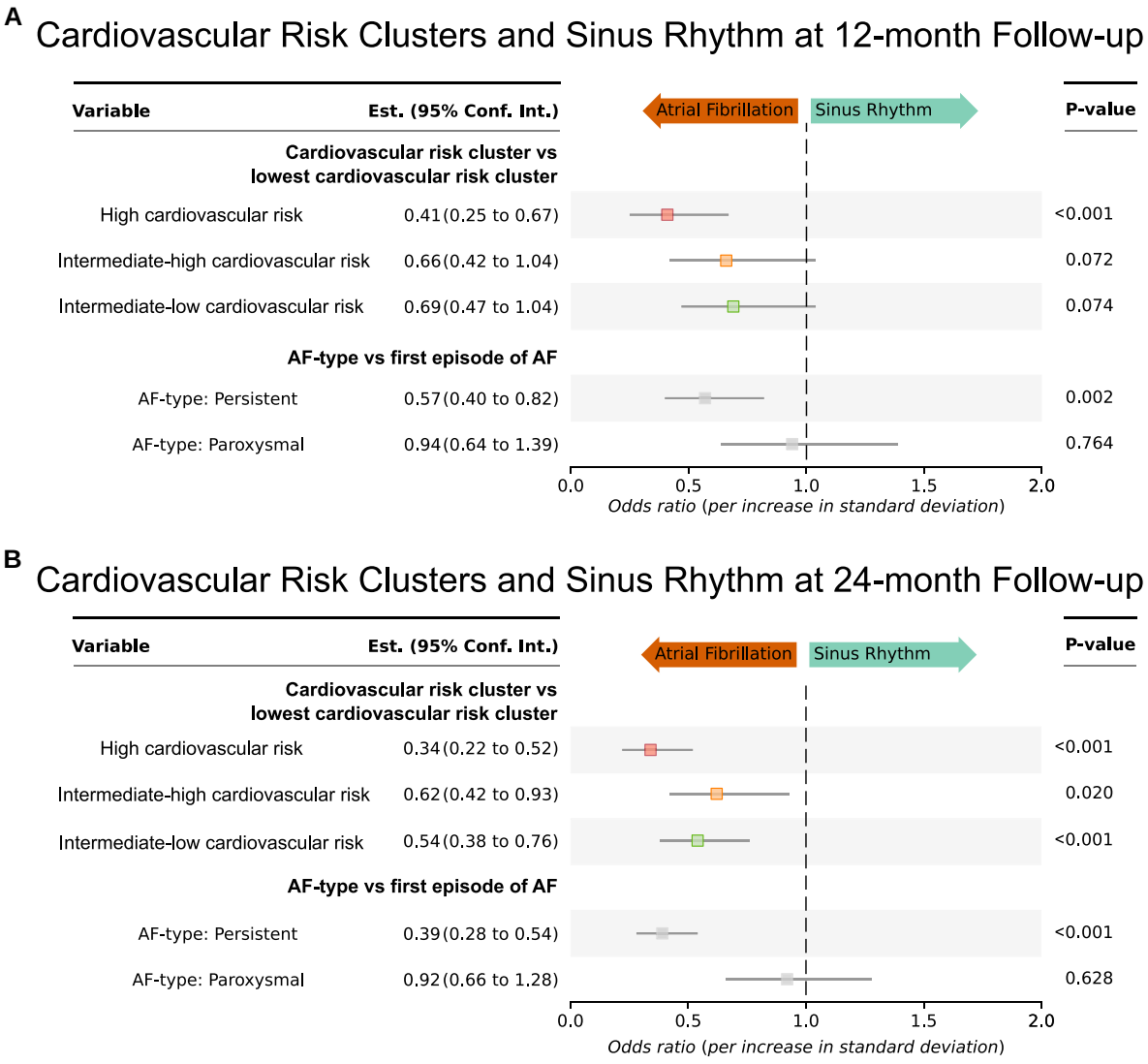


Figure 5 Validation applying biomarker-based clusters indicating cardiovascular outcome risk: patients at high risk of cardiovascular complications as estimated by biomarker-based clusters have reduced odds of sinus rhythm at 12-month and 24-month follow-up. Odds ratio for the high cardiovascular outcome risk (red) and intermediate cardiovascular outcome risk biomarker clusters (orange and green) for sinus rhythm at 12-month follow-up (*A*, above) and at 24-month follow-up (*B*, bottom) tested against the low cardiovascular risk cluster (not depicted as used as reference). All odds ratios are corrected for age, sex, study centre, rhythm at baseline, atrial fibrillation type (depicted in grey odds ratios below the cluster odds ratios), randomized treatment group (early rhythm control or usual care), as well as body mass index, diastolic blood pressure, and left ventricular ejection fraction, the clinical features that were associated with outcomes including sinus rhythm in the main EAST-FNET 4 trial.[4] AF, atrial fibrillation

consistent findings at both time points may suggest that the effects can be extrapolated to even longer follow-up, but this would require validation. Another strength of the analysis is the collection of samples in a broad range of care settings in adequately treated patients with AF, and external validation both in a controlled clinical trial and in cohorts of patients with AF enrolled in routine care settings. Validation of the findings using the same assays in different clinical datasets is a strength, but also limits the findings to the assays used in this study.

The study has important limitations. Although the statistical analysis plan was prespecified and validation was possible in different datasets, all results are explorative. This study is limited to 14 preselected biomarkers. Selected biomarkers intentionally reflect overlapping disease processes, creating redundancy that enables robust definition of disease pathways. Collinearity of biomarkers was more deeply investigated in a previous study defining biomarker-based patient clusters agnostic to clinical features.[17]

Additional biomarkers in AF may emerge from hypothesis-free quantification of many molecules at once e.g. by RNA-sequencing of cardiac tissue,[70] quantification of circulating RNAs, and by proteomics.[71,72] Repeat blood samples were not obtained and no information on changes over time is available. Some data on the changes of BMP10 and NT-proBNP over time have been published.[20,36]

While NT-proBNP can be measured in clinical routine as *in vitro* diagnostic devices with regulatory approval, the assays for ANGPT2 and BMP10 are not approved for clinical use, restricting them to research settings. Only the consenting portion of the total EAST-AFNET study

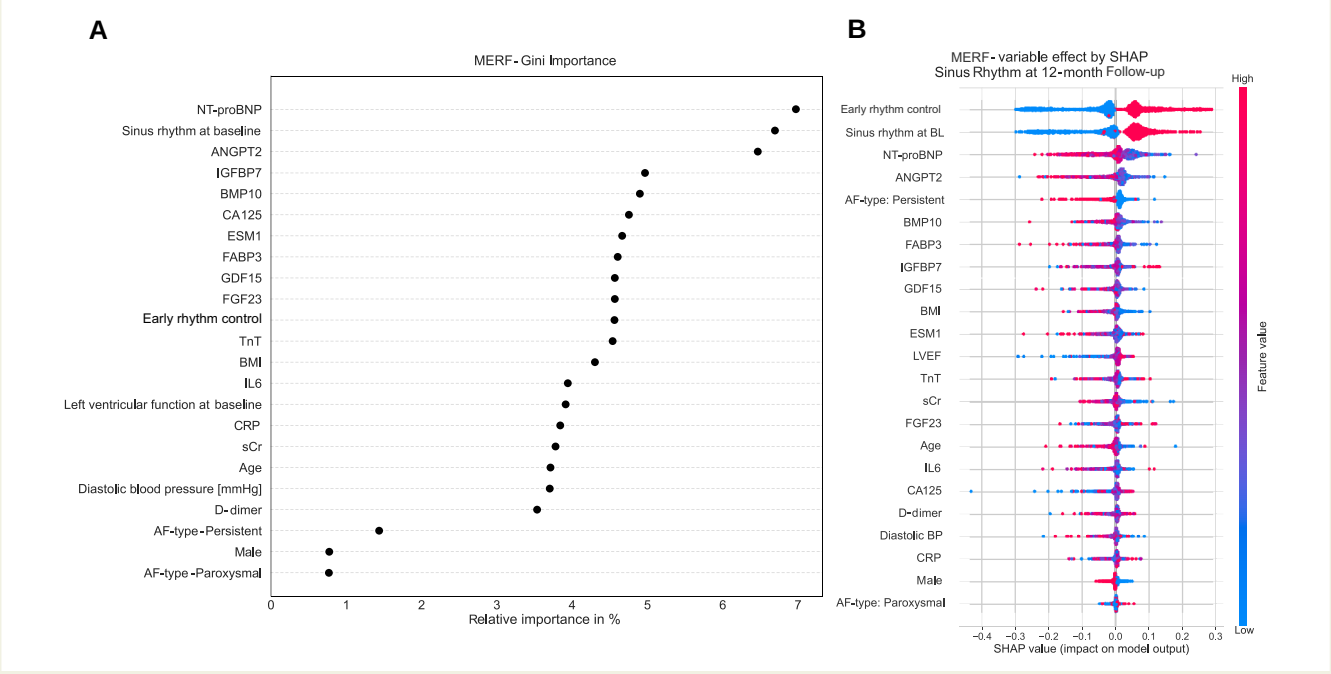


Figure 6 Validation by random forest analyses identified highest importance for similar biomarkers, alongside rhythm at baseline and AF pattern, as predictors of sinus rhythm at 12-month follow-up (*A*—importance, *B*—SHAP value). AF, atrial fibrillation; ANGPT2, angiopoietin 2; BL, baseline; BMI, body mass index; BMP10, bone morphogenetic protein 10; CA125, cancer antigen 125; CRP, C-reactive protein; D-dimer, ESM1, endothelial specific molecule 1; FABP3, fatty acid binding protein 3; FGF23, fibroblast growth factor 23; GDF15, growth differentiation factor 15; IGFBP7, insulin-like growth factor binding protein 7; IL-6, interleukin-6; MERF, mixed effect random forest; NT-proBNP, N-terminal pro-B-type natriuretic peptide; TnT, cardiac troponin; sCr, serum creatinine; SHAP, SHapley Additive exPlanations

Table 4 Threshold concentrations for NT-proBNP, BMP10, and ANGPT2 determined in the derivation dataset (EAST-AFNET 4 biomolecule study)

Biomarker	Low threshold (>80% sinus rhythm at 12 months)	High threshold (>40% AF at 12 months)
NT-proBNP (pg/mL)	<1000	>1500
BMP10 (ng/mL)	<2	>3
ANGPT2 (ng/mL)	<3.5	>3.5

The lower threshold was defined as the nearest round concentration below which 80% of patients attained sinus rhythm at 12 months. The higher threshold was defined as the nearest rounded concentration above which 40% of patients were in AF at 12 months. AF, atrial fibrillation; ANGPT2, angiopoietin 2; BMP10, bone morphogenetic protein 10; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

participants was included in the biomarker study (two-thirds), hence there could be a considerable selection bias. Due to time required to setup the biobank, the first 400 patients were not invited to participate in the biomarker study.

The present study used serum creatinine rather than estimated glomerular filtration rate in the analyses as the formulas used to estimate kidney function rely on clinical parameters that are used in the regression model, including age, sex, and body mass index. Serum creatinine was not a major predictor of sinus rhythm. Whether estimated kidney function is a better predictor of sinus rhythm was not studied.

Validation datasets were smaller than the derivation dataset and therefore did not allow for multiple confounding. *Post hoc* subgroup analysis by baseline rhythm in EAST-AFNET 4 may have underestimated effects due to smaller group sizes. Almost all patients received guideline-recommended anticoagulation, rate and rhythm control, and often effective treatment of concomitant conditions. Twenty-four hour blood pressure may provide more granular prognostic information than office-based blood pressure, but 24 h blood pressure readings were not available for this analysis.

Left atrial size was used in the clinical score rather than left atrial volume. Indexed left atrial volume can provide more detailed information on left atrial size compared to left atrial diameter, but the predictive value of left atrial volume for recurrent AF is less well established than left atrial size.[28] Indexed left atrial volume was not available in a sufficient number of patients to be assessed in this study. The predictive ability of the different biomarker-based models is only valid for the specific AF prevalences in the cohorts studied. Further research into the clinical utility of the biomarkers identified here is warranted.

The blood samples studied here stem from patients with predominantly Caucasian ethnicity, which may limit the generalizability of the findings to other ethnic groups. Validation in other ethnicities is therefore needed.

Testing the relationship between specific blood biomarker levels and a remote outcome observed 12 months later is challenging. In order to limit acute effects of the specific biomarker levels at baseline, we corrected for the acute rhythm at baseline, among other clinical parameters. Prediction of future rhythm by biomarkers depends on several factors, including the underlying biology of each biomarker,

Table 5 Estimated clinical utility of adding NT-proBNP, BMP10, and ANGPT2 alone or in combination to a clinical risk score to predict sinus rhythm at 12 months

	Patients reclassified as high risk of not attaining sinus rhythm at 12 M (*N*)	Confusion matrix			
		Predicted sinus rhythm (actual patients in SR: *N* = 1081)		Predicted: AF (actual patients in AF: *N* = 365)	
		Patients in sinus rhythm at 12 M	Patients in AF at 12 M	Patients in AF at 12 M	Patients in sinus rhythm at 12 M
Clinical model (LA size > 50 mm, persistent AF, age > 75 years)	Reference	813 (77%)	245 (23%)	75 (40%)	112 (60%)
+NT-proBNP	135	743 (79%)	201 (21%)	129 (40%)	191 (60%)
+BMP10	240	670 (79%)	175 (21%)	161 (37%)	279 (63%)
+ANGPT2	301	650 (82%)	145 (18%)	198 (40%)	303 (60%)
+NT-proBNP and BMP10	298	638 (80%)	158 (20%)	183 (37%)	315 (63%)
+NT-proBNP and ANGPT2	345	625 (83%)	130 (17%)	215 (39%)	332 (61%)
+ANGPT2 and BMP10	410	570 (82%)	125 (18%)	223 (36%)	394 (64%)
+NT-proBNP and BMP10 and ANGPT2	441	551 (83%)	115 (17%)	234 (36%)	416 (64%)

Sinus rhythm at 12 months was initially predicted by a clinical risk score based on three validated clinical features (LA size > 50 mm, persistent AF, age > 75 years) alone. This reference score was then combined with one, a combination of two, or all three binarized predictive biomarkers (biomarker thresholds: NT-proBNP < 1000 pg/mL or >1500 pg/mL, ANGPT2 < 3.5 ng/mL or >3.5 ng/mL, BMP10 < 2 ng/mL or >3 ng/mL, *Table 4*). If either the clinical risk score is ≥2 or any of the biomarkers added to the model surpasses its threshold, the model predicts failure to attain sinus rhythm at 12-month follow-up and predicts AF instead. All numbers indicate number of patients with percentages of the predicted class in brackets. There were 140 missing values in outcomes and 225 missing values in LA size. The additional use of biomarkers for prediction can lead to differing missing values in predictions made for participants with available outcome data.

AF, atrial fibrillation; ANGPT2, angiopoietin 2; BMP10, bone morphogenetic protein 10; NT-proBNP, N-terminal pro-B-type natriuretic peptide; SR, sinus rhythm; 12 M, 12-month follow-up.

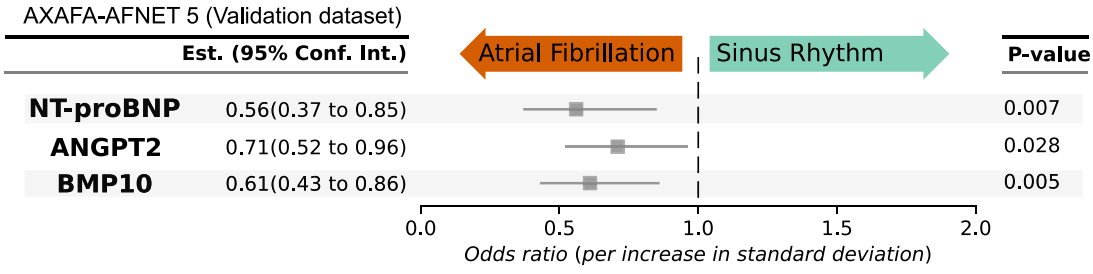


Figure 7 External validation of the prediction of sinus rhythm at the end of follow-up by baseline biomarkers in AXAFA-AFNET 5. AXAFA-AFNET 5 enrolled 674 patients undergoing a first AF ablation with at least one stroke risk factor. Patients were randomized to apixaban or vitamin K antagonist therapy without affecting rhythm. Individual models with rhythm at baseline, age, and sex were constructed to determine whether each biomarker predicts sinus rhythm at the end of follow-up 120 days after randomization, 549 patients with sinus rhythm, 71 patients with atrial fibrillation, 620 patients with baseline biomarkers completed follow-up. **P*-values were calculated using logistic regression, adjusted for sex, age, rhythm at baseline, and treatment group. ANGPT2, angiopoietin 2; BMP10, bone morphogenetic protein 10; NT-proBNP, N-terminal pro-B-type natriuretic peptide

spontaneous variations in concentrations, and assay quality. Lack of predictive ability in this study does not rule out relevant biological function of a given molecule. The proposed interventions countering the disease processes associated with biomarkers require further testing.

Conclusion

In conclusion, these findings suggest that NT-proBNP, ANGPT2, and BMP10 can be combined to identify patients with AF at high risk of not attaining sinus rhythm. The disease processes related to ANGPT2 and BMP10 emerge as likely contributors to future rhythm in patients with and without rhythm control therapy. NT-proBNP elevations interact with early rhythm control, potentially suggesting repeat assessment of NT-proBNP to monitor the effectiveness of rhythm control.

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Supplementary data

Supplementary data are available at *European Heart Journal* online.

Declarations

Disclosure of Interest

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Data Availability

Anonymized data may be provided upon reasonable request. Please contact AFNET info:info@kompetenznetz-vorhofflimmern.de. Source code will be made available at GitHub under UCCSHH by the University Center of Cardiovascular Science, Hamburg (https://github.com/UCCSHH).

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Ethical Approval

Derivation and validation cohorts have ethical approval. EAST-AFNET 4: Ethikkommission der Ärztekammer Westfalen-Lippe und der Medizinischen Fakultät der Westfälischen Wilhelms-Universität Münster, 09/02/2011, ref: 2010-274-f-A AXAFA-AFNET 5: Ethics Committee at the Medical Faculty of the University of Leipzig (Ethik-Kommission an der Medizinischen Fakultät der Universität Leipzig), 20/01/2015, ref: 341/14-ff BBC-AF: National Research Ethics Service Committee, Integrated Research Application System Identification: (IRAS ID 97753), REC12/WM/0344; 3 Dec 2012 TRUST: Ethikkommission der Ärztekammer Hamburg, 2020-10066-BO-ff, initial vote of 2021-01-25, first patient recruited 2021-03-17, 2nd amendment voted at 2023-5-11.

Pre-registered Clinical Trial Number

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