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

Larissa Fabritz[1,2,3,4,5,6], Christoph Al-Taie[1,2,3,6], Katrin Borof[2], Günter Breithardt[4,7], A John Camm[8], Harry JGM Crijns[9], Victor Roth Cardoso[5,10], Winnie Chua[5,6], Silke van Elferen[1,2,11], Lars Eckardt[4,7], George Gkoutos[1,5,6,10], Andreas Goette[4,6,12,13], Eduard Guasch[14], Stéphane Hatem[6,15], Andreas Metzner[2], Lluís Mont[14], Vaishnavi Ameya Murukutla[1,3,6] , Julius Obergassel[1,2,6], Andreas Rillig[2,3], Moritz F Sinner[16], Renate B Schnabel[2,3,4], Ulrich Schotten[4,6,17], Laura C Sommerfeld[1,3,5,6] , Ursula-Henrike Wienhues-Thelen[18], Antonia Zapf[3,4,19], Tanja Zeller[1,2,3], Paulus Kirchhof[2,3,4,5,6]

[1]University Center of Cardiovascular Science, University Heart and Vascular Center Hamburg, University Medical Center Hamburg Eppendorf, Hamburg, Germany

[2]Department of Cardiology, University Heart and Vascular Center Hamburg, University Medical Center Hamburg Eppendorf, Hamburg, Germany

[3]German Center for Cardiovascular Research (DZHK), partner site Hamburg/Kiel/Lübeck, Germany

[4]AFNET, Münster, Germany

[5] Institute of Cardiovascular Sciences, University of Birmingham, UK

[6] MAESTRIA Consortium, European Union´s Horizon 2020 research and innovation programme, agreement number 965386

[7]Department of Cardiology II (Electrophysiology), University Hospital Münster, Germany

[8]Clinical Sciences, St George´s University, London, UK

[9]Department of Cardiology, University Hospital Maastricht, Maastricht, The Netherlands

ACCEPTED MANUSCRIPT

© The Author(s) 2024. Published by Oxford University Press on behalf of the European Society of Cardiology. This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (https://creativecommons.org/licenses/by-nc/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact reprints@oup.com for reprints and translation rights for reprints. All other permissions can be obtained through our RightsLink service via the Permissions link on the article page on our site—for further information please contact journals.permissions@oup.com.

[10]MRC Health Data Research UK (HDR), Midlands Site, UK and Institute of Cancer and Genomic Sciences, University of Birmingham, Birmingham, UK.

[11]Computational and Systems Biology at Hamburg University, Germany

[12]Department of Cardiology and Intensive Care Medicine, St. Vincenz Hospital, Paderborn, Germany

[13] Otto-von-Guericke University, Medical Faculty, Magdeburg, Germany

[14]Hospital Clinic de Barcelona, Institute of Biomedical Research August Pi Sunyer (IDIBAPS), Hospital Clinic, CIBERCV, University of Barcelona, Catalonia, Spain

[15]Department of Cardiology, Sorbonne Universités, Faculté de médecine UPMC, Assistance Publique-Hôpitaux de Paris, Pitié-Salpêtrière Hospital, Paris, France.

[16]Department of Medicine I, University Hospital Munich, Ludwig Maximilian University of Munich (LMU), Munich, Germany and German Centre for Cardiovascular Research (DZHK), partner site Munich Heart Alliance, Munich, Germany.

[17]Department of Physiology, Maastricht University, Maastricht, The Netherlands

[18]Roche Diagnostics, Penzberg, Germany

[19]Institute of Medical Biometry and Epidemiology, University Medical Center Hamburg Eppendorf, Hamburg, Germany

Short title: Biomolecules predict sinus rhythm: EAST-AFNET 4

Corresponding author:
Prof. Dr. Paulus Kirchhof
Department of Cardiology
University Heart and Vascular Centre Hamburg Eppendorf
University Medical Center Hamburg Eppendorf
Martinist. 52
20246 Hamburg
Phone: +49 (0)40 7410-52438
Fax: +49 (0)40 7410-55862
Email: p.kirchhof@uke.de
www.uhz.de

ACCEPTED MANUSCRIPT

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, 46% 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.

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] 0.76 [95% confidence interval 0.65-0.89], p=0.001), bone morphogenetic protein 10 (BMP10) (OR 0.83 [0.71-0.97], p=0.017) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) (OR 0.73 [0.60–0.88], p=0.001). Analysis of rhythm at 24 months confirmed the results. Early rhythm control interacted with the predictive potential of NT-proBNP ($p_{interaction}$=0.033). The predictive effect of NT-proBNP was reduced in patients randomized to early rhythm control (usual care: OR 0.64 [0.51-0.80], p<0.001; early rhythm control: OR 0.90 [0.69-1.18], p=0.453). 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.

Key words: atrial fibrillation; blood biomarker; sinus rhythm; rhythm control; natriuretic peptides; bone morphogenetic protein 10; angiopoietin 2; risk prediction; risk score

ACCEPTED MANUSCRIPT

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[6]. 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 estimated (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 the molecules modify future rhythm in patients with AF has not been investigated.

This analysis of the EAST-AFNET 4 biomolecule study embedded into the **E**arly treatment of **A**trial fibrillation for **ST**roke prevention (EAST-AFNET 4) trial[2] quantified 14 biomarkers reflecting different disease processes in AF that were defined *a priori*[9]. The

ACCEPTED MANUSCRIPT

ability of each biomarker to predict sinus rhythm at 12-month follow-up in patients with and without early rhythm control therapy was evaluated.

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

ACCEPTED MANUSCRIPT

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]

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 (GP) 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.

ACCEPTED MANUSCRIPT

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: 1) Members of the consortium identified candidate biomarkers reflecting disease processes known to contribute to AF and its complications, 2) Deep literature and patent searches for candidate biomarkers and additional novel biomarkers were performed, 3) Expert discussion and Delphi-like votes by the consortium defined most promising candidates, and 4) 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 hours transport time). Upon arrival at UHZ, samples were spun, shock-frozen and stored at -80C 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.

Statistical methods. As this is a secondary outcome analysis of the EAST-AFNET 4 trial, all results are exploratory. Biomarker concentrations were one-percent 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 fourteen 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

ACCEPTED MANUSCRIPT

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 (**Supplementary Table S1**). Rhythm at time of blood sampling was included as a confounder in all subsequent analyses in addition to the features predicting rhythm at 12 months in the main EAST-AFNET 4 data set.[4]

Mixed logistic regression models were used to assess the predictive value of the 14 biomarkers on rhythm at 12 months, with study center as a random intercept. The lme4 R package[24] was used. Each biomarker was assessed in a separate model adjusted for sex, age, 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). Those features are associated with rhythm at 12 months in the EAST-AFNET 4 trial[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 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 odds ratios (OR) 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.

ACCEPTED MANUSCRIPT

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 center 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 (**Supplementary Table S2**). As many patients with one of these three features attain sinus rhythm at 12 months, the score was considered positively 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 were 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 preprocessing and

ACCEPTED MANUSCRIPT

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 Figure S1**).

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=0.033) 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 versus AF at 12 months. Numbers of mean biomarker concentrations by rhythm at 12-month follow-up and by randomized treatment group are given (**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 Table S1**. Post-hoc subgroup analyses by rhythm at the time of baseline

ACCEPTED MANUSCRIPT

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**).

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 (**Supplementary 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 high-risk 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**).

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 Figures S2, S3, S4**). To compare them to clinical features predicting sinus rhythm, a score combining clinical features predicting recurrent AF was created[28] (**Supplementary Table S2**). Adding

ACCEPTED MANUSCRIPT

biomarkers using these thresholds improved identification of patients at risk of not attaining sinus rhythm at 12-month follow-up (**Table 5, Supplementary Table S4**).

External validation. Several separate validation datasets (AXAFA-AFNET 5 trial, BBC-AF and TRUST cohort snapshot **Supplementary Tables S5, S6, 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**). The clinical utility of adding the biomolecules to clinical predictors was validated in both cohorts using the thresholds derived in EAST-AFNET 4 (**Supplementary Table S8 and S9**).

Discussion

Main findings. Three out of fourteen 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

ACCEPTED MANUSCRIPT

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, and the predictive ability of BMP10 decreased in the subgroup of patients who were in sinus rhythm, but not in the subgroup of patients who were in AF, similar to rhythm-dependent decrease of predictive ability of NT-proBNP[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 processes in patients with AF, probably explaining its interaction with rhythm. 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 without AF and heart

ACCEPTED MANUSCRIPT

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 signaling 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 remodeling[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.

ACCEPTED MANUSCRIPT

BMP10 is selectively expressed in and released by atrial cardiomyocytes[16,62]. BMP10 is part of the TGFß 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 left 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 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 previously defining these patient clusters.[17] The biomarker-based clusters show a certain risk gradient for sinus rhythm at 12 months (**Figure 5**). At difference to the prior study that defined patient clusters based on 14 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 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.

ACCEPTED MANUSCRIPT

NT-proBNP, ANGPT2 and BMP10 can refine that selection. 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[69] could have beneficial effects in patients with elevated BMP10 and ANGPT2 concentrations.[16,67,70] 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 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 provided for 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].

ACCEPTED MANUSCRIPT

Additional biomarkers in AF may emerge from hypothesis-free quantification of many molecules at once e.g. by RNA-sequencing of cardiac tissue[71], quantification of circulating RNAs, and by proteomics[72,73]. 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 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. 24-hour blood pressure may provide more granular prognostic information than office-based blood pressure, but 24-hour 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 sufficient

ACCEPTED MANUSCRIPT

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, 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.

Acknowledgement: We thank AFNET staff.

ACCEPTED MANUSCRIPT

References

1. Hindricks G, Potpara T, Dagres N, Arbelo E, Bax JJ, Blomstrom-Lundqvist C*, et al.* 2020 ESC Guidelines for the diagnosis and management of atrial fibrillation developed in collaboration with the European Association for Cardio-Thoracic Surgery (EACTS): The Task Force for the diagnosis and management of atrial fibrillation of the European Society of Cardiology (ESC) Developed with the special contribution of the European Heart Rhythm Association (EHRA) of the ESC. Eur Heart J 2021;**42**(5):373-498.
2. Kirchhof P, Camm AJ, Goette A, Brandes A, Eckardt L, Elvan A*, et al.* Early Rhythm-Control Therapy in Patients with Atrial Fibrillation. N Engl J Med 2020;**383**(14):1305-1316.
3. Linz D, Andrade JG, Arbelo E, Boriani G, Breithardt G, Camm AJ*, et al.* Longer and better lives for patients with atrial fibrillation: the 9th AFNET/EHRA consensus conference. Europace 2024;**26**(4).
4. Eckardt L, Sehner S, Suling A, Borof K, Breithardt G, Crijns H*, et al.* Attaining sinus rhythm mediates improved outcome with early rhythm control therapy of atrial fibrillation: the EAST-AFNET 4 trial. Eur Heart J 2022;**43**(40):4127-4144.
5. Becher N, Metzner A, Toennis T, Kirchhof P, Schnabel RB. Atrial fibrillation burden: a new outcome predictor and therapeutic target. Eur Heart J 2024.
6. Andrade JG, Deyell MW, Khairy P, Champagne J, Leong-Sit P, Novak P*, et al.* Atrial fibrillation progression after cryoablation versus radiofrequency ablation: the CIRCA-DOSE trial. Eur Heart J 2023.
7. Kuck KH, Lebedev DS, Mikhaylov EN, Romanov A, Geller L, Kalejs O*, et al.* Catheter ablation or medical therapy to delay progression of atrial fibrillation: the randomized controlled atrial fibrillation progression trial (ATTEST). Europace 2021;**23**(3):362-369.
8. Andrade JG, Deyell MW, Macle L, Wells GA, Bennett M, Essebag V*, et al.* Progression of Atrial Fibrillation after Cryoablation or Drug Therapy. N Engl J Med 2022.
9. Fabritz L, Guasch E, Antoniades C, Bardinet I, Benninger G, Betts TR*, et al.* Expert consensus document: Defining the major health modifiers causing atrial fibrillation: a roadmap to underpin personalized prevention and treatment. Nat Rev Cardiol 2016;**13**(4):230-7.
10. Nielsen JB, Thorolfsdottir RB, Fritsche LG, Zhou W, Skov MW, Graham SE*, et al.* Biobank-driven genomic discovery yields new insight into atrial fibrillation biology. Nat Genet 2018;**50**(9):1234-1239.
11. Schnabel RB, Yin X, Gona P, Larson MG, Beiser AS, McManus DD*, et al.* 50 year trends in atrial fibrillation prevalence, incidence, risk factors, and mortality in the Framingham Heart Study: a cohort study. Lancet 2015;**386**(9989):154-62.
12. Billing AM, Kim YC, Gullaksen S, Schrage B, Raabe J, Hutzfeldt A*, et al.* Metabolic Communication by SGLT2 Inhibition. Circulation 2023.
13. Reyat J, Sommerfeld L, O'Reilly M, Roth Cardoso V, Thiemann E, Khan A*, et al.* PITX2-deficiency leads to atrial mitochondrial dysfunction. Cardiovasc Res 2024;**in press, doi 10.1093/cvr/cvae169**.
14. Hu YF, Chen YJ, Lin YJ, Chen SA. Inflammation and the pathogenesis of atrial fibrillation. Nat Rev Cardiol 2015;**12**(4):230-43.
15. Spronk HM, De Jong AM, Verheule S, De Boer HC, Maass AH, Lau DH*, et al.* Hypercoagulability causes atrial fibrosis and promotes atrial fibrillation. Eur Heart J 2017;**38**(1):38-50.
16. Reyat JS, Chua W, Cardoso VR, Witten A, Kastner PM, Kabir SN*, et al.* Reduced left atrial cardiomyocyte PITX2 and elevated circulating BMP10 predict atrial fibrillation after ablation. JCI Insight 2020;**5**(16).
17. Fabritz L, Chua W, Cardoso VR, Al-Taie C, Borof K, Suling A*, et al.* Blood-based cardiometabolic phenotypes in atrial fibrillation and their associated risk: EAST-AFNET 4 biomolecule study. Cardiovasc Res 2024.

ACCEPTED MANUSCRIPT

18. Kany S, Al-Taie C, Roselli C, Pirruccello JP, Borof K, Reinbold C*, et al.* Association of genetic risk and outcomes in patients with atrial fibrillation: interactions with early rhythm control in the EAST-AFNET4 trial. Cardiovasc Res 2023;**119**(9):1799-1810.
19. Kirchhof P, Haeusler KG, Blank B, De Bono J, Callans D, Elvan A*, et al.* Apixaban in patients at risk of stroke undergoing atrial fibrillation ablation. Eur Heart J 2018;**39**(32):2942-2955.
20. Chua W, Khashaba A, Canagarajah H, Nielsen JC, di Biase L, Haeusler KG*, et al.* Disturbed atrial metabolism, shear stress, and cardiac load contribute to atrial fibrillation after ablation: AXAFA biomolecule study. Europace 2024;**26**(2).
21. Chua W, Purmah Y, Cardoso VR, Gkoutos GV, Tull SP, Neculau G*, et al.* Data-driven discovery and validation of circulating blood-based biomarkers associated with prevalent atrial fibrillation. Eur Heart J 2019;**40**(16):1268-1276.
22. Brady PF, Chua W, Nehaj F, Connolly DL, Khashaba A, Purmah YJV*, et al.* Interactions Between Atrial Fibrillation and Natriuretic Peptide in Predicting Heart Failure Hospitalization or Cardiovascular Death. J Am Heart Assoc 2022;**11**(4):e022833.
23. Dixon WJ, Yuen KK. Trimming and winsorization: A review. Statistische Hefte 1974;**15**(2):157-170.
24. Bates D MM, Bolker B, Walker S. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software 2015;**67(1)**:1–48.
25. Figueiras A, Domenech-Massons JM, Cadarso C. Regression models: calculating the confidence interval of effects in the presence of interactions. Stat Med 1998;**17**(18):2099-105.
26. White IR, Royston P, Wood AM. Multiple imputation using chained equations: Issues and guidance for practice. Stat Med 2011;**30**(4):377-99.
27. van Buuren S G-OK. mice: Multivariate Imputation by Chained Equations in R. Journal of Statistical Software 2011;**45(3)**:1-67.
28. Dretzke J, Chuchu N, Agarwal R, Herd C, Chua W, Fabritz L*, et al.* Predicting recurrent atrial fibrillation after catheter ablation: a systematic review of prognostic models. Europace 2020;**22**(5):748-760.
29. Team RC. R: A Language and Environment for Statistical Computin. In: R Foundation for Statistical Computing; 2022.
30. Ruff CT, Giugliano RP, Braunwald E, Hoffman EB, Deenadayalu N, Ezekowitz MD*, et al.* Comparison of the efficacy and safety of new oral anticoagulants with warfarin in patients with atrial fibrillation: a meta-analysis of randomised trials. Lancet 2014;**383**(9921):955-62.
31. Sohns C, Fox H, Marrouche NF, Crijns H, Costard-Jaeckle A, Bergau L*, et al.* Catheter Ablation in End-Stage Heart Failure with Atrial Fibrillation. N Engl J Med 2023;**389**(15):1380-1389.
32. Whitlock RP, Belley-Cote EP, Paparella D, Healey JS, Brady K, Sharma M*, et al.* Left Atrial Appendage Occlusion during Cardiac Surgery to Prevent Stroke. N Engl J Med 2021;**384**(22):2081-2091.
33. Hijazi Z, Lindback J, Alexander JH, Hanna M, Held C, Hylek EM*, et al.* 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**(20):1582-90.
34. Hijazi Z, Oldgren J, Lindback J, Alexander JH, Connolly SJ, Eikelboom JW*, et al.* The novel biomarker-based ABC (age, biomarkers, clinical history)-bleeding risk score for patients with atrial fibrillation: a derivation and validation study. Lancet 2016;**387**(10035):2302-11.
35. Pol T, Hijazi Z, Lindback J, Oldgren J, Alexander JH, Connolly SJ*, et al.* Using multimarker screening to identify biomarkers associated with cardiovascular death in patients with atrial fibrillation. Cardiovasc Res 2022;**118**(9):2112-2123.
36. Gkarmiris KI, Lindback J, Alexander JH, Granger CB, Kastner P, Lopes RD*, et al.* Repeated Measurement of the Novel Atrial Biomarker BMP10 (Bone Morphogenetic Protein 10) Refines Risk Stratification in Anticoagulated Patients With Atrial Fibrillation: Insights From the ARISTOTLE Trial. J Am Heart Assoc 2024;**13**(7):e033720.

ACCEPTED MANUSCRIPT

37. Yamaji T, Ishibashi M, Nakaoka H, Imataka K, Amano M, Fujii J. Possible role for atrial natriuretic peptide in polyuria associated with paroxysmal atrial arrhythmias. Lancet 1985;**1**(8439):1211.
38. Ravelli F, Allessie M. Effects of atrial dilatation on refractory period and vulnerability to atrial fibrillation in the isolated Langendorff-perfused rabbit heart. Circulation 1997;**96**(5):1686-95.
39. Walters TE, Lee G, Spence S, Larobina M, Atkinson V, Antippa P*, et al.* Acute atrial stretch results in conduction slowing and complex signals at the pulmonary vein to left atrial junction: insights into the mechanism of pulmonary vein arrhythmogenesis. Circ Arrhythm Electrophysiol 2014;**7**(6):1189-97.
40. Wachter R, Lahno R, Haase B, Weber-Kruger M, Seegers J, Edelmann F*, et al.* Natriuretic peptides for the detection of paroxysmal atrial fibrillation in patients with cerebral ischemia--the Find-AF study. PLoS One 2012;**7**(4):e34351.
41. Zografos T, Maniotis C, Katsivas A, Katritsis D. Relationship between brain natriuretic peptides and recurrence of atrial fibrillation after successful direct current cardioversion: a meta-analysis. Pacing Clin Electrophysiol 2014;**37**(11):1530-7.
42. Asselbergs FW, van den Berg MP, Bakker SJ, Signorovitch JE, Hillege HL, van Gilst WH, van Veldhuisen DJ. N-terminal pro B-type natriuretic peptide levels predict newly detected atrial fibrillation in a population-based cohort. Neth Heart J 2008;**16**(3):73-8.
43. Hwang HJ, Son JW, Nam BH, Joung B, Lee B, Kim JB*, et al.* Incremental predictive value of pre-procedural N-terminal pro-B-type natriuretic peptide for short-term recurrence in atrial fibrillation ablation. Clin Res Cardiol 2009.
44. Freestone B, Gustafsson F, Chong AY, Corell P, Kistorp C, Hildebrandt P, Lip GY. Influence of atrial fibrillation on plasma von willebrand factor, soluble E-selectin, and N-terminal pro B-type natriuretic peptide levels in systolic heart failure. Chest 2008;**133**(5):1203-8.
45. Xu X, Tang Y. Relationship between Brain Natriuretic Peptide and Recurrence of Atrial Fibrillation after Successful Electrical Cardioversion: an Updated Meta-Analysis. Braz J Cardiovasc Surg 2017;**32**(6):530-535.
46. Darkner S, Goetze JP, Chen X, Henningsen K, Pehrson S, Svendsen JH. Natriuretic Propeptides as Markers of Atrial Fibrillation Burden and Recurrence (from the AMIO-CAT Trial). Am J Cardiol 2017;**120**(8):1309-1315.
47. den Uijl DW, Delgado V, Tops LF, Ng AC, Boersma E, Trines SA*, et al.* Natriuretic peptide levels predict recurrence of atrial fibrillation after radiofrequency catheter ablation. Am Heart J 2011;**161**(1):197-203.
48. Cushman M, Judd SE, Howard VJ, Kissela B, Gutierrez OM, Jenny NS*, et al.* N-terminal pro-B-type natriuretic peptide and stroke risk: the reasons for geographic and racial differences in stroke cohort. Stroke 2014;**45**(6):1646-50.
49. Schrage B, Geelhoed B, Niiranen TJ, Gianfagna F, Vishram-Nielsen JKK, Costanzo S*, et al.* Comparison of Cardiovascular Risk Factors in European Population Cohorts for Predicting Atrial Fibrillation and Heart Failure, Their Subsequent Onset, and Death. J Am Heart Assoc 2020;**9**(9):e015218.
50. Svennberg E, Henriksson P, Engdahl J, Hijazi Z, Al-Khalili F, Friberg L, Frykman V. N-terminal pro B-type natriuretic peptide in systematic screening for atrial fibrillation. Heart 2017;**103**(16):1271-1277.
51. Patton KK, Ellinor PT, Heckbert SR, Christenson RH, DeFilippi C, Gottdiener JS, Kronmal RA. N-terminal pro-B-type natriuretic peptide is a major predictor of the development of atrial fibrillation: the Cardiovascular Health Study. Circulation 2009;**120**(18):1768-74.
52. Engdahl J, Svennberg E, Friberg L, Al-Khalili F, Frykman V, Kemp Gudmundsdottir K*, et al.* Stepwise mass screening for atrial fibrillation using N-terminal pro b-type natriuretic peptide: the STROKESTOP II study design. Europace 2017;**19**(2):297-302.

ACCEPTED MANUSCRIPT

53. Parwani AS, Kaab S, Friede T, Tilz RR, Bauersachs J, Frey N*, et al.* Catheter-based ablation to improve outcomes in patients with atrial fibrillation and heart failure with preserved ejection fraction: Rationale and design of the CABA-HFPEF-DZHK27 trial. Eur J Heart Fail 2024.
54. Gale NW, Thurston G, Hackett SF, Renard R, Wang Q, McClain J*, et al.* Angiopoietin-2 is required for postnatal angiogenesis and lymphatic patterning, and only the latter role is rescued by Angiopoietin-1. Dev Cell 2002;**3**(3):411-23.
55. Chen JX, Zeng H, Reese J, Aschner JL, Meyrick B. Overexpression of angiopoietin-2 impairs myocardial angiogenesis and exacerbates cardiac fibrosis in the diabetic db/db mouse model. Am J Physiol Heart Circ Physiol 2012;**302**(4):H1003-12.
56. Huang YQ, Li JJ, Hu L, Lee M, Karpatkin S. Thrombin induces increased expression and secretion of angiopoietin-2 from human umbilical vein endothelial cells. Blood 2002;**99**(5):1646-50.
57. Chua W, Cardoso VR, Guasch E, Sinner MF, Al-Taie C, Brady P*, et al.* An angiopoietin 2, FGF23, and BMP10 biomarker signature differentiates atrial fibrillation from other concomitant cardiovascular conditions. Sci Rep 2023;**13**(1):16743.
58. Bontekoe J, Lee J, Bansal V, Syed M, Hoppensteadt D, Maia P*, et al.* Biomarker Profiling in Stage 5 Chronic Kidney Disease Identifies the Relationship between Angiopoietin-2 and Atrial Fibrillation. Clin Appl Thromb Hemost 2018;**24**(9_suppl):269S-276S.
59. Bhandari V, Choo-Wing R, Lee CG, Zhu Z, Nedrelow JH, Chupp GL*, et al.* Hyperoxia causes angiopoietin 2-mediated acute lung injury and necrotic cell death. Nat Med 2006;**12**(11):1286-93.
60. Kumpers P, Lukasz A, David S, Horn R, Hafer C, Faulhaber-Walter R*, et al.* Excess circulating angiopoietin-2 is a strong predictor of mortality in critically ill medical patients. Crit Care 2008;**12**(6):R147.
61. Benz AP, Hijazi Z, Lindback J, Connolly SJ, Eikelboom JW, Kastner P*, et al.* Plasma angiopoietin-2 and its association with heart failure in patients with atrial fibrillation. Europace 2023;**25**(7).
62. Kahr PC, Piccini I, Fabritz L, Greber B, Scholer H, Scheld HH*, et al.* Systematic Analysis of Gene Expression Differences between Left and Right Atria in Different Mouse Strains and in Human Atrial Tissue. PLoS ONE 2011;**6**(10):e26389.
63. Wang L, Rice M, Swist S, Kubin T, Wu F, Wang S*, et al.* BMP9 and BMP10 Act Directly on Vascular Smooth Muscle Cells for Generation and Maintenance of the Contractile State. Circulation 2021;**143**(14):1394-1410.
64. Hodgson J, Swietlik EM, Salmon RM, Hadinnapola C, Nikolic I, Wharton J*, et al.* Characterization of GDF2 Mutations and Levels of BMP9 and BMP10 in Pulmonary Arterial Hypertension. Am J Respir Crit Care Med 2020;**201**(5):575-585.
65. Steimle JD, Grisanti Canozo FJ, Park M, Kadow ZA, Samee MAH, Martin JF. Decoding the PITX2-controlled genetic network in atrial fibrillation. JCI Insight 2022;**7**(11).
66. Hennings E, Aeschbacher S, Coslovsky M, Paladini RE, Meyre PB, Voellmin G*, et al.* Association of bone morphogenetic protein 10 and recurrent atrial fibrillation after catheter ablation. Europace 2023;**25**(6).
67. Hennings E, Blum S, Aeschbacher S, Coslovsky M, Knecht S, Eken C*, et al.* Bone Morphogenetic Protein 10-A Novel Biomarker to Predict Adverse Outcomes in Patients With Atrial Fibrillation. J Am Heart Assoc 2023;**12**(6):e028255.
68. Winters J, Kawczynski MJ, Gilbers MD, Isaacs A, Zeemering S, Bidar E*, et al.* Circulating BMP10 Levels Associate With Late Postoperative Atrial Fibrillation and Left Atrial Endomysial Fibrosis. JACC Clin Electrophysiol 2024.
69. Billing AM, Kim YC, Gullaksen S, Schrage B, Raabe J, Hutzfeldt A*, et al.* Metabolic Communication by SGLT2 Inhibition. Circulation 2024;**149**(11):860-884.
70. Hijazi Z, Benz AP, Lindback J, Alexander JH, Connolly SJ, Eikelboom JW*, et al.* Bone morphogenetic protein 10: a novel risk marker of ischaemic stroke in patients with atrial fibrillation. Eur Heart J 2023;**44**(3):208-218.

ACCEPTED MANUSCRIPT

71. Winters J, Isaacs A, Zeemering S, Kawczynski M, Maesen B, Maessen J*, et al.* Heart Failure, Female Sex, and Atrial Fibrillation Are the Main Drivers of Human Atrial Cardiomyopathy: Results From the CATCH ME Consortium. J Am Heart Assoc 2023;**12**(22):e031220.
72. Barallobre-Barreiro J, Gupta SK, Zoccarato A, Kitazume-Taneike R, Fava M, Yin X*, et al.* Glycoproteomics Reveals Decorin Peptides With Anti-Myostatin Activity in Human Atrial Fibrillation. Circulation 2016;**134**(11):817-32.
73. Ko D, Benson MD, Ngo D, Yang Q, Larson MG, Wang TJ*, et al.* Proteomics Profiling and Risk of New-Onset Atrial Fibrillation: Framingham Heart Study. J Am Heart Assoc 2019;**8**(6):e010976.

ACCEPTED MANUSCRIPT

Figure legends

Graphical Abstract. 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

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 center, 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.

Angiopoietin 2 (ANGPT2), bone morphogenetic protein 10 (BMP10), cancer antigen 125 (CA125), C-reactive protein (CRP), D-dimer, endothelial specific molecule 1 (ESM1), fatty acid binding protein 3 (FABP3), fibroblast growth factor 23 (FGF23), growth differentiation factor 15 (GDF15), insulin-like

ACCEPTED MANUSCRIPT

growth factor binding protein 7 (IGFBP7), interleukin-6 (IL-6), N-terminal pro–B-type natriuretic peptide (NT-proBNP), cardiac troponin (TnT) and serum creatinine (sCr).

Figure 2. Biomarker concentration distributions at baseline in patients with sinus rhythm (teal) or atrial fibrillation (orange) 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, study site, 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.

Angiopoietin 2 (ANGPT2), bone morphogenetic protein 10 (BMP10), cancer antigen 125 (CA125), C-reactive protein (CRP), D-dimer, endothelial specific molecule 1 (ESM1), fatty acid binding protein 3 (FABP3), fibroblast growth factor 23 (FGF23), growth differentiation factor 15 (GDF15), insulin-like growth factor binding protein 7 (IGFBP7), interleukin-6 (IL-6), N-terminal pro–B-type natriuretic peptide (NT-proBNP), cardiac troponin (TnT) and serum creatinine (sCr).

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.

ACCEPTED MANUSCRIPT

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.

Angiopoietin 2 (ANGPT2), bone morphogenetic protein 10 (BMP10), N-terminal pro–B-type natriuretic peptide (NT-proBNP)

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 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.

Angiopoietin 2 (ANGPT2), bone morphogenetic protein 10 (BMP10), cancer antigen 125 (CA125), C-reactive protein (CRP), D-dimer, endothelial specific molecule 1 (ESM1), fatty acid binding protein 3 (FABP3), fibroblast growth factor 23 (FGF23), growth differentiation factor 15 (GDF15), insulin-like growth factor binding protein 7 (IGFBP7), interleukin-6 (IL-6), N-terminal pro–B-type natriuretic peptide (NT-proBNP), cardiac troponin (TnT) and serum creatinine (sCr).

Figure 5. Validation applying biomarker based clusters indicating cardiovascular outcome risk: Patients at high risk of cardiovascular complications as estimated by biomarker-based

ACCEPTED MANUSCRIPT

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-months 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 center, rhythm at baseline, atrial fibrillation type (depicted) 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)

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 (Figure 6A – importance, Figure 6B – SHAP value).

Atrial fibrillation (AF), Angiopoietin 2 (ANGPT2), baseline (BL), body mass index (BMI), bone morphogenetic protein 10 (BMP10), cancer antigen 125 (CA125), C-reactive protein (CRP), D-dimer, endothelial specific molecule 1 (ESM1), fatty acid binding protein 3 (FABP3), fibroblast growth factor 23 (FGF23), growth differentiation factor 15 (GDF15), insulin-like growth factor binding protein 7 (IGFBP7), interleukin-6 (IL-6), mixed-effects random forest (MERF), N-terminal pro–B-type natriuretic peptide (NT-proBNP), cardiac troponin (TnT), serum creatinine (sCr) and SHapley Additive exPlanations (SHAP)

ACCEPTED MANUSCRIPT

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.

** p-values were calculated using logistic regression, adjusted for sex, age, rhythm at baseline and treatment group. Abbreviations: Angiopoietin 2 (ANGPT2), bone morphogenetic protein 10 (BMP10), N-terminal pro–B-type natriuretic peptide (NT-proBNP).*

ACCEPTED MANUSCRIPT

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%)	0.639
Age (years)		71 [66, 75]	71 [66, 76]	0.711
BMI		28.7 [25.6, 32.1]	29.0 [25.6, 32.5]	0.699
Blood pressure (systolic) (mmHg)		135 [123, 150]	135 [125, 148]	0.730
Blood pressure (diastolic) (mmHg)		80 [74, 90]	80 [74, 90]	0.716
LVEF (%)		60 [55, 65]	60 [55, 65]	0.873
AF type (First episode)		290 (36%)	270 (34%)	
AF type (Paroxysmal)		302 (38%)	288 (37%)	0.839
AF type (Persistent)		208 (26%)	228 (29%)	0.202
Other Clinical Characteristics				
Diabetes		207 (26%)	189 (24%)	0.400
Hypertension		494 (62%)	512 (65%)	0.170
Chronic kidney disease		98 (12%)	97 (12%)	0.956
Estimated Glomerular Filtration Rate (ml/min1.73 m²)		75 [63 - 87]	76 [64 - 87]	0.734
Previous stroke or transient ischemic attack		114 (14%)	81 (10%)	0.017
Chronic obstructive pulmonary disease		63 (8%)	61 (8%)	0.991
Diastolic LA diameter (mm)		42 [38, 47]	43 [39, 47]	0.730
NYHA class				
No heart failure		523 (65%)	509 (65%)	
I		82 (10%)	88 (11%)	0.555
II		164 (21%)	160 (20%)	0.985
III		31 (4%)	29 (4%)	0.882
EHRA-score				
I		232 (29%)	236 (30%)	
II		386 (48%)	374 (48%)	0.679
III		122 (15%)	122 (15%)	0.914
IV		8 (1%)	9 (1%)	0.839
Missing		52 (7%)	45 (6%)	
Biomarker (unit)	Coefficient of variation			
NT-proBNP (pg/ml)	1.51	441 [175 - 966]	467 [187 - 1036]	0.537
ANGPT2 (ng/ml)	0.70	2.53 [1.87 - 3.65]	2.53 [1.87 - 3.75]	0.456
BMP10 (ng/ml)	0.24	2.10 [1.82 - 2.41]	2.11 [1.83 - 2.45]	0.507
FGF23 (pg/ml)	1.27	155 [115 - 218]	153 [115 - 211]	0.244
ESM1 (ng/ml)	0.76	2.04 [1.64 - 2.59]	2.05 [1.63 - 2.63]	0.818
GDF15 (pg/ml)	0.80	1333 [990 – 2000]	1359 [971 - 2005]	0.078
IGFBP7 (ng/ml)	0.26	102 [90.7 – 117]	102 [90.1 – 117]	0.457
IL-6 (pg/ml)	6.62	2.56 [1.64 – 4.04]	2.68 [1.67 – 4.18]	0.479
FABP3 (ng/ml)	0.50	32.0 [26.3 – 39.6]	31.9 [26.4 – 39.6]	0.837
D-dimer (µg/ml)	1.74	0.17 [0.09 – 0.34]	0.16 [0.08 – 0.36]	0.506

ACCEPTED MANUSCRIPT

TnT (ng/l)	2.26	11.1 [8.02 – 16.6]	11.4 [8.21 – 16.7]	0.337
CRP (mg/l)	3.28	2.02 [0.96 – 4.99]	2.38 [1.04 – 4.75]	0.392
sCr (µmol/l)	0.29	81.7 [70.7 – 95.5]	80.4 [70.0 - 94.5]	0.771
CA125 (U/ml)	1.51	11.5 [8.08 - 15.9]	11.1 [7.93 - 16.1]	0.433

** 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.*

Abbreviations: Atrial Fibrillation (AF), sinus rhythm (SR), early rhythm control (ERC), usual care (UC), body mass index (BMI), left ventricular ejection fraction (LVEF), left atrium (LA), New York Heart Association Functional Classification of heart failure (NYHA), European Heart Rhythm Association score (EHRA), angiopoietin 2 (ANGPT2), bone morphogenetic protein 10 (BMP10), cancer antigen 125 (CA125), C-reactive protein (CRP), D-dimer, endothelial specific molecule 1 (ESM1), fatty acid binding protein 3 (FABP3), fibroblast growth factor 23 (FGF23), growth differentiation factor 15 (GDF15), insulin-like growth factor binding protein 7 (IGFBP7), interleukin-6 (IL-6), N-terminal pro–B-type natriuretic peptide (NT-proBNP), cardiac troponin (TnT) and serum creatinine (sCr). Estimated Glomerular Filtration (eGFR) rate was calculated as CKD EPI, Chronic Kidney Disease Epidemiology Collaboration

ACCEPTED MANUSCRIPT

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

Randomisation group	Early rhythm control		Usual care		SR vs. AF 12 month
Rhythm at 12-month follow-up	Sinus rhythm 12-month FU	AF 12-month FU	Sinus rhythm 12-month FU	AF 12-month FU	p-value*
NT-proBNP (pg/ml)	377 [164 - 859]	750 [376 - 1351]	294 [127 - 700]	782 [437 - 1454]	0.001
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]	0.001
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]	0.010
FGF23 (pg/ml)	151 [112 - 209]	179 [125 - 238]	141 [108 - 197]	168 [129 - 226]	0.429
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]	0.218
GDF15 (pg/ml)	1304 [958 - 1934]	1441 [997 - 2008]	1254 [911 - 1782]	1589 [1071 - 2347]	0.461
IGFBP7 (ng/ml)	100 [89 - 114]	108 [93 - 126]	98.8 [88.5 - 110]	104 [94.7 - 119]	0.487
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]	0.417
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]	0.151
D-dimer (µg/ml)	0.17 [0.08 - 0.33]	0.19 [0.1 - 0.36]	0.16 [0.08 - 0.32]	0.16 [0.08 - 0.32]	0.638
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]	0.415
CRP (mg/l)	2 [0.95 - 4.65]	1.97 [0.9 - 4.63]	2.07 [0.93 - 4.37]	2.52 [1.12 - 4.87]	0.910
sCr (µmol/l)	81.3 [70 - 95]	83 [72.7 - 94.8]	79.5 [68.0 - 91.9]	84.4 [72 - 97.2]	0.541
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]	0.779

** 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 and randomization group. Values are shown as median [IQR].*

Abbreviations: Atrial fibrillation (AF), sinus rhythm (SR), follow-up (FU), angiopoietin 2 (ANGPT2), bone morphogenetic protein 10 (BMP10), cancer antigen 125 (CA125), C-reactive protein (CRP), D-dimer, endothelial specific molecule 1 (ESM1), fatty acid binding protein 3 (FABP3), fibroblast growth factor 23 (FGF23), growth differentiation factor 15 (GDF15), insulin-like growth factor binding protein 7 (IGFBP7), interleukin-6 (IL-6), N-terminal pro–B-type natriuretic peptide (NT-proBNP), cardiac troponin (TnT) and serum creatinine (sCr).

ACCEPTED MANUSCRIPT

Table 3: Baseline clinical characteristics used as confounders and biomarker concentrations in the derivation data set (EAST-AFNET 4 biomolecule study) at baseline by randomized group and by baseline rhythm. Rhythm at time of blood sampling was included as a fix factor in the analyses of outcome.

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%)	<0.001
Age, years	70 [65 - 75]	71 [67- 76]	71 [66 - 75]	72 [66 - 76]	0.035
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]	0.022
Blood pressure (diastolic) (mmHg)	80 [72, 87]	80 [76, 90]	80 [71, 89]	80 [76, 90]	<0.001
LVEF (%)	60 [57, 65]	59 [50, 64]	60 [59, 65]	60 [51, 64]	<0.001
AF type: First episode	172 (38%)	118 (34%)	155 (35%)	115 (33%)	
AF type: Paroxysmal	235 (52%)	67 (19%)	223 (51%)	65 (19%)	<0.001
AF type: Persistent or long-standing persistent	45 (10%)	163 (47%)	60 (14%)	168 (48%)	<0.001
Biomarker concentrations					
NT-proBNP (pg/ml)	228 [121 - 467]	890 [506 - 1496]	253 [124 - 504]	934 [529 - 1603]	<0.001
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]	<0.001
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]	<0.001
FGF23 (pg/ml)	139 [106 - 194]	178 [128 - 247]	140 [110 - 192]	170 [130 - 243]	0.003
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]	0.002
GDF15 (pg/ml)	1251 [938 - 1847]	1478 [1058 - 2188]	1259 [914 - 1761]	1585 [1065 - 2272]	<0.001
IGFBP7 (ng/ml)	99.0 [89.3 - 111.2]	106.8 [93.6 - 125]	99.2 [87.9 - 111]	105 [93.8 - 123]	<0.001
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]	0.041
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]	0.020
D-dimer (µg/ml)	0.17 [0.08 - 0.32]	0.18 [0.09 - 0.36]	0.15 [0.08 - 0.32]	0.18 [0.09 - 0.4]	0.267
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]	0.436
CRP (mg/l)	1.76 [0.87 - 4.29]	2.48 [1.09 - 5.78]	2.08 [0.93 - 4.52]	2.58 [1.26 - 5.03]	0.130

ACCEPTED MANUSCRIPT

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]	0.296
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]	0.052

** 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].*

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 or AF during the baseline visit.

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

ACCEPTED MANUSCRIPT

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

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

Abbreviations: Atrial Fibrillation (AF), angiopoietin 2 (ANGPT2), bone morphogenetic protein 10 (BMP10), N-terminal pro–B-type natriuretic peptide (NT-proBNP)

ACCEPTED MANUSCRIPT

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. Sinus rhythm at 12 months was initially predicted by a clinical risk score based on three validated clinical features (LA size >50mm, persistent AF, age >75 years) alone. This reference score was then combined with one, a combination of two or all three binarized predictive biomarkers (see Table 3 on 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). 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. By definition, there was no reclassification into low-risk groups. All numbers indicate number of patients with percentages of the predicted class in brackets.

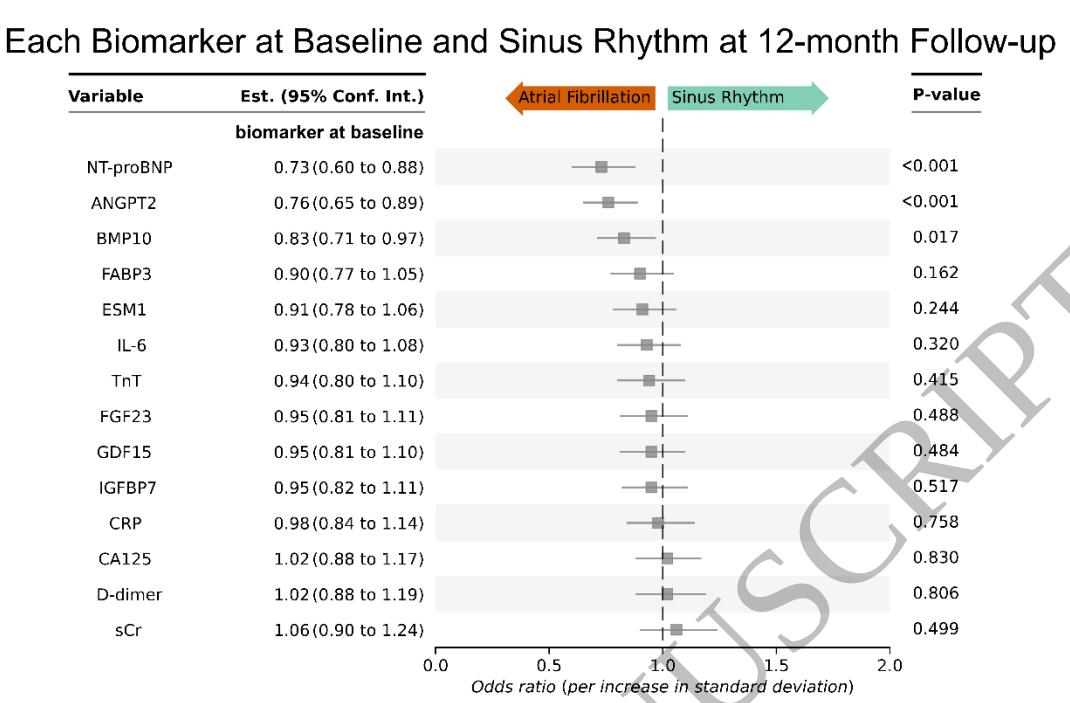
	Patients reclassified as high risk of not attaining Sinus Rhythm at 12M (N)	Confusion matrix			
		Predicted Sinus Rhythm (Actual patients in SR: N=1081)		Predicted: AF (Actual patients in AF: N=365)	
		Patients in Sinus Rhythm at 12M	Patients in AF at 12M	Patients in AF at 12M	Patients in Sinus Rhythm at 12M
Clinical model*	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 (36%)	279 (64%)
+ ANGPT2	301	650 (81%)	145 (19%)	198 (39%)	303 (61%)
+ NT-proBNP and BMP10	298	638 (80%)	158 (20%)	183 (36%)	315 (64%)
+ NT-proBNP and ANGPT2	345	625 (82%)	130 (18%)	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%)

Abbreviations: Atrial Fibrillation (AF), angiopoietin 2 (ANGPT2), bone morphogenetic protein 10 (BMP10), N-terminal pro–B-type natriuretic peptide (NT-proBNP), Sinus Rhythm (SR), 12-month Follow-up (12M)

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.

ACCEPTED MANUSCRIPT

A



B

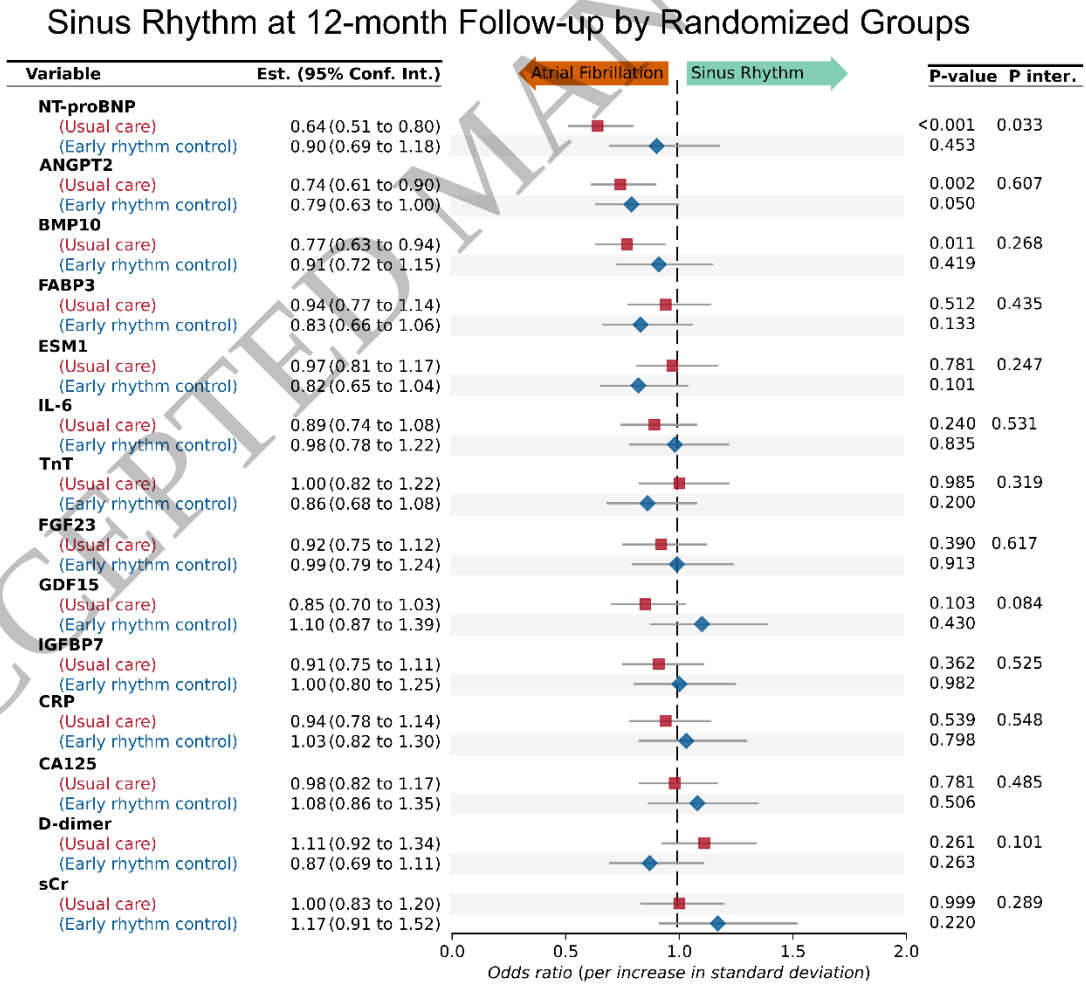


Figure 1
159x227 mm (x DPI)

ACCEPTED MANUSCRIPT

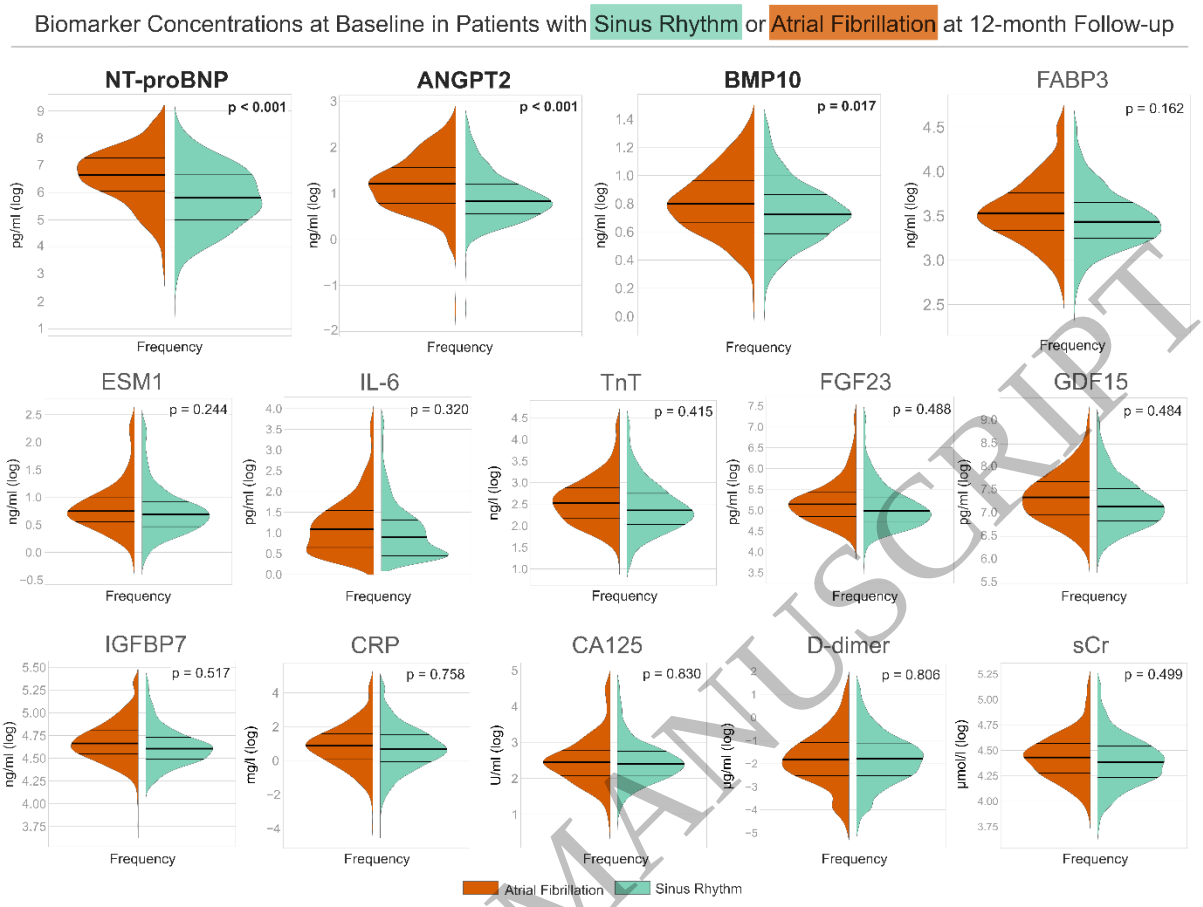


Figure 2
159x121 mm (x DPI)

ACCEPTED MANUSCRIPT

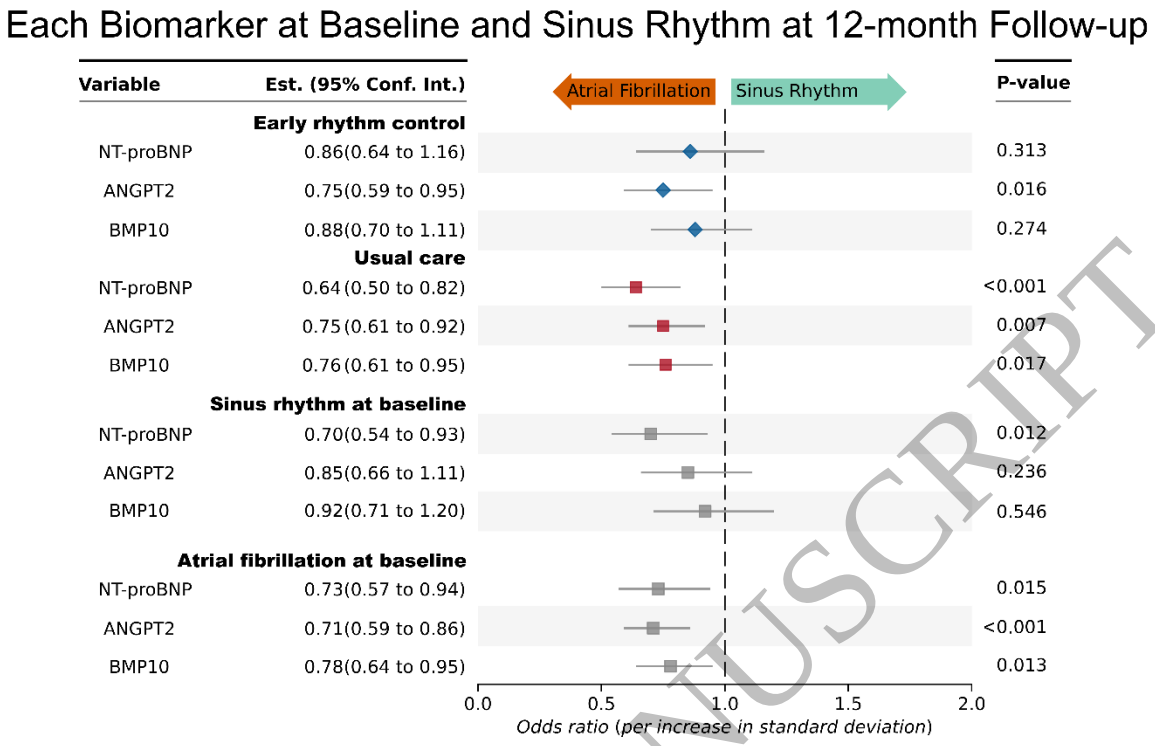


Figure 3
159x102 mm (x DPI)

ACCEPTED MANUSCRIPT

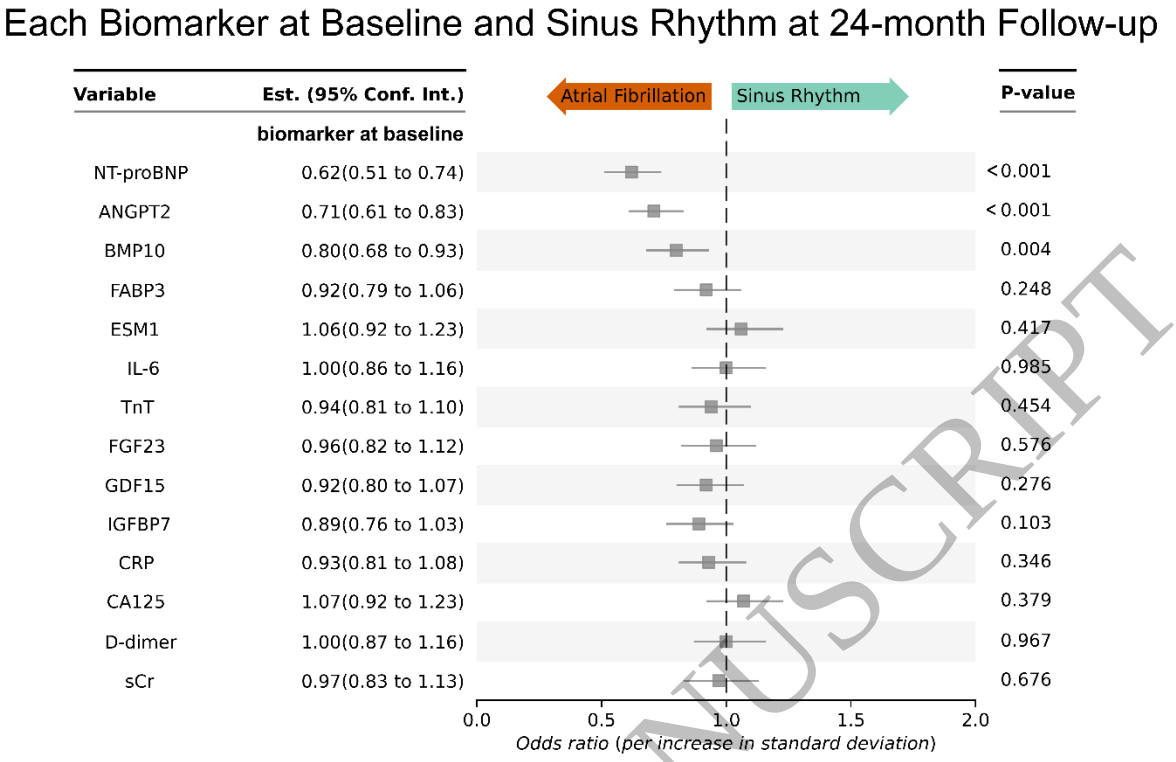


Figure 4
159x102 mm (x DPI)

ACCEPTED MANUSCRIPT

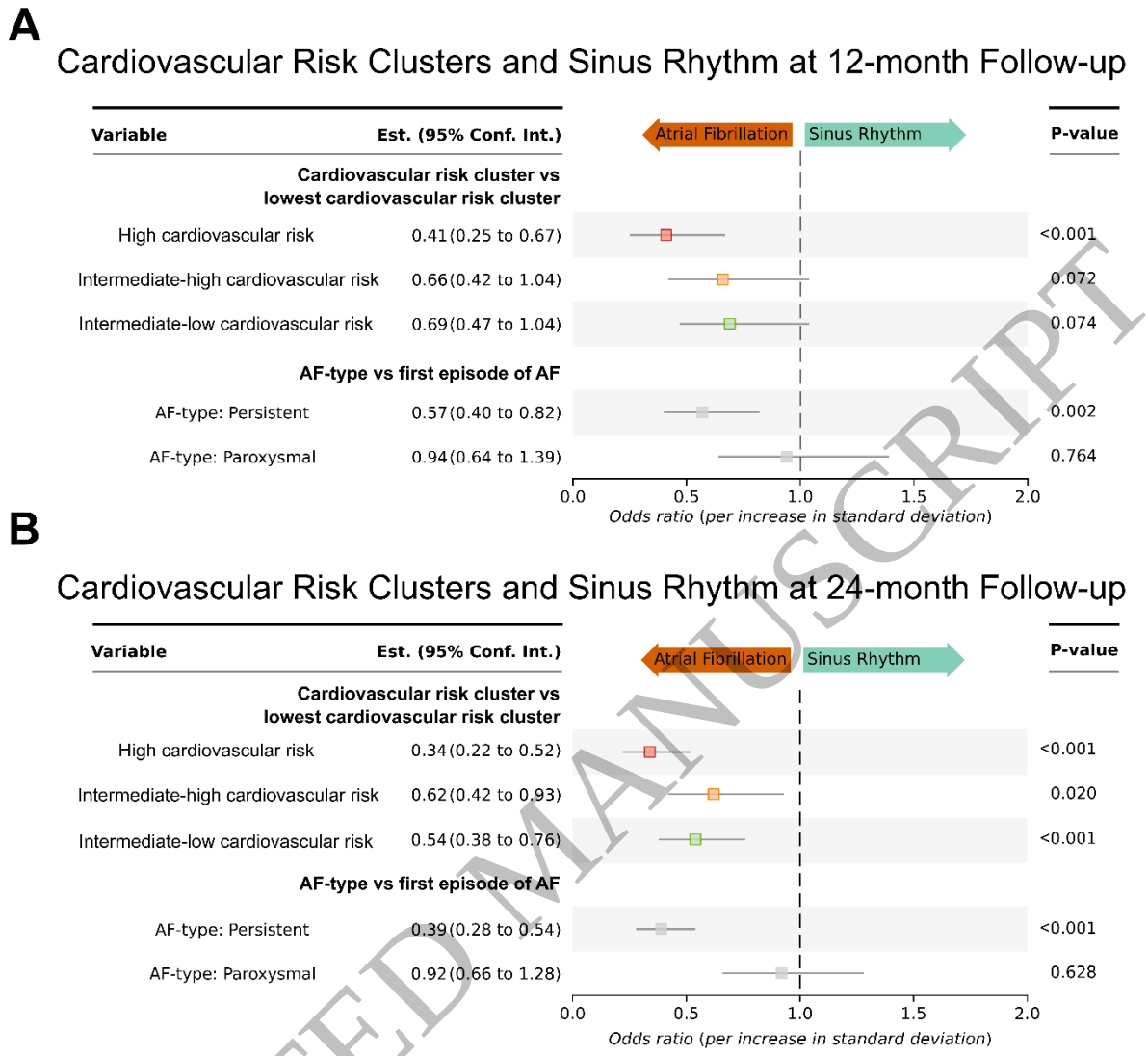


Figure 5
159x147 mm (x DPI)

ACCEPTED MANUSCRIPT

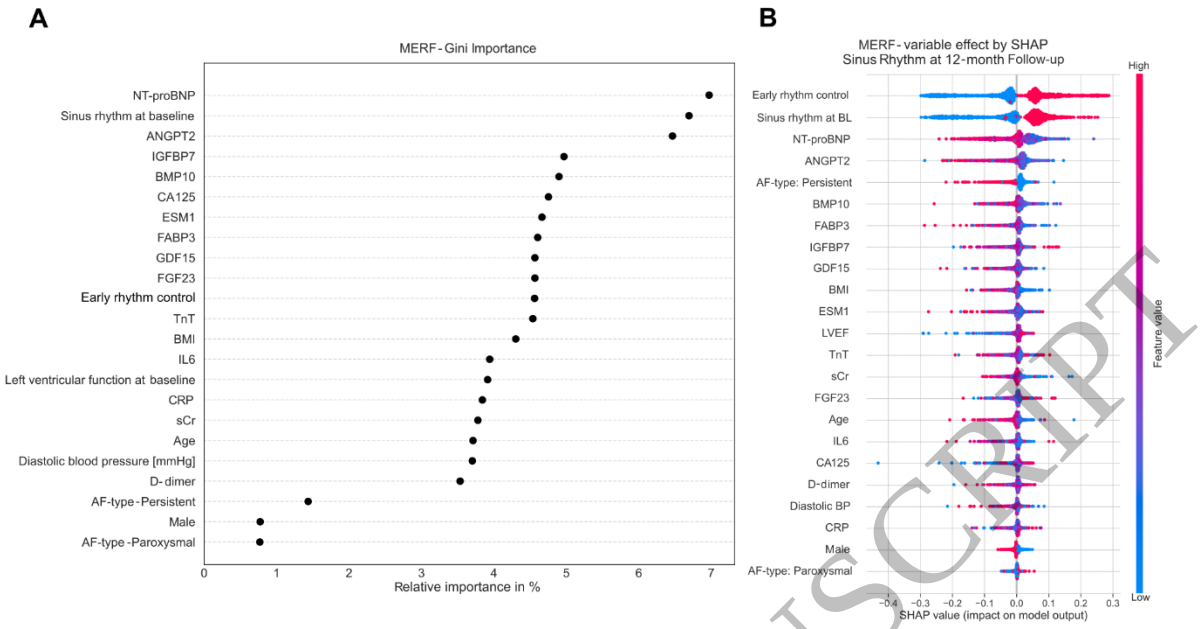


Figure 6
159x86 mm (x DPI)

ACCEPTED MANUSCRIPT

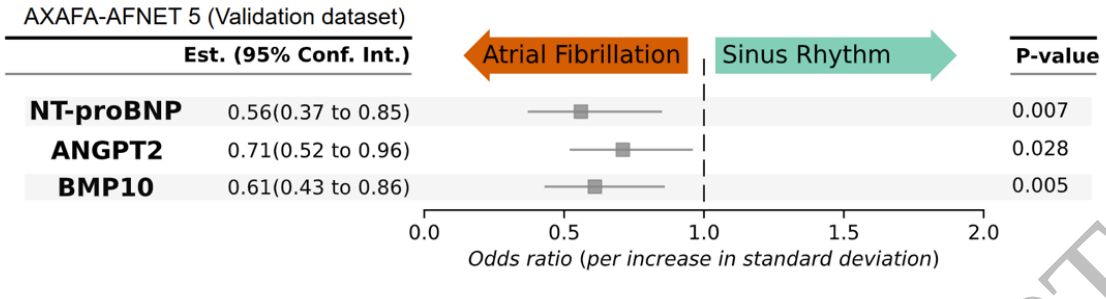


Figure 7
159x45 mm (x DPI)

ACCEPTED MANUSCRIPT

Structured Graphical Abstract

Key Question

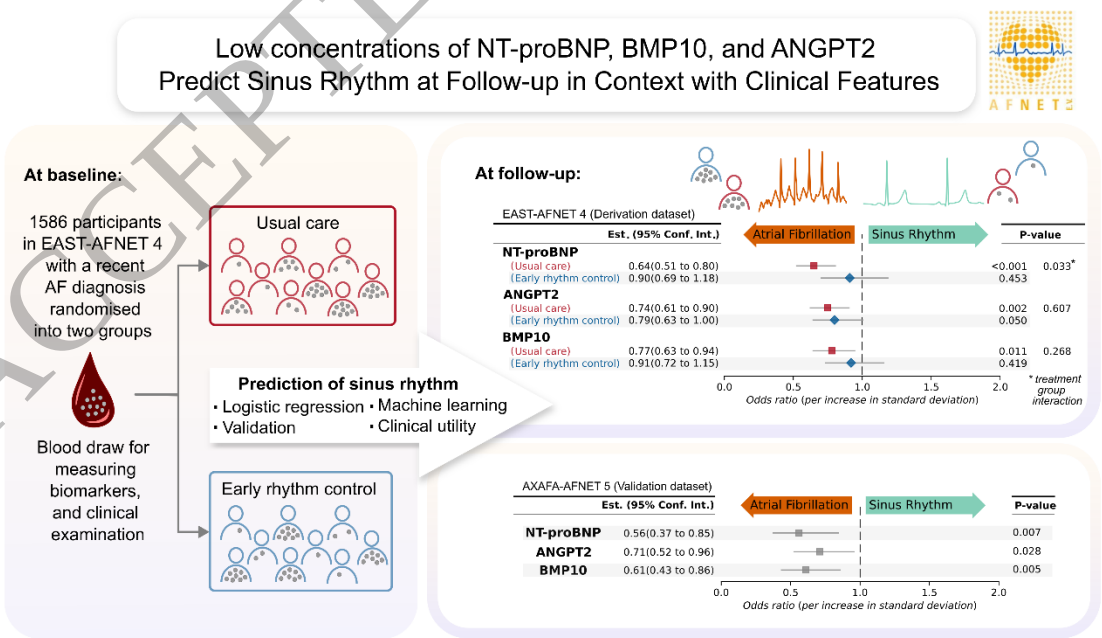
Can clinical features or circulating biomarkers measured at baseline predict sinus rhythm during mid-term follow-up in patients with atrial fibrillation (AF)? Which biomarkers interact with early rhythm control therapy?

Key Finding

Low baseline concentrations of angiopoietin 2 (ANGPT2), bone morphogenetic protein 10 (BMP10), and N-terminal pro-B-type natriuretic peptide (NT-proBNP) predicted sinus rhythm at follow-up in the EAST-AFNET 4 trial and in two external validation datasets. NT-proBNP had reduced predictive value in patients treated with early rhythm control. ANGPT2 and BMP10 added most information in patients who were in AF when blood samples were taken. The three biomarkers refined prediction of sinus rhythm compared to a clinical risk score.

Take Home Message

Low concentrations of NT-proBNP (<1000 pg/ml), ANGPT2 (<3.5 ng/ml) and BMP10 (<2 ng/ml) identified patients with a high chance of attaining sinus rhythm during follow-up when added to a clinical risk score. Combining NT-proBNP with ANGPT2 and BMP10 is particularly useful in patients in AF at the time of blood sampling and in patients on rhythm control.



ACCEPTED MANUSCRIPT