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CLINICAL RESEARCH

Disturbed atrial metabolism, shear stress, and cardiac load contribute to atrial fibrillation after ablation: AXAFA biomolecule study

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Aims Different disease processes can combine to cause atrial fibrillation (AF). Their contribution to recurrent AF after ablation in patients is not known. Cardiovascular processes associated with recurrent AF after AF ablation were determined by quantifying biomolecules related to inflammation, metabolism, proliferation, fibrosis, shear stress, atrial pressure, and others in the AXAFA biomolecule study.

Methods and results Twelve circulating cardiovascular biomolecules (ANGPT2, BMP10, CA125, hsCRP, ESM1, FABP3, FGF23, GDF15, IGFBP7, IL6, NT-proBNP, and hsTnT) were quantified in plasma samples obtained prior to a first AF ablation using high-throughput, high-precision assays. Cox regression was used to identify biomolecules associated with recurrent AF during the first 3 months after AF ablation. In 433 patients (64 years [58, 70]; 33% women), baseline concentrations of ANGPT2, BMP10, hsCRP, FGF23, FABP3, GDF15, and NT-proBNP were elevated in patients with recurrent AF (120/433; 28%). After adjustment for 11 clinical features and randomized treatment, elevated NT-proBNP [hazard ratio (HR) 1.58, 95% confidence interval (1.29, 1.94)], ANGPT2 [HR 1.37, (1.12, 1.67)], and BMP10 [HR 1.24 (1.02, 1.51)] remained associated with recurrent AF. Concentrations of ANGPT2, BMP10, and NT-proBNP decreased in patients who remained arrhythmia free, but not in patients with recurrent AF, highlighting their connection to AF. The other eight biomarkers showed unchanged concentrations.

Conclusion Elevated concentrations of ANGPT2, BMP10, and NT-proBNP are associated with recurrent AF after a first AF ablation, suggesting that processes linked to disturbed cardiomyocyte metabolism, altered atrial shear stress, and increased load contribute to AF after AF ablation in patients.

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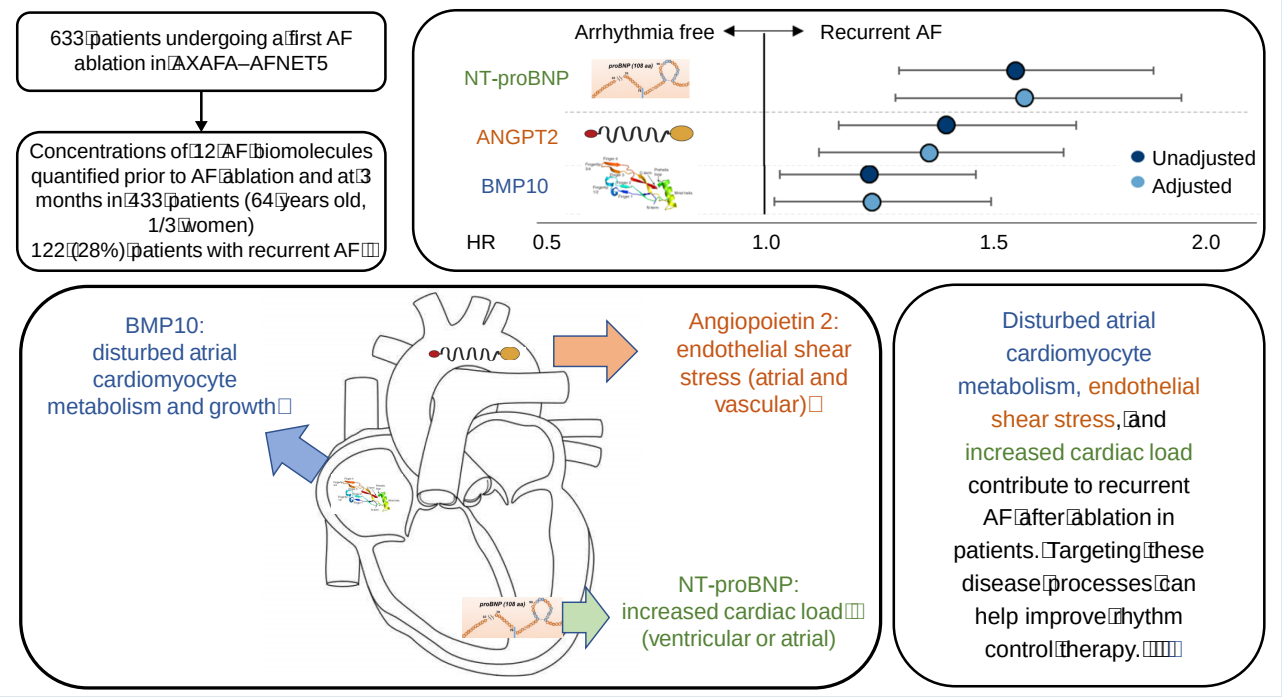
Graphical Abstract



Twelve biomolecules selected to reflect different disease processes were quantified in 433 patients undergoing a first atrial fibrillation (AF) ablation (64 years; 33% women) in the AXAFA–AFNET5 biomolecule study. Biomolecule concentrations were associated with recurrent AF after AF ablation. After adjustment for clinical features and randomized treatment, NT-proBNP [Hazard Ratio (HR) 1.58, 95% CI (1.29, 1.94)], angiopoietin 2 [ANGPT2, HR 1.37 (1.12, 1.67)], and bone morphogenetic protein 10 [BMP10, HR 1.24 (1.02, 1.51)] remained associated with recurrent AF. Concentrations of ANGPT2, BMP10, and NT-proBNP decreased in patients who remained arrhythmia free, but not in patients with recurrent AF. These results suggest that the disease processes leading to elevation of these biomolecules, including disturbed atrial cardiomyocyte metabolism, altered shear stress, and increased load, contribute to recurrent AF after ablation in patients.

Keywords Atrial fibrillation • Rhythm control • Ablation • Angiopoietin 2 • Bone morphogenetic protein 10 • N-Terminal pro-B-type natriuretic peptide

What's new?

- Three out of 12 biomolecules reflecting disease processes that are deemed important in atrial fibrillation (AF) are associated with recurrent AF after a first AF ablation.
- These biomolecules, bone morphogenetic protein 10, angiopoietin 2, and NT-proBNP, reflect different disease processes associated with AF.
- The same biomolecules are reduced in AF-free patients during follow-up but remain elevated in patients with recurrent AF, linking them to the arrhythmia.
- The findings shed light into processes leading to early recurrences after AF ablation that can help stratify therapy and risk prediction.

Introduction

Early rhythm control is emerging as an important component of atrial fibrillation (AF) therapy.[1–3] Atrial fibrillation ablation is the most effective rhythm-controlling therapy available.[4,5] But even with optimal techniques, 20–45% of patients experience recurrent AF in the first months after AF ablation.[6] These early recurrences are associated with later recurrences and with cardiovascular events.[7,8] The mechanisms leading to recurrent AF after ablation are not well understood,[9] and prediction of these events remains difficult in clinical practice.[6]

Several biological processes have been suggested to contribute to recurrent AF, including loss of atrial cardiomyocytes, atrial stretch, fibrosis, inflammation, metabolic imbalance, endothelial dysfunction, and altered cell proliferation.[9] Circulating biomolecules provide quantifiable proxies of cardiovascular disease processes. Their measurement can quantify disease processes leading to AF.[10] To quantify disease processes leading to recurrent AF after a first AF ablation, we measured the concentrations of 12 cardiovascular biomolecules reflecting disease processes that have been associated with AF and analysed their contribution to recurrent AF after a first AF ablation in context with clinical parameters.[6] Using repeat sampling, we also assessed changes in biomolecule concentrations associated with rhythm (AF or sinus rhythm) during follow-up.

Methods

This paper reports the first results from the biomolecule study embedded into the AXAFA–AFNET5 trial (*Anticoagulation using the direct factor Xa inhibitor apixaban during Atrial Fibrillation catheter Ablation: Comparison to vitamin K antagonist therapy*[11]). Briefly, it was an investigator-led, prospective, international, randomized, blinded outcome assessment study that compared the use of continuous vitamin K antagonist therapy to apixaban

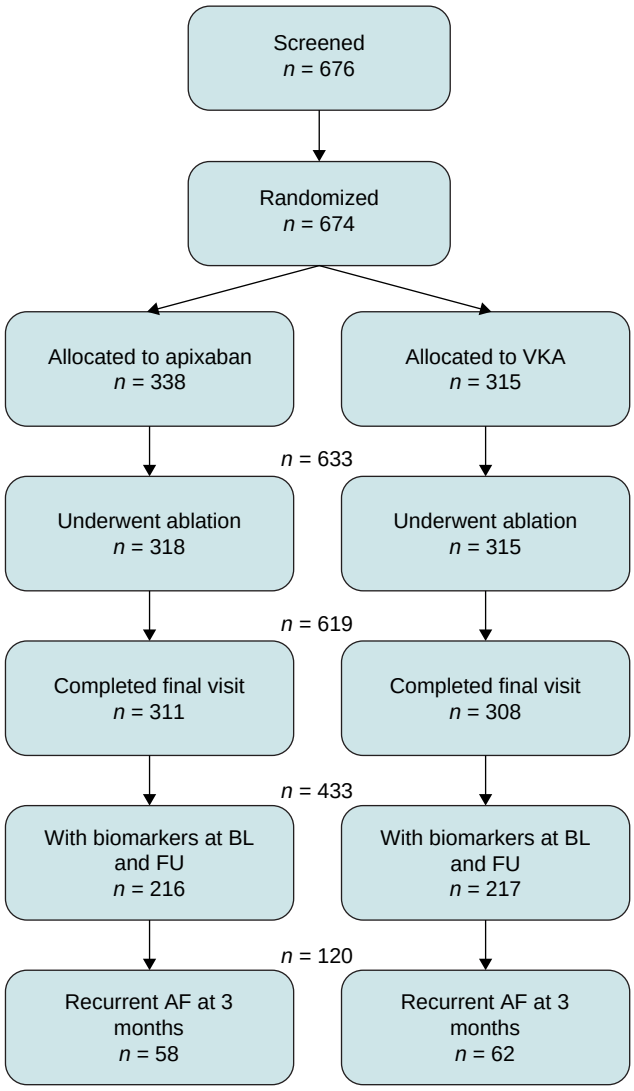


Figure 1 Flowchart of patients included in analysis. Patients without blood samples and quantified biomarkers at both time points (pre-ablation and 3 months post-ablation) were excluded. Apart from cohort descriptives, complete cases were used as there were no missing data for variables of interest. AF, atrial fibrillation; BL, baseline; FU follow-up; VKA vitamin K antagonist.

in 633 patients undergoing a first AF ablation in 49 European and US-American study sites. Outcomes were not different between randomized groups. The Atrial Fibrillation NETwork (AFNET e.V.), Münster, Germany (www.af-net.eu), was the sponsor of the trial and of its biosample substudy. All patients provided written informed consent for their participation.

Study population

AXAFA–AFNET5 enrolled patients undergoing a first AF ablation who had a prior stroke, age ≥75 years, heart failure, hypertension, or diabetes. The analysis population in this study included all patients in the trial who also consented to, and donated, blood samples at baseline and at the end of follow-up (*Figure 1*). Centres participating in the biomolecule study were encouraged to consecutively enrol all study patients into the biomolecule study as well.

Selection of biomolecules

Several mechanisms can contribute to recurrent AF.[9] The selection of biomolecules was conducted prior to the completion of AXAFA–AFNET5 as part of the CATCH ME consortium.[9] A modified Delphi process was conducted to select mechanisms that can contribute to AF. Biomolecules reflecting these disease processes were then selected via a modified three-stage Delphi process integrating a literature review and expert consensus within the partners of the CATCH ME consortium. Details of the process have been published.[10] The biomolecules included secreted biomolecules that can be quantified in peripheral blood reflecting cardiomyocyte loss (troponin), atrial stretch [N-terminal pro-B-type natriuretic peptide (NT-proBNP)], inflammation (C-reactive protein, interleukin 6), fibrosis (fibroblast growth factor 23), atrial metabolic dysfunction [bone morphogenetic protein 10 (BMP10), insulin-dependent growth factor–binding protein 7, and fatty acid–binding protein 3], cellular aging and proliferation (CA-125, GDF-15), and endothelial shear stress [angiopoietin 2 (ANGPT2), endothelial surface molecule 1].

Biomolecule quantification

After informed consent, blood samples were collected at the baseline visit and during the final in-person follow-up 3 months after ablation. ECG monitoring for recurrent AF was done during the entire follow-up time using ECG and

Table 1 Clinical characteristics of the cohort, stratified by rhythm outcome

	All patients $n = 433$	No recurrence $n = 313$	Recurrence $n = 120$	*P*-value
Clinical characteristics				
Sex (males), *n* (%)	290 (67.0%)	215 (68.7%)	75 (62.5%)	0.220
Age (years), median (Q1, Q3)	64 (58, 70)	64 (57, 70)	65 (60, 70)	0.134
Height (cm), mean (SD)	175.07 (9.705)	175.05 (9.709)	175.11 (9.734)	0.956
Body mass index (mg/kg^2), *median* (Q1, Q3)	28 (25, 31)	28 (25, 31)	29 (25, 32)	0.065
Heart rate (b.p.m.), median (Q1, Q3)	61 (53, 75)	60 (53, 70)	63 (52, 82)	0.210
Systolic blood pressure (mmHg), median (Q1, Q3)	138 (125, 150)	138 (125, 151)	137 (125, 150)	0.726
Diastolic blood pressure (mmHg), median (Q1, Q3)	81 (75, 90)	80 (75, 90)	84 (75, 92)	0.732
CHA_2D_2-VASc score, median (Q1, Q3)	2 (1, 3)	2 (1, 3)	3 (2, 3)	**<0.001**
CHA_2D_2-VASc score, *n* (%)				**0.010**
1	112 (25.9%)	91 (29.1%)	21 (17.5%)	
2	145 (33.5%)	112 (35.8%)	33 (27.5%)	
3	109 (25.2%)	70 (22.4%)	39 (32.5%)	
4	51 (11.8%)	30 (9.6%)	21 (17.5%)	
5	10 (2.3%)	7 (2.2%)	3 (2.5%)	
6	4 (0.9%)	2 (0.6%)	2 (1.7%)	
7	1 (0.2%)	0 (0.0%)	1 (0.8%)	
8	1 (0.2%)	1 (0.3%)	0 (0.0%)	
Randomization, *n* (%)				0.976
Apixaban (1)	216 (49.9%)	156 (49.8%)	60 (50.0%)	
VKA (2)	217 (50.1%)	157 (50.2%)	60 (50.0%)	
Medical history, *n* (%)				
Hypertension	392 (90.5%)	283 (90.4%)	109 (90.8%)	0.894
Diabetes mellitus	47 (10.9%)	29 (9.3%)	18 (15.0%)	0.086
Chronic obstructive lung disease	22 (5.1%)	18 (5.8%)	4 (3.3%)	0.305
Prior stroke or TIA	32 (7.4%)	19 (6.1%)	13 (10.8%)	0.090
Clinical history of major bleeding	8 (1.8%)	4 (1.3%)	4 (3.3%)	0.155
History of coronary artery disease				
Myocardial infarction	17 (3.9%)	10 (3.2%)	7 (5.8%)	0.206
Percutaneous coronary intervention	30 (6.9%)	17 (5.4%)	13 (10.8%)	0.048
Coronary artery bypass graft surgery	7 (1.6%)	7 (2.2%)	0 (0.0%)	0.099
Symptomatic heart failure (NYHA II–IV)	88 (20.3%)	59 (18.8%)	29 (24.2%)	0.218
NYHA I	34 (7.9%)	23 (7.3%)	11 (9.2%)	
NYHA II	76 (17.6%)	54 (17.3%)	22 (18.3%)	
NYHA III	12 (2.8%)	5 (1.6%)	7 (5.8%)	
NYHA IV	—	-	-	
Valvular heart disease	50 (11.5%)	37 (11.8%)	13 (10.8%)	0.773
AF-related symptoms and ablation parameters, *n* (%)				
AF pattern				0.095
Paroxysmal	269 (62.1%)	202 (64.5%)	67 (55.8%)	
Persistent or long-standing persistent	164 (37.9%)	111 (35.5%)	53 (44.2%)	
Modified EHRA scale				0.880
mEHRA I	34 (7.9%)	24 (7.7%)	10 (8.3%)	
mEHRA IIa	118 (27.3%)	89 (28.4%)	29 (24.2%)	
mEHRA IIb	140 (32.3%)	101 (32.3%)	39 (32.5%)	
mEHRA III	130 (30.0%)	92 (29.4%)	38 (31.7%)	
mEHRA IV	11 (2.5%)	7 (2.2%)	4 (3.3%)	

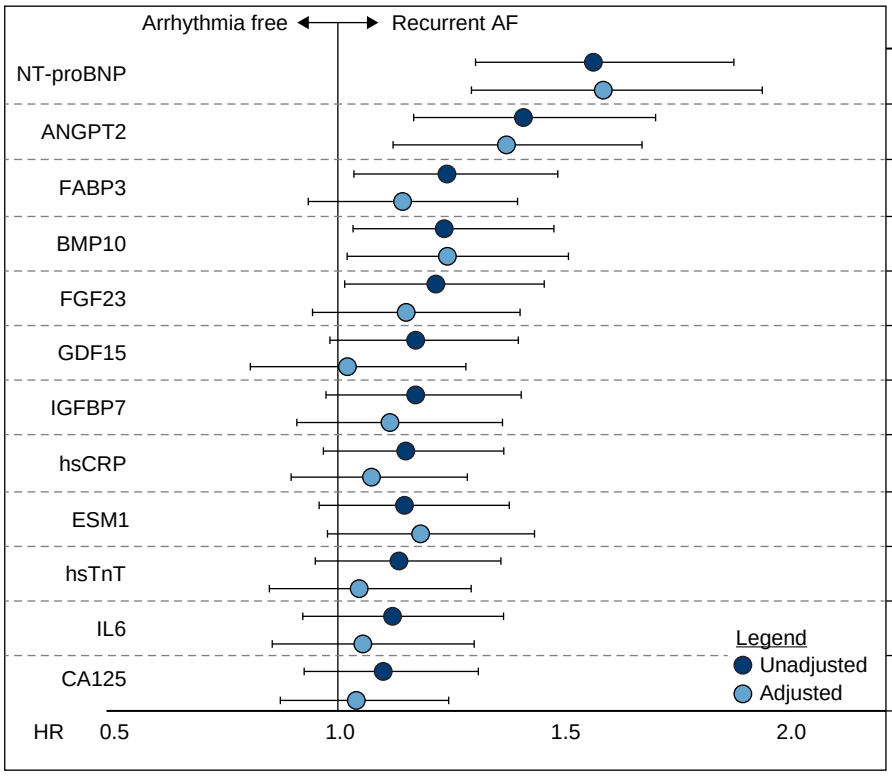
Continued

Table 1 ***Continued***

	All patients $n = 433$	No recurrence $n = 313$	Recurrence $n = 120$	*P*-value
Ablation type				0.513
PVI	398 (91.9%)	285 (91.1%)	113 (94.2%)	
PVI + other	1 (0.2%)	1 (0.3%)	0 (0.0%)	
Other	34 (7.9%)	27 (8.6%)	7 (5.8%)	
Ablation energy				0.243
Radiofrequency	266 (61.4%)	189 (60.4%)	77 (64.2%)	
Cryoablation	134 (30.9%)	96 (30.7%)	38 (31.7%)	
Other	33 (7.6%)	28 (8.9%)	5 (4.2%)	
Cardioversion during ablation				**<0.001**
0	301 (69.5%)	238 (76.0%)	63 (52.5%)	
1	104 (24.0%)	66 (21.1%)	38 (31.7%)	
2	19 (4.4%)	9 (2.9%)	10 (8.3%)	
3	4 (0.9%)	0 (0.0%)	4 (3.3%)	
4	5 (1.2%)	0 (0.0%)	5 (4.2%)	
Biomarkers (pre-ablation), median (Q1, Q3)				
ANGPT2 (ng/mL)	2.166 (1.700, 2.988)	2.039 (1.658, 2.779)	2.456 (1.824, 3.550)	**<0.001**
BMP10 (ng/mL)	2.053 (1.804, 2.367)	2.031 (1.769, 2.301)	2.146 (1.900, 2.434)	**0.013**
CA125 (per 10 U/mL)	11.450 (8.255, 16.240)	11.330 (8.070, 15.700)	11.645 (8.625, 16.760)	0.138
hsCRP (mg/L)	1.640 (0.660, 3.235)	1.540 (0.620, 3.100)	1.985 (1.045, 3.410)	**0.030**
ESM1 (ng/mL)	1.864 (1.507, 2.213)	1.857 (1.494, 2.151)	1.871 (1.554, 2.268)	0.395
FGF23 (per 100 pg/mL)	153.830 (123.550, 200.380)	149.040 (120.580, 193.180)	169.610 (134.065, 231.225)	**0.003**
FABP3 (per 10 ng/mL)	28.943 (23.956, 34.889)	28.490 (22.953, 34.163)	30.035 (25.595, 35.963)	**0.028**
GDF15 (per 100 pg/mL)	1079.000 (793.400, 1503.00)	1060.000 (761.200, 1477.000)	1161.500 (860.45, 1514.000)	**0.042**
IGFBP7 (ng/mL)	96.646 (86.938, 107.830)	96.427 (86.531, 106.780)	97.834 (87.912, 112.460)	0.121
IL6 (pg/mL)	1.660 (1.500, 2.800)	1.550 (1.500, 2.710)	1.825 (1.500, 3.320)	0.120
NT-proBNP (pg/mL)	219.00 (85.730, 574.100)	168.00 (72.765, 361.450)	458.200 (150.800, 770.725)	**<0.001**
hsTnT (per 100 pg/mL)	8.840 (6.495, 11.930)	8.750 (6.360, 11.850)	9.515 (6.852, 12.587)	0.113
Biomarkers (post-ablation), median (Q1, Q3)				
ANG2 (ng/mL)	1.830 (1.460, 2.300)	1.720 (1.430, 2.180)	2.100 (1.580, 3.063)	**<0.001**
BMP10 (ng/mL)	1.990 (1.760, 2.260)	1.940 (1.740, 2.180)	2.130 (1.903, 2.418)	**<0.001**
CA125 (U/mL)	11.840 (8.420, 16.575)	11.620 (8.315, 16.510)	12.050 (8.970, 17.120)	0.285
hsCRP (mg/L)	1.540 (0.675, 2.950)	1.480 (0.610, 3.050)	1.615 (0.890, 2.580)	0.339
ESM1 (ng/mL)	1.830 (1.525, 2.230)	1.790 (1.500, 2.205)	1.915 (1.610, 2.358)	0.063
FGF23 (pg/mL)	150.930 (123.145, 189.160)	145.350 (120.510, 178.600)	163.730 (132.820, 227.870)	**0.001**
FABP3 (ng/mL)	29.110 (24.615, 35.195)	28.920 (23.915, 35.195)	29.225 (25.875, 35.328)	0.130
GDF15 (pg/mL)	1080.000 (782.400, 1500.500)	1041.000 (725.950, 1442.500)	1161.500 (864.075, 1661.500)	**0.009**
IGFBP7 (ng/mL)	95.490 (86.730, 108.790)	94.730 (86.430, 106.485)	96.260 (87.868, 113.578)	**0.026**
IL6 (pg/mL)	1.610 (1.500, 2.675)	1.600 (1.500, 2.720)	1.625 (1.500, 2.560)	0.779
NT-proBNP (pg/mL)	124.800 (67.340, 268.675)	105.200 (60.150, 209.800)	233.200 (108.025, 599.325)	**<0.001**
TnT (pg/mL)	8.690 (6.330, 11.685)	8.530 (6.075, 11.470)	9.530 (7.450, 12.693)	**0.008**

Categorical variables are reported as *n* (%), and continuous variables are reported as mean (standard deviation) or median (quartile 1, quartile 3) for skewed distributions. The independent *t*-test (or Mann–Whitney *U* test for skewed distributions) and χ^2 tests were used to compare characteristics between patients. Italicized clinical characteristics were included in the multivariate analysis for predictors of recurrent AF.

ANGPT2, angiopoietin 2; BMI, body mass index; BMP10, bone morphogenetic protein 10; CA125, cancer antigen 125; ECV, electrical cardioversion; ESM1, endothelial cell-specific molecule 1; FABP3, fatty acid–binding protein 3; FGF23, fibroblast growth factor 23; GDF15, growth differentiation factor 15; hsCRP, high-sensitivity C-reactive protein; IGFBP7, insulin-like growth factor–binding protein 7; IL6, interleukin 6; NT-proBNP, N-terminal pro-B-type natriuretic peptide; hsTnT, high-sensitivity cardiac troponin T; TIA, transient ischaemic attack.



Biomarkers	HR	95% CI	
NT-proBNP	1.564	1.303	1.876
	1.584	1.294	1.938
ANGPT2	1.409	1.167	1.701
	1.370	1.121	1.673
FABP3	1.238	1.033	1.484
	1.141	0.932	1.396
BMP10	1.234	1.032	1.475
	1.241	1.020	1.509
FGF23	1.214	1.014	1.454
	1.149	0.943	1.400
GDF15	1.170	0.980	1.397
	1.016	0.806	1.281
IGFBP7	1.168	0.971	1.405
	1.111	0.907	1.361
hsCRP	1.149	0.967	1.365
	1.072	0.895	1.285
ESM1	1.147	0.956	1.377
	1.182	0.975	1.433
hsTnT	1.135	0.947	1.360
	1.046	0.846	1.295
IL6	1.120	0.920	1.364
	1.053	0.853	1.300
CA125	1.100	0.924	0.309
	1.040	0.872	1.242

Figure 2 ANGPT2, BMP10, and NT-proBNP predict recurrent AF. These three biomarkers were significantly predictive of AF post-ablation after adjustment for randomized treatment and 11 clinical parameters (age, sex, BMI, hypertension, diabetes, chronic obstructive pulmonary disorder, stroke, heart failure, ablation type, ablation energy, and cardioversion during ablation). Unadjusted FGF23 was also predictive of recurrent AF; however, this effect was not present after adjustment. Hazard ratios and corresponding 95% confidence intervals calculated with rank normalized Blom transformed biomarkers using Cox regression. AF, atrial fibrillation; ANGPT2, angiopoietin 2; BMP10, bone morphogenetic protein 10; CA125, cancer antigen 125; ESM1, endothelial cell-specific molecule 1; FABP3, fatty acid–binding protein 3; FGF23, fibroblast growth factor 23; GDF15, growth differentiation factor 15; hsCRP, high-sensitivity C-reactive protein; hsTnT, high-sensitivity cardiac troponin T; IGFBP7, insulin-like growth factor–binding protein 7; IL6, interleukin 6; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

completed using a Holter ECG recorded at the same visit as the follow-up blood sample. Blood samples were shipped from the sites using courier services at ambient temperature. Courier services were available 7 days a week, and transport time from the site to the central biosample storage facility at the University Heart and Vascular Center Hamburg was around 2 days. Upon arrival at the central facility, all samples were spun, frozen, fractionated, and stored at −80°C. Biomolecule concentrations were centrally quantified in EDTA plasma. Commercially available immunoassays were used to quantify six proteins: CA125, GDF-15, IL-6, NT-proBNP, troponin T, C-reactive protein, GDF-15, IL-6, NT-proBNP II, and high-sensitivity troponin T (cobas c 501 for high-sensitivity C-reactive protein; cobas Elecsys® Roche Diagnostics, Mannheim, Germany). Pre-commercial, high-throughput, high-precision sandwich immunoassays developed using monoclonal antibodies were used to quantify ANGPT2, BMP10, ESM1, FABP3, FGF23, and IGFBP7 (Roche Diagnostics, Mannheim, Germany). Details of the method have been published.[12] Run controls and calibrators were measured twice each run, and staff involved were blinded to clinical status and outcomes.

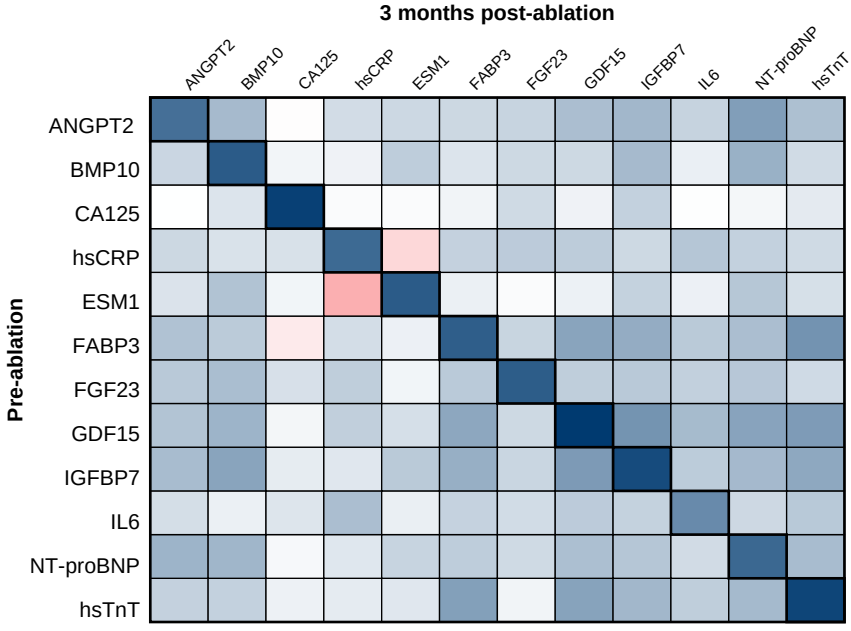
Study outcomes

The primary outcome of this analysis was recurrent AF during follow-up after ablation. Recurrent AF was detected using site reports of symptomatic and clinical recurrences, ECGs recorded at each in-person visit, and a 24-h ECG recorded at the 3-month visit. As a secondary outcome, the change in biomarker concentrations at follow-up was also evaluated, again split between patients with and without recurrent AF.

Statistical analysis

Descriptive statistics for continuous variables were summarized as means (standard deviations), medians (25th, 75th percentiles), or counts (percentages). Continuous variables were compared using Student's *t*-test or Mann–Whitney *U* test after checking for normality using the Kolmogorov–Smirnov test. Categorical variables were compared using Pearson's χ^2 test. Corrections for multiple testing were not applied. Blood biomolecule concentrations were analysed as original values in univariate analyses. For multivariate analyses, concentrations were rank normalized by Blom transform and included as continuous parameters. Associations between biomolecule concentrations and outcomes were computed for each biomolecule on its own. Multivariate models were constructed including all biomolecules and all biomolecules together with clinical characteristics. Clinical features associated with recurrent AF were extracted from a recent meta-analysis on this topic.[6] We also assessed how well the CHA_2DS_2-VASc score at baseline was predictive of the outcomes of interest. Furthermore, we compared concentrations of the biomolecules quantified at the end of follow-up between patients with and without recurrent AF to detect AF-related changes. As the intervention did not affect other clinical conditions, changes between baseline and follow-up concentrations were attributed to the AF ablation. To assess whether these changes were related to recurrent AF, changes in biomolecule concentrations were compared between patients with and without recurrent AF. To characterize pre- and post-ablation concentration changes, Spearman's rank correlation and the paired *t*-test or the Wilcoxon signed-rank test was used. Change values (pre-ablation values

A



B

	ANGPT2	BMP10	CA125	hsCRP	ESM1	FABP3	FGF23	GDF15	IGFBP7	IL6	NT-proBNP	hsTnT
ANGPT2	0.653**	0.313**	–0.002	0.160*	0.180**	0.181**	0.199**	0.295**	0.327**	0.201**	0.441**	0.288**
BMP10	0.187**	0.745**	0.048	0.059	0.228**	0.123*	0.176**	0.221**	0.313**	0.074	0.357**	0.166**
CA125	0.004	0.123*	0.866**	0.02	0.019	0.051	0.170**	0.059	0.21**	0.008	0.039	0.096*
hsCRP	0.181**	0.133**	0.139*	0.679**	–0.051	0.209**	0.236**	0.232**	0.176**	0.260**	0.210**	0.169**
ESM1	0.129*	0.276**	0.049	–0.107*	0.774**	0.070	0.019	0.067	0.208**	0.068	0.257**	0.144*
FABP3	0.279**	0.238**	–0.027	0.154*	0.068	0.730**	0.194**	0.414**	0.376**	0.244**	0.296**	0.492**
FGF23	0.248**	0.298**	0.143*	0.226**	0.049	0.243**	0.737**	0.226**	0.245**	0.216**	0.254**	0.168**
GDF15	0.270**	0.343**	0.045	0.219**	0.147*	0.402**	0.176**	0.892**	0.488**	0.312**	0.417**	0.454**
IGFBP7	0.305**	0.413**	0.090	0.111*	0.244**	0.364**	0.190**	0.458**	0.816**	0.235**	0.317**	0.397**
IL6	0.152*	0.070	0.122*	0.296**	0.076	0.204**	0.162*	0.237**	0.208**	0.530**	0.177*	0.249**
NT-proBNP	0.343**	0.335**	0.033	0.112*	0.199**	0.229**	0.17**	0.292**	0.26**	0.164*	0.687**	0.305**
hsTnT	0.209**	0.211**	0.066	0.092	0.111*	0.434**	0.052	0.42**	0.327**	0.225**	0.316**	0.845**

*$P < 0.05$ **$P < 0.001$

Figure 3 Stability of biomolecule concentrations over time. Pre-ablation biomarker concentrations were highly correlated to concentrations of the same biomolecules quantified 3 months after AF ablation, although to varying degrees (*A*, *B*), demonstrating the dynamic nature of biomarker measurements post-procedure. Correlations were calculated using original biomarker values with Spearman's rank correlation. Colour coding (A): Blue indicates positive correlations, pink inverse correlations. Darker shades indicate stronger correlations, lighter shades indicated weaker correlations. ANGPT2, angiopoietin 2; BMP10, bone morphogenetic protein 10; CA125, cancer antigen 125; ESM1, endothelial cell-specific molecule 1; FABP3, fatty acid–binding protein 3; FGF23, fibroblast growth factor 23; GDF15, growth differentiation factor 15; hsCRP, high-sensitivity C-reactive protein; hsTnT, high-sensitivity cardiac troponin T; IGFBP7, insulin-like growth factor–binding protein 7; IL6, interleukin 6; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

subtracted from post-ablation) were calculated, and differences were determined using one-sample *t*-tests (difference from 0).

All analyses were completed using IBM SPSS (version 25 and higher, IBM Corporation, USA).

Results

Patient characteristics

Of the 633 patients undergoing a first AF ablation in AXAFA–AFNET5,[11] blood samples were available and analysable in 433 patients (*Figure 1*). The characteristics of these patients were similar to the overall cohort (see Supplementary material online, *Table S1*). In brief, the median age was 64 years [interquartile range (58, 70)], 120 (33%) were female, median BMI was 28 (25, 31), and average CHA_2DS_2-VASc score was 2 [median 2 (1, 3)]. Concentrations of all biomolecules were quantified in all patients. Recurrent AF was detected in 120/433 patients (28%) after AF ablation (*Table 1*). Clinical factors associated with recurrent AF were few and included a higher CHA_2DS_2-VASc score, presence of AF at the baseline visit, and intraprocedural cardioversion (*Table 1*).

Table 2 Changes in biomarker concentrations pre- and 3 months post-ablation

Biomarker	Difference, median [Q1, Q3]	*P*-value (Wilcoxon signed rank)
ANGPT2 (ng/mL)	−0.236 [−0.701, 0.025]	**<0.001**
BMP10 (ng/mL)	−0.065 [−0.265, 0.123]	**<0.001**
CA125 (U/L)	0.000 [−1.240, 1.220]	0.797
hsCRP (mg/L)	−0.060 [−0.735, 0.500]	0.078
ESM1 (ng/mL)	0.041 [−0.207, 0.221]	0.488
FABP3 (ng/mL)	0.639 [−2.885, 4.513]	**0.003**
FGF23 (pg/mL)	1.200 [−28.045, 24.825]	0.801
GDF15 (pg/mL)	−1.900 [−125.500, 105.950]	0.703
IGFBP7 (ng/mL)	0.390 [−5.937, 6.252]	0.970
IL6 (pg/mL)	0.000 [−0.375, 0.335]	0.790
NT-proBNP (pg/mL)	−28.890 [−230.775, 22.550]	**<0.001**
hsTnT (pg/mL)	−0.130 [−1.410, 1.180]	0.205

Negative values denote a reduction from baseline to follow-up whereas positive values denote an increase. Eight biomarker levels remained relatively unchanged whereas ANGPT2, BMP10, NT-proBNP, and FABP3 changed significantly at follow-up. ANGPT2, BMP10, and NT-proBNP decreased whereas there was an increase in FABP3. Bold values indicate significant changes to baseline.

ANGPT2, angiopoietin 2; BMP10, bone morphogenetic protein 10; CA125, cancer antigen 125; ESM1, endothelial cell-specific molecule 1; FGF23, fibroblast growth factor 23; FABP3, fatty acid–binding protein 3; GDF15, growth differentiation factor 15; hsCRP, high-sensitivity C-reactive protein; hsTnT, high-sensitivity cardiac troponin T; IGFBP7, insulin-like growth factor–binding protein 7; IL6, interleukin 6; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

Three biomolecules reflecting different disease processes are associated with recurrent atrial fibrillation after a first atrial fibrillation ablation

Concentrations of ANGPT2, BMP10, hsCRP, FGF23, FABP3, GDF15, and NT-proBNP were elevated in patients with recurrent AF (*Table 1*). As expected from a prior meta-analysis,[6] clinical risk scores such as the CHA_2DS_2-VASc score were predictive of recurrent AF [CHA_2DS_2-VASc score hazard ratio (HR) 1.288 (95% CI 1.128, 1.472)]. After adjustment for 11 clinical features (age, sex, BMI, hypertension, diabetes, coronary artery disease, chronic obstructive pulmonary disease, stroke, heart failure, ablation type, and ablation energy, highlighted in bold in *Table 1*) and randomized group, elevated NT-proBNP {HR 1.584 [95% confidence intervals (CIs) 1.294, 1.938]}, ANGPT2 [HR 1.370 (95% CI 1.121, 1.673)], and BMP10 [HR 1.241 (95% CI 1.020, 1.509)] remained significantly predictive of recurrent AF (*Figure 2*).

Changes in biomolecule concentrations by rhythm outcome at follow-up

Pre- and post-ablation biomolecule concentrations were highly correlated (*Figure 3*, each $P < 0.001$, Spearman correlation coefficients 0.5–0.9). Concentrations of eight biomolecules remained unchanged between baseline and follow-up, irrespective of recurrent AF status (*Table 2*). The concentrations of ANGPT2, BMP10, and NT-proBNP decreased at the follow-up visit compared to baseline, whereas FABP3 increased. Changes in biomolecule concentrations were driven by patients who were arrhythmia free without relevant changes in patients who had recurrent AF (*Figure 4*). Comorbidities and age, summarised in the CHADSVASc score, only showed weak associations with recurrent AF and with biomolecule concentrations (see Supplementary material online, *Figure S1*). Our findings are summarized in *Figure 5*.

Discussion

Main findings

This hypothesis-generating analysis of the plasma of over 400 patients undergoing a first AF ablation in seven European countries and the USA identified three circulating biomolecules that predict recurrent AF after ablation: BMP10, ANGPT2, and NT-proBNP. Three of the key disease processes associated with these biomolecules are (*Figure 5*): disturbed atrial metabolism and proliferation (BMP10), endothelial shear stress (ANGPT2), and cardiac load (NT-proBNP). The same biomolecules showed lower concentrations 3 months after successful AF ablation, underpinning that their blood concentrations are related to AF. These biomolecules predicted recurrent AF in context with known clinical parameters that are associated with recurrent AF after AF ablation.[6,13] They also highlight mechanisms contributing to stroke and to periprocedural brain lesions in patients undergoing a first AF ablation.[14,15]

NT-proBNP is an inactive polypeptide that is released by cardiomyocytes in response to myocardial stretch. BNP and NT-proBNP are used in clinical routine to rule out heart failure. NT-proBNP is also an established biomarker for AF.[16] Elevated concentrations of NT-proBNP predict incident and prevalent AF,[17–19] recurrent AF post-ablation,[20] and recurrent AF after cardioversion.[21] In addition, midregional pro-ANP has been suggested as a biomarker for undiagnosed AF in patients with stroke.[22] Our analysis confirms this association and identifies two additional biomolecules associated with AF after AF ablation.

ANGPT2 is a growth factor belonging to the angiopoietin/Tie signalling pathway, with roles in angiogenesis and vascular regression, increasing permeability.[23] One study in patients with cryptogenic stroke suggested that elevated ANGPT2 concentrations are associated with undiagnosed AF.[24] To our knowledge, this is the first time ANGPT2 has been demonstrated to be predictive of recurrent AF after ablation. In patients with AF, ANGPT2 and the ANGPT2-regulated VEGF were elevated.[25] Changes in endothelial shear stress and lack of regular pulsatile flow were postulated as contributing factors to atrial endothelial injury, increasing ANGPT2 release in atrial endothelium and activating coagulation in atria in AF. Our data suggest that ANGPT2 could also regulate atrial cardiac cells. This calls for research into endothelial–myocardial interactions in the atria. Restoration of pulsatile, regular blood flow after successful AF ablation can reduce shear stress and endothelial injury. These effects can explain reduced ANGPT2 concentrations in patients without recurrent AF after ablation.

BMP10 is a polypeptide belonging to the TGF-β superfamily. BMP10 mutations have been associated with cardiovascular disease.[26] While BMP10 has an overarching role in cardiovascular development, it is selectively secreted by atrial cardiomyocytes in the adult heart. Elevated BMP10 blood concentrations were associated with reduced left atrial PITX2 and with recurrent AF after ablation in another study.[27] A similar elevation was found in patients with recurrent AF after a cardioversion.[21] Our results confirm the association of BMP10 and recurrent AF. BMP10 is uniquely expressed and secreted by atrial cardiomyocytes[28] and, therefore, is a promising atrial-specific circulating

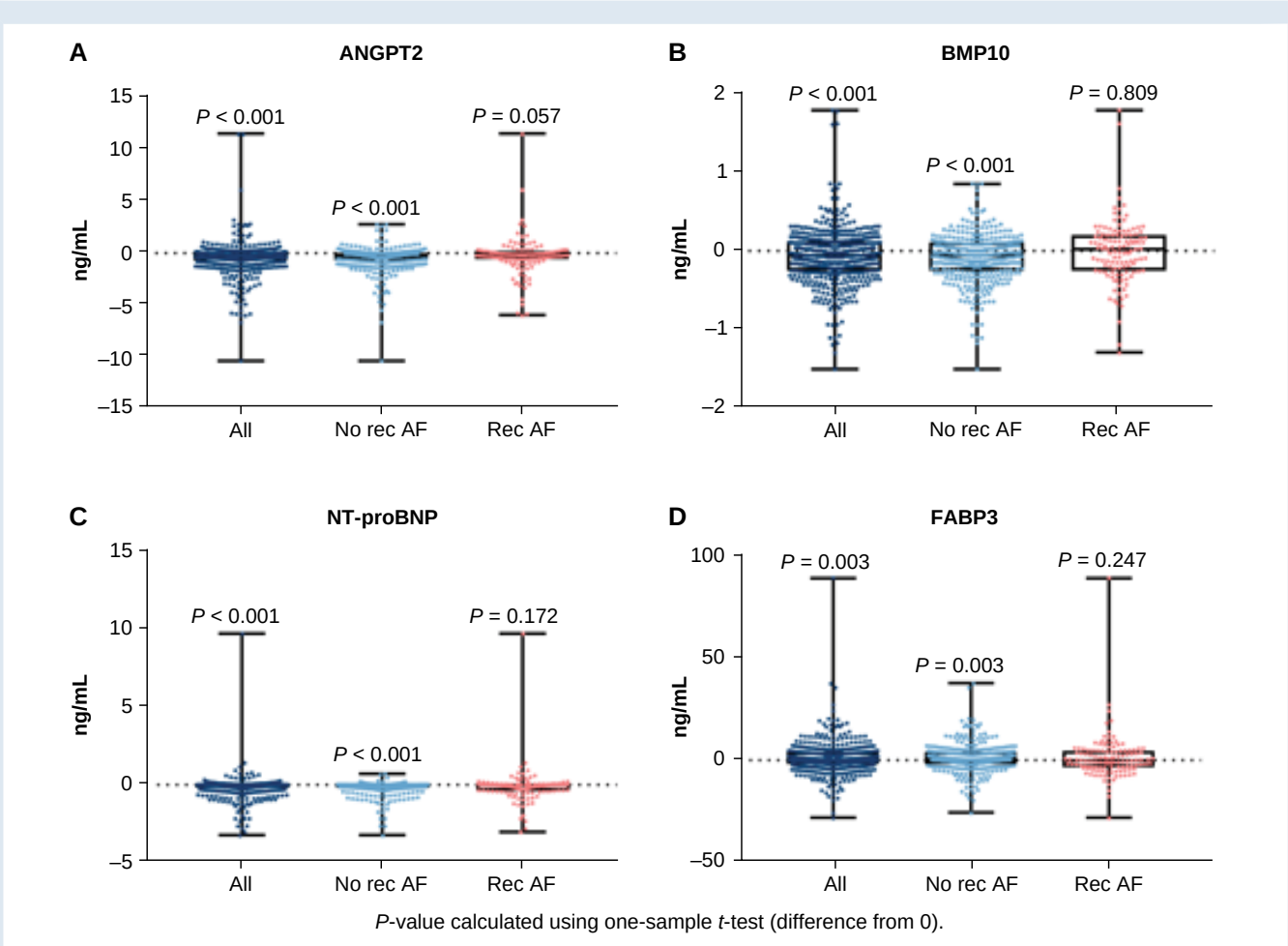


Figure 4 Reduced ANGPT2, BMP10, FABP3, and NT-proBNP concentrations during follow-up in patients who remain in sinus rhythm. In comparison with pre-ablation concentrations, ANGPT2 (*A*), BMP10 (*B*), and NT-proBNP (*C*) decreased significantly whereas FABP3 (*D*) increased (all patients, dark blue, left column). When stratified by rhythm outcome, the changes were only noted in the arrhythmia-free group (light blue, middle columns) but not in patients who experienced recurrent episodes (red, rightmost columns). Change difference from 0 calculated using one-sample *t*-test. Absolute biomolecule concentrations are given in *Table 1*. ANGPT2, angiopoietin 2; BMP10, bone morphogenetic protein 10; FABP3, fatty acid–binding protein 3; NT-proBNP, N-terminal pro-B-type natriuretic peptide; Rec AF, recurrent atrial fibrillation.

biomolecule for AF, including in patients with heart failure.[29] In view of its association with stroke in anticoagulated patients with AF,[30] reduced BMP10 concentrations in patients without recurrent AF after ablation could contribute to the outcome-reducing effect of early rhythm control therapy.[1,2,31]

Measuring multiple biomolecules in patients with atrial fibrillation

Pending validation in other data sets, our findings demonstrate that a combination of biomolecules can be used to identify patients at risk of recurrent AF after a first AF ablation. Despite clear collinearity of all biomolecule concentrations, the combination of ANGPT2, BMP10, and NT-proBNP enabled prediction of recurrent AF. The biomolecule combination identified here underpins that different mechanisms contribute to recurrent AF after ablation in patients. A similar biomolecule-based assessment for stroke and bleeding risk was developed[16] and is currently undergoing testing in a controlled clinical trial (ABC-AF study, NCT03753490). Furthermore, a combination of biomolecules can provide information that may enable development and testing of stratified, mechanism-oriented prevention of recurrent AF after ablation in the future. Clearly, independent validation of our findings is required. Our hypothesis-generating data suggest that the mechanisms leading to secretion of NT-proBNP, ANGPT2, and BMP10 are potential candidates for treatable drivers of AF after ablation.

Change of biomolecule concentrations after atrial fibrillation ablation

Generally, all biomolecules showed stable concentrations upon repeat measurement after 3 months. This is expected as they reflect long-term disease processes. The observed stability of biomolecule concentrations found here (*Figure 4*) confirms reports from the ARISTOTLE trial.[32] Longer term changes were recently described in the ENGAGE AF-TIMI 48 trial, which quantified changes in NT-proBNP, GDF15, and hsTnT from baseline to 12 months.[33] Unlike our analysis, neither ENGAGE nor ARISTOTLE captured rhythm status during follow-up.

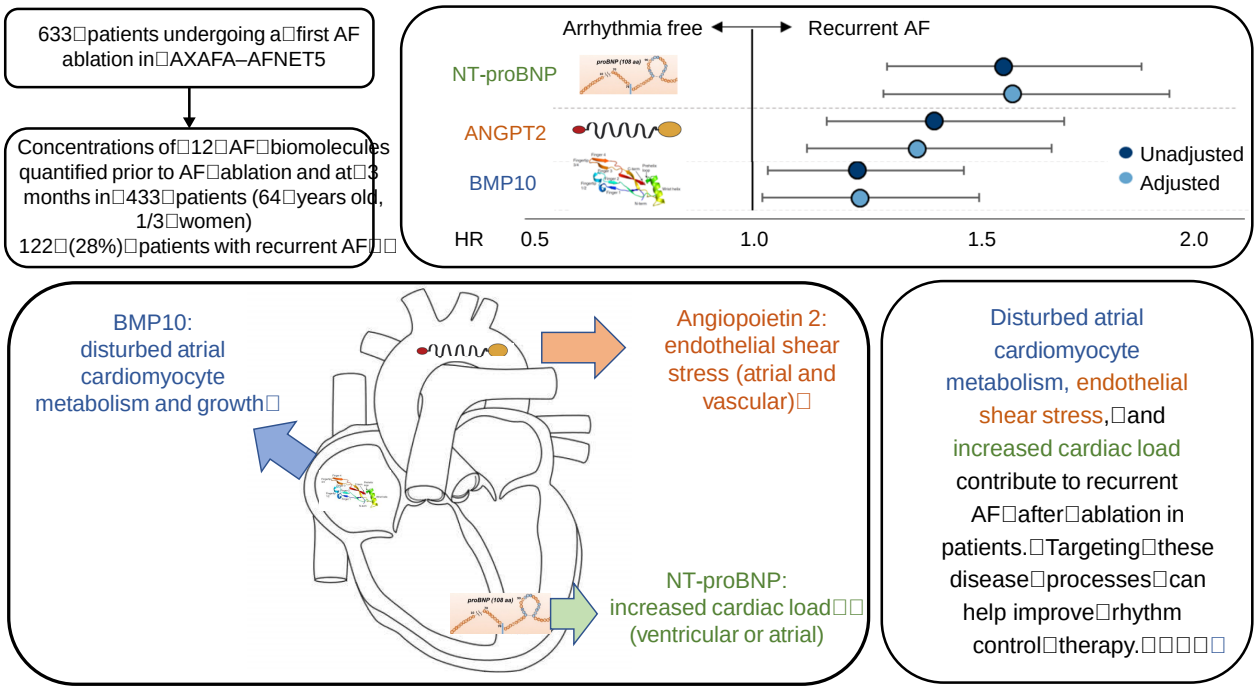


Figure 5 Biomolecule-quantified mechanisms of recurrent AF after AF ablation. Our analysis of 12 biomolecules representing different disease processes relevant for AF identify three disease processes associated with recurrent AF after a first AF ablation: altered endothelial shear stress (ANGPT2, orange), altered atrial metabolism (BMP10, blue), and increased cardiac load (NT-proBNP, green). These biomolecules can be used to identify patients at high risk of recurrent AF and provide information on the vascular, atrial, and cardiac processes contributing to recurrent AF. They may also be useful to develop stratified prevention and stratified rhythm control therapy for patients with AF. AF, atrial fibrillation; ANGPT2, Angiopoietin 2; BMP10 bone morphogenetic protein 10; NT-proBNP N_terminal pro-brain natriuretic peptide; HR Hazard Ratio.

Our hypothesis-generating results suggest that the disease processes leading to the release of ANGPT2, BMP10, and NT-proBNP are modified by successful rhythm control therapy as evident in the decrease in patients without recurrent AF while concentrations remained constant in patients with recurrent AF. In view of the emerging role of arrhythmia burden for outcomes in patients with AF,[34,35] and the outcome-reducing impact of attaining sinus rhythm through early rhythm control therapy,[1,2] rhythm-related changes could help to explain the outcome modification observed in ENGAGE[33] and the outcome-reducing effect of early rhythm control therapy.[1] More research is needed to characterize the potential outcome-mediating effect of arrhythmia burden and the possible contribution of circulating, rhythm-dependent biomolecules. This can be studied in *post hoc* analyses of biosamples collected in interventional trials with rhythm outcomes that may have effects on AF, e.g. SGLT-2 or RAAS inhibitors, and in prospective studies integrating rhythm outcomes and biosamples. Interestingly, a similar biomolecule signature is also found in patients with prevalent AF,[36] suggesting that similar changes are found outside of the ablation setting.

Conclusions

Using quantification of 12 biomolecules selected to estimate the activity of different disease processes relevant for recurrent AF after ablation, this hypothesis-generating analysis identified ANGPT2 reflecting altered endothelial shear stress, BMP10 reflecting defective atrial cardiomyocyte metabolism and proliferation, and NT-proBNP reflecting increased cardiac load as quantifiable proxies predicting recurrent AF after a first AF ablation. Furthermore, the concentrations of these biomolecules decreased after successful AF ablation, suggesting a connection of their concentrations with the arrhythmia. Pending validation, these circulating biomolecules provide quantifiable proxies for mechanisms that can be targeted to improve prevention of recurrent AF after ablation.

Strengths and limitations

Strengths of the data are inclusion of an international cohort of patients undergoing a first AF ablation, central quantification of biomolecule concentrations using high-precision, high-throughput assays, and rhythm follow-up in the context of a clinical trial. Our multicentre data set provides a robust reference for concentration ranges and AF ablation–induced changes 3 months after a first AF ablation that can be used for comparison and calibration. While the *a priori* selection of 12 biomolecules relevant for AF guided by a semi-formalized expert consensus process can be seen as a strength,[10] this analysis is also limited to the biomolecules quantified. There are several other limitations: One, the genetic predisposition to AF,[37] an independent contributor to recurrent AF, and biomolecules related to coagulation (affected by the randomized treatment in the trial, but not by recurrent AF[38]) were not considered in this analysis. Two, the shipment of blood samples to the central biosample facility at room temperature may have affected concentrations of selected biomolecules, introducing imprecision in the measurements. On the other hand, this is a practical strength as the biomolecules identified here can be quantified after ambient temperature courier shipment. Three, two of the three biomolecules identified in our study (ANGPT2 and BMP10) can so far only be measured in

research contexts. More research is needed to establish their clinical utility. Four, while left atrial size, a decent surrogate for atrial structural remodelling, was included in this analysis, we did not have access to other echocardiographic parameters, MRI imaging, or intracardiac mapping. Further studies are warranted to determine whether the biomolecules identified here are associated with altered cardiac structure and function as seen on imaging and on atrial electrical function as measured using mapping. The biomolecules proposed here provide a quantitative tool to evaluate such associations. Five, our hypothesis-generating data call for independent validation in other data sets. Six, the AXAFA–AFNET5 data set captured clinical recurrences of AF combined with systematic ECG and Holter ECG monitoring, but did not employ continuous monitoring. This will have reduced the number of patients with recurrent AF, but is unlikely to affect the main findings of the analysis.[39] Seven, follow-up for recurrent AF was limited to 120 days after AF ablation. Further studies are warranted to assess the association of biomolecule concentrations with later recurrences of AF after ablation. Eight, small effects of biomolecules on recurrent AF cannot be excluded from this analysis. Nine this analysis is limited to the selected biomolecules. Other biomolecules may further aid in predicting recurrent AF after ablation. Ten, we did not correct for multiple testing and there is a possibility of false-positive findings. The internal consistency of our hypothesis-generating results renders them plausible in our view. Eleven, these findings call for validation in independent cohorts with available biosamples and rhythm information. Twelve, kidney function as estimated by creatinine concentrations was normal in the majority of the patients studied here (mean estimated creatinine clearance 78 ± 19 mL/min at baseline, no change during follow-up). It is furthermore not a clear predictor for recurrent AF.[6] Creatinine was therefore not included in the models. Results may differ in patients with chronic kidney disease.

Clinical perspectives

Competency in medical knowledge

Translational research identified multiple mechanisms that can lead to AF. Their relative contribution to recurrent AF in patients undergoing AF ablation is not well understood. This study quantified multiple circulating biomolecules related to different cardiovascular disease processes. Three biomolecules reflecting disturbed cardiomyocyte metabolism and growth (BMP10), altered endothelial shear stress (ANGPT2), and increased atrial and ventricular load (NT-proBNP) were associated with recurrent AF after a first AF ablation. External validation of these hypothesis-generating findings is needed.

Translational outlook

The biomolecules identified here can be used to quantify disease processes contributing to recurrent AF using blood samples. This can be applied to translate mechanistic knowledge into clinical research. BMP10, ANGPT2, and NT-proBNP can be combined to identify patients at highest risk for recurrent AF after AF ablation. Following external validation, the disease processes reflected by these biomolecules, disturbed cardiomyocyte metabolism and growth, endothelial shear stress, and increased atrial and ventricular load, provide promising therapeutic targets to improve rhythm control in patients with AF.°

Supplementary material

Supplementary material is available at *Europace* online.

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Data availability

The AXAFA–AFNET5 trial data set and the measured biomolecule concentrations can be made available upon reasonable request after the check of

consent and review by the trial sponsor, AFNET e.V. Please send requests to info@kompetenznetz-vorhofflimmern.de.

References

1. 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**:1305–16.
2. 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**:4127–44.
3. Van Gelder IC, Ekrami NK, Borof K, Fetsch T, Magnussen C, Mulder BA *et al.* Sex differences in early rhythm control of atrial fibrillation in the EAST-AFNET 4 trial. *J Am Coll Cardiol* 2023;**81**:845–7.
4. Nielsen JC, Johannessen A, Raatikainen P, Hindricks G, Walfridsson H, Kongstad O *et al.* Radiofrequency ablation as initial therapy in paroxysmal atrial fibrillation. *N Engl J Med* 2012;**367**:1587–95.
5. Andrade JG, Wells GA, Deyell MW, Bennett M, Essebag V, Champagne J *et al.* Cryoablation or drug therapy for initial treatment of atrial fibrillation. *N Engl J Med* 2021;**384**:305–15.
6. 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**:748–60.
7. Kim YG, Boo KY, Choi JI, Choi YY, Choi HY, Roh SY *et al.* Early recurrence is reliable predictor of late recurrence after radiofrequency catheter ablation of atrial fibrillation. *JACC Clin Electrophysiol* 2021;**7**:343–51.
8. Nalliah CJ, Lim TW, Kizana E, Qian P, Kovoor P, Thiagalingam A *et al.* Clinical significance of early atrial arrhythmia type and timing after single ring isolation of the pulmonary veins. *Europace* 2015;**17**:1038–44.
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**:230–7.
10. Chua W, Easter CL, Guasch E, Sitch A, Casadei B, Crijns H *et al.* Development and external validation of predictive models for prevalent and recurrent atrial fibrillation: a protocol for the analysis of the CATCH ME combined dataset. *BMC Cardiovasc Disord* 2019;**19**:120.
11. 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**:2942–55.
12. Chua W, Law JP, Cardoso VR, Purmah Y, Neculau G, Jawad-Ul-Qamar M *et al.* Quantification of fibroblast growth factor 23 and N-terminal pro-B-type natriuretic peptide to identify patients with atrial fibrillation using a high-throughput platform: a validation study. *PLoS Med* 2021;**18**:e1003405.
13. Zink MD, Chua W, Zeemering S, di Biase L, Antoni BL, David C *et al.* Predictors of recurrence of atrial fibrillation within the first 3 months after ablation. *Europace* 2020;**22**:1337–44.
14. Haeusler KG, Eichner FA, Heuschmann P, Fiebach J, Engelhorn T, Callans D *et al.* Detection of brain lesions after catheter ablation depends on imaging criteria—insights from AXAFA-AFNET 5 trial. *Europace* 2023;**25**:euad323.
15. Haeusler KG, Eichner FA, Heuschmann PU, Fiebach JB, Engelhorn T, Blank B *et al.* MRI-detected brain lesions and cognitive function in patients with atrial fibrillation undergoing left atrial catheter ablation in the randomized AXAFA-AFNET 5 trial. *Circulation* 2022;**145**:906–15.
16. Hijazi Z, Oldgren J, Siegbahn A, Wallentin L. Application of biomarkers for risk stratification in patients with atrial fibrillation. *Clin Chem* 2017;**63**:152–64.
17. Kemp Gudmundsdottir K, Fredriksson T, Svennberg E, Al-Khalili F, Friberg L, Frykman V *et al.* Stepwise mass screening for atrial fibrillation using N-terminal B-type natriuretic peptide: the STROKESTOP II study. *Europace* 2020;**22**:24–32.
18. Staerk L, Preis SR, Lin H, Lubitz SA, Ellinor PT, Levy D *et al.* Protein biomarkers and risk of atrial fibrillation: the FHS. *Circ Arrhythm Electrophysiol* 2020;**13**:e007607.
19. 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**:1268–76.
20. 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**:197–203.
21. Meyre PB, Aeschbacher S, Blum S, Voellmin G, Kastner PM, Hennings E *et al.* Biomarkers associated with rhythm status after cardioversion in patients with atrial fibrillation. *Sci Rep* 2022;**12**:1680.
22. Schweizer J, Arnold M, Konig IR, Bicvic A, Westphal LP, Schutz V *et al.* Measurement of midregional pro-atrial natriuretic peptide to discover atrial fibrillation in patients with ischemic stroke. *J Am Coll Cardiol* 2022;**79**:1369–81.
23. Akwii RG, Sajib MS, Zahra FT, Mikelis CM. Role of angiopoietin-2 in vascular physiology and pathophysiology. *Cells* 2019;**8**:471.
24. Pala E, Bustamante A, Pagola J, Juega J, Francisco-Pascual J, Penalba A *et al.* Blood-based biomarkers to search for atrial fibrillation in high-risk asymptomatic individuals and cryptogenic stroke patients. *Front Cardiovasc Med* 2022;**9**:908053.
25. Freestone B, Chong AY, Lim HS, Blann A, Lip GY. Angiogenic factors in atrial fibrillation: a possible role in thrombogenesis? *Ann Med* 2005;**37**:365–72.
26. Morrell NW, Bloch DB, ten Dijke P, Goumans MJ, Hata A, Smith J *et al.* Targeting BMP signalling in cardiovascular disease and anaemia. *Nat Rev Cardiol* 2016;**13**:106–20.
27. 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**:e139179.
28. Litvinukova M, Talavera-Lopez C, Maatz H, Reichart D, Worth CL, Lindberg EL *et al.* Cells of the adult human heart. *Nature* 2020;**588**:466–72.
29. Staszewsky L, Meessen J, Novelli D, Wienhues-Thelen UH, Disertori M, Maggioni AP *et al.* Total NT-proBNP, a novel biomarker related to recurrent atrial fibrillation. *BMC Cardiovasc Disord* 2021;**21**:553.
30. 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**:208–18.
31. Metzner A, Suling A, Brandes A, Breithardt G, Camm AJ, Crijns H *et al.* Anticoagulation, therapy of concomitant conditions, and early rhythm control therapy: a detailed analysis of treatment patterns in the EAST-AFNET 4 trial. *Europace* 2022;**24**:552–64.
32. Hijazi Z, Lindahl B, Oldgren J, Andersson U, Lindback J, Granger CB *et al.* Repeated measurements of cardiac biomarkers in atrial fibrillation and validation of the ABC stroke score over time. *J Am Heart Assoc* 2017;**6**:e004851.
33. Oyama K, Giugliano RP, Berg DD, Ruff CT, Jarolim P, Tang M *et al.* Serial assessment of biomarkers and the risk of stroke or systemic embolism and bleeding in patients with atrial fibrillation in the ENGAGE AF-TIMI 48 trial. *Eur Heart J* 2021;**42**:1698–706.
34. Kirchhof P, Toennis T, Goette A, Camm AJ, Diener HC, Becher N *et al.* Anticoagulation with edoxaban in patients with atrial high-rate episodes. *N Engl J Med* 2023;**389**:1167–79.
35. Toennis T, Bertaglia E, Brandes A, Dichtl W, Fluschnik N, de Groot JR *et al.* The influence of atrial high rate episodes on stroke and cardiovascular death—an update. *Europace* 2023;**25**:euad166.
36. 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**:16743.
37. 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**:1799–810.
38. Kirchhof P, Ezekowitz MD, Purmah Y, Schiffer S, Meng IL, Camm AJ *et al.* Effects of rivaroxaban on biomarkers of coagulation and inflammation: a post hoc analysis of the X-VeRT trial. *TH Open* 2020;**4**:e20–32.
39. Kirchhof P, Auricchio A, Bax J, Crijns H, Camm J, Diener HC *et al.* Outcome parameters for trials in atrial fibrillation: recommendations from a consensus conference organized by the German Atrial Fibrillation Competence NETwork and the European Heart Rhythm Association. *Europace* 2007;**9**:1006–23.