

ORIGINAL RESEARCH

Combined Effect of Homocysteine and Uric Acid to Identify Patients With High Risk for Subclinical Atrial Fibrillation

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BACKGROUND: Subclinical atrial fibrillation (SCAF) is often asymptomatic nonetheless harmful. In patients with cardiac implantable electronic devices, we evaluated the combined performance of homocysteine and uric acid (UA) biomarkers to discriminate high-risk patients for SCAF.

METHODS AND RESULTS: We enrolled 1224 consecutive patients for evaluation of SCAF in patients with cardiac implantable electronic devices in Dalian, China, between January 2013 and December 2019. Clinical data and blood samples were obtained from patients selected according to the absence or presence of atrial high-rate episodes >6 minutes. Blood samples were obtained, and homocysteine and UA biomarkers were tested in all patients to distinguish their prognostic performance for SCAF. Homocysteine and UA biomarkers were significantly different in SCAF versus no SCAF. On multivariable Cox regression analysis with potential confounders, elevated homocysteine and UA biomarkers were significantly associated with an increased risk of SCAF. A rise of 1 SD in homocysteine (5.7 $\mu\text{mol/L}$) was associated with an increased risk of SCAF in men and women regardless of their UA levels. Similarly, a 1-SD increase in UA (91 $\mu\text{mol/L}$) was associated with an increased risk of SCAF among the patients with high levels of homocysteine in men (hazard ratio, 1.81; 95% CI, 1.43–2.30) and women (hazard ratio, 2.11; 95% CI, 1.69–2.62). The addition of homocysteine and UA to the atrial fibrillation risk factors recommended by the 2020 European Society of Cardiology Guidelines significantly improved risk discrimination for SCAF.

CONCLUSIONS: Homocysteine and UA biomarkers were strongly associated with SCAF. The prediction performance of the European Society of Cardiology model for SCAF was increased by the addition of the selected biomarkers.

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Key Words: continuous monitoring ■ homocysteine ■ pacemaker ■ subclinical atrial fibrillation ■ uric acid

Nonvalvular atrial fibrillation (AF) is the most common cardiac arrhythmia in clinical practice, and it is considered to be one of the most important causes of stroke. AF manifests itself with or without clinical manifestations. Subclinical atrial fibrillation (SCAF) refers to individuals without symptoms attributable to AF, in whom AF episodes are detected by insertable cardiac monitor or wearable monitor and

confirmed by visually reviewed intracardiac electrograms or ECG-recorded rhythm.¹ SCAF accounts for at least 1 in 3 patients with AF,² and current evidence suggests that the presence of device-detected SCAF among patients with no history of AF increases the risk of thromboembolism,^{3,4} heart failure,⁵ and cardiovascular mortality.⁴ Though cardiac implantable electronic devices (CIEDs), including dual-chamber permanent

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CLINICAL PERSPECTIVE

What Is New?

- Elevated plasma homocysteine and uric acid (UA) levels are strongly associated with an increased incidence of subclinical atrial fibrillation (SCAF) in patients with cardiac implantable electronic devices.
- A significant interaction effect of homocysteine and UA for increasing the incidence of SCAF.
- Application of both homocysteine and UA biomarkers combined with 2020 European Society of Cardiology risk factors improves the prediction of SCAF more accurately in the clinical practice.

What Are the Clinical Implications?

- An increase in homocysteine and UA values was markedly associated with an increased risk for SCAF in both men and women, suggesting that a substantial SCAF risk was present among those patients classified at the high homocysteine (>14 $\mu\text{mol/L}$ in men and >12 $\mu\text{mol/L}$ in women) and high UA (>420 $\mu\text{mol/L}$ in men and >320 $\mu\text{mol/L}$ in women) levels.
- Therefore, it may be reasonable to interpret in a way that cardiac implantable electronic device–implanted patients with increased homocysteine and UA as candidates for more intensive rhythm monitoring.

Nonstandard Abbreviations and Acronyms

CIED	cardiac implantable electronic device
ESC	European Society of Cardiology
IDI	integrated discrimination improvement
SCAF	subclinical atrial fibrillation
UA	uric acid

pacemakers, implantable cardioverter defibrillators, and cardiac resynchronization therapy devices improved management of multiple types of arrhythmias, it is important to explore early indicators of SCAF in patients with CIEDs.

Several pathophysiological mechanisms have been implicated to contribute to AF, including atrial fibrosis, atrial dilatation, inflammation, and oxidative stress. Plasma biomarkers of AF-related processes may improve risk prediction in addition to clinical stratification models. Cardiac biomarkers routinely measured in clinical practice, such as homocysteine and uric acid (UA) plasma biomarkers have been found elevated in patients with AF.^{6,7}

Homocysteine, a nonproteinogenic sulfur-containing amino acid, involves in the metabolism of cysteine and methionine. An increased level of homocysteine is widely considered as a long-standing biomarker associated with a range of disorders related to oxidative stresses,⁸ such as hypertension, heart failure, and stroke.^{9,10} Also, UA, an end product in the degradation of the purine nucleotides adenine and guanine, is speculated to participate in the pathogenesis of AF.^{11–13} Importantly, experimental and clinical data indicate that UA is implicated in the pathophysiology of AF via activation of inflammation, oxidative stress, and fibrosis-induced atrial remodeling.

Bearing in mind that SCAF compared with sustained AF carries a similar risk for transient ischemic attack/stroke and non–central nervous system embolism, early detection and management are critical to prevent AF-related complications. Consensus statements are evidence based and derived primarily from multiple sources. In contrast with the current status of evidence, European Society of Cardiology (ESC) recommended/indicated risk factors of AF, we believe these risk factors (the risk factors defined in the 2020 ESC guideline for AF, provided in Table S1) are worth establishing clinical stratification models. Therefore, we evaluated the performance of the risk factors alone and its combined prognostic performance with homocysteine and UA biomarkers for identifying patients at high risk for SCAF. The present study aimed to evaluate the combined performance of homocysteine and UA biomarkers to discriminate the high-risk population for SCAF in patients with CIEDs. We hypothesized that homocysteine and UA biomarkers are elevated in patients with SCAF as validated by continuous rhythm monitoring. We further hypothesized that homocysteine and UA biomarkers may provide additional discriminative power when compared with the ESC model in identifying patients at high risk for SCAF.

METHODS

Study Design

The data that support the findings of this study are available from the corresponding author upon reasonable request. A prospective cohort study was conducted in patients who underwent a CIED procedure between January 2013 and December 2019. The prospective cohort study was established in January 2013 at First Affiliated Hospital of Dalian Medical University, Dalian, P.R. China to assess the risk factors for AF among the CIED-implanted population. Briefly, this is an ancillary study conducted to explore the relationship between plasma homocysteine and UA concentration and SCAF occurrence. Our study protocol was registered on the Chinese Clinical Trial Registry on September 21,

2020 (<https://www.chictr.org.cn>, registration number: Chi-CTR2000038377).

Study Population

This study recruited 2783 patients with newly implanted CIEDs. Patients who received regular physical examinations for >6 months with complete electrogram recordings were eligible for this study. A total of 2550 patients were screened after satisfying the requirements of a minimum age of 18 years and complete clinical data. To monitor SCAF, implanted CIEDs without algorithms for the detection of atrial tachycardia/AF episodes were excluded (n=279). Patients with rheumatic heart disease were excluded because they were expected to have a higher risk of AF (n=47). Also, patients with a prior diagnosis of AF, atrial flutter, and atrial tachycardia evidenced by ECG or Holter monitoring were excluded (n=981). Additionally, we excluded 19 patients who presented with early SCAF incidence during the first month of CIED implantation. Finally, a total of 1224 patients were included in the study (Figure 1). The research was conducted in accordance with the Declaration of Helsinki guidelines and was approved by the institutional review board of the First Affiliated Hospital of Dalian Medical University. All participants provided informed consent, and all procedures listed here were carried out in compliance with the approved guidelines.

Clinical Measurements and Definition of Explanatory Variables

At baseline, basal data and various clinical details related to pacing indication, type of CIEDs, clinical history, smoking/drinking status, blood pressure, body mass index,

medication use, 12-lead ECG, 24-hour Holter, transthoracic echocardiography, coronary computed tomography angiography, and components of the CHA₂DS₂-VASc score were recorded following a detailed assessment. The estimated glomerular filtration rate was calculated using the creatinine-based Chronic Kidney Disease Epidemiology Collaboration equation.¹⁴ Diabetes has been defined as fasting 7.0 mmol/L plasma glucose or current use of antidiabetic treatments. Hypertension has been defined as systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg or active use of antihypertensive drugs. Smoking and drinking status were self-reported and classified as never smokers/drinkers, past smokers/drinkers, or current smokers/drinkers. Participants were deemed current smokers if reported they are currently smoking or registered smoking at least 100 cigarettes during their lifetime.^{15,16}

Biological Sampling and Biochemical Measurements

A sample of fasting blood from the brachial vein was collected, and it was biochemically examined for lipid panel profiles and plasma concentration of homocysteine and UA levels. The serum UA concentrations were examined with an autoanalyzer using the uricase-peroxidase process (BECKMAN COULTER AU680 Chemistry Analyzer). Homocysteine levels were determined by using an enzymatic cycling method (Hitachi7600 series automatic analyzer). Blood glucose levels were measured using the standard protocols. The laboratory personnel were not aware of the subjects' health status, and all samples were treated identically throughout the processing and analysis.

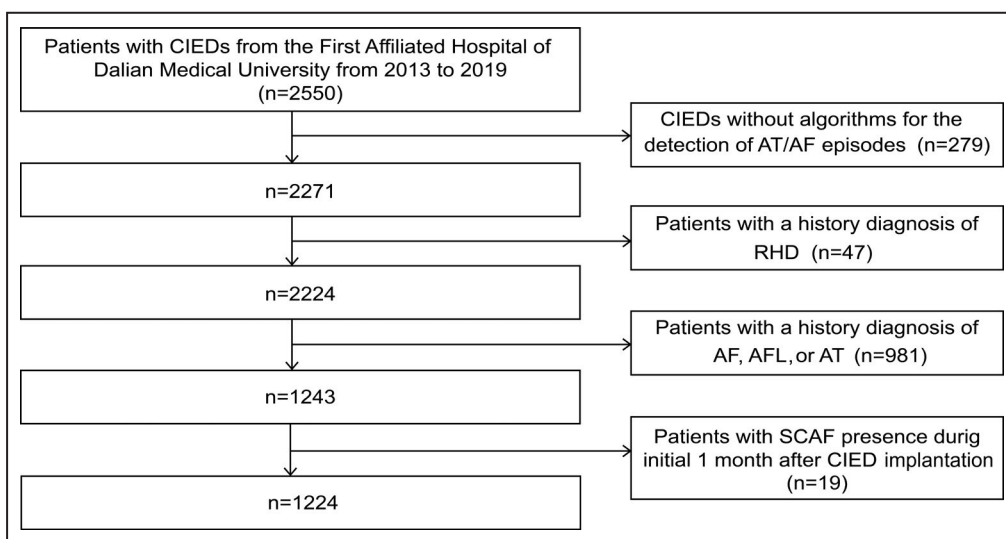


Figure 1. Flow chart of patients included in the analysis.

Median duration of atrial arrhythmia monitoring was 643 (319–1277) days. AF indicates atrial fibrillation; AFL, atrial flutter; AT, atrial tachycardia. CIED, cardiac implantable electronic device; RHD, rheumatic heart disease; and SCAF, subclinical atrial fibrillation.

Device Characteristics, Implantation Procedure, and Interpretation of SCAF Episodes

All patients underwent implantation of a dual-chamber permanent pacemaker, implantable cardioverter defibrillator, or cardiac resynchronization therapy devices, which were programmed to a DDI(R) or a DDD(R) pacing mode at the discretion of the implanting physician. The atrial tachycardia detection feature was programmed “on,” and the AF suppression algorithm was programmed “off” in all patients. The device was programmed so that atrial tachycardia was detected when the heart rate reached 190 beats per minute, and electrogram storage was activated. SCAF was defined as the presence of at least 1 episode of atrial tachyarrhythmia episodes lasting >6 minutes. The stored atrial and ventricular electrograms were reviewed and adjudicated by 2 experienced electrophysiologists, who were blinded for other study data. All inappropriately detected SCAF episodes were excluded. Finally, SCAF was confirmed as AF, atrial flutter, or atrial tachycardia episodes.

Follow-Up and Outcome Assessment

Follow-up visits, including physical examination, assessment of potential AF symptoms, 12-lead ECG, and routine device checkups were performed at 1, 3, and 6 months, and then every 6 months regularly since CIED implantation. The primary outcome of this study was an occurrence of an SCAF event. All SCAF events were confirmed using intracardiac electrograms. Patients with <6 minutes of atrial tachyarrhythmia episodes were considered “no SCAF.” During follow-up, SCAF episodes occurring within 1 month of device implantation were excluded because of a concern that the arrhythmia could be related to atrial lead implantation. All patients were followed up until the occurrence of SCAF or December 31, 2019, whichever came first.

Statistical Analysis

The statistical analyses were performed using SPSS software (version 22, IBM) and R software (version 3.3.0). Because of sex-specific differences in metabolism of homocysteine and UA,^{17,18} all analyses were stratified by gender. Categorical data were presented as count and percentage and checked for differences using the chi-square test. For continuous data, normality was assessed by both the D’Agostino and Pearson’s test and the Shapiro-Wilk test. The Mann-Whitney *U* test was used to analyze the nonnormally distributed continuous variables, and data were expressed as the median and interquartile range.

We first calculated the hazard ratios (HRs) for SCAF associated with homocysteine and UA to establish the

association between these biomarkers and SCAF. The pattern of quantitatively assessed homocysteine or UA linking SCAF occurrence was analyzed by restricted cubic splines that display the HR on the *y* axis versus the metric used to quantify homocysteine or UA level on the *x* axis. To balance best fit and overfitting in the splines for SCAF, the number of knots, between 3 and 7, was chosen as the lowest value for the Akaike information criterion. Finally, we chose 4 knots located at the 5th, 35th, 65th, and 95th percentiles of homocysteine/UA. A HR of 1 indicates reference value (cut point), HR >1 represents a higher risk of SCAF, and HR <1 indicates a lower risk of SCAF.

We further calculated HR and corresponding 95% CIs for the occurrence of SCAF associated with 1 SD increase in homocysteine and UA. To investigate the potential interaction effect between homocysteine and UA on incident SCAF risk, the likelihood ratio tests in subgroups were performed using the cut point of homocysteine/UA to classify the population into 2 groups. On subgroup analyses, 2 distinct Cox models were employed independently. The first Cox proportional hazard model was employed to investigate the association of homocysteine with an increase in 1 SD in UA, modeled as a continuous variable, for the occurrence of SCAF. Whereas the second Cox model was established to explore the relationship of UA with an increase in 1 SD in homocysteine, modeled as a continuous variable, for the occurrence of SCAF. To account for potential confounding effects, we adjusted these risk factors for incident AF defined in the 2020 ESC guideline, including age, smoking, drinking, body mass index, systolic blood pressure, diastolic blood pressure, estimated glomerular filtration rate, coronary heart disease, diabetes, congestive heart failure, chronic obstructive pulmonary disease, and left ventricular ejection fraction. Left atrial dimension was also included as an indicator of atrial fibrotic remodeling. We used 3 different models: a crude model, an age-adjusted model, and a fully adjusted model (adjusted for all the aforementioned variables). The log-rank test for trend and Kaplan-Meier methods were used to compare the freedom (from SCAF) distributions and study the differences in SCAF freedom as stratified by homocysteine and UA status, respectively. The proportional hazards assumption was tested and satisfied in all cases using Schoenfeld residuals.

To evaluate the discriminatory power of SCAF incidence at 5 years among ESC model, ESC model+UA, ESC model+homocysteine, and ESC model+UA+homocysteine, we executed time-dependent receiver operating characteristics, Harrell’s concordance statistics, and integrated discrimination improvement (IDI).¹⁹

To evaluate the performance of the final combined model (ESC model+UA+ homocysteine), we used the calibration plot. Also, we determined the bootstrap

method (with 1000 bootstrap samples) to decrease the overfit bias and variance errors for the observed calibration of SCAF against the predicted probability of SCAF to improve consistency and reduce bias. In addition, we performed a decision curve analysis to evaluate the clinical benefit of our final model. Statistical tests were 2-sided, and $P < 0.05$ was considered statistically significant.

RESULTS

Baseline Characteristics

A total of 1224 patients were included in the study, 225 patients with detected SCAF and 999 patients without SCAF. The median age was 69 years with a median time of rhythm monitoring of 642 days. Sixty-nine percent of the patients were monitored for at least 1 year, with a maximum monitoring time of 2684 days. The proportion of women (50.6%) and men (49.4%) was similar. The clinical and demographic characteristics of the patients are shown in Table. Indications for permanent pacemaker implantation in the no SCAF group and the SCAF group were sinus-node disease, (40% versus 34% in men and 55% versus 57% in women), atrioventricular node disease (45% versus 41% in men and 34% versus 37% in women), both sinus and atrioventricular node disease (5% versus 7% in men and 2% versus 1% in women), and other indication (11% versus 19% in men and 9% versus 5% in women). Compared with patients who were free of SCAF, patients with SCAF received more statin and angiotensin-converting enzyme inhibitor/angiotensin receptor blocker medication in both men and women (all $P < 0.05$). Patients who experience SCAF had larger left atrial diameter compared with the no SCAF group. In both men and women, there were no significant differences in terms of age, body mass index, hypertension, diabetes, coronary artery disease, chronic heart failure and previous stroke/transient ischemic attack, estimated glomerular filtration rate, CHA₂DS₂-VASc score between the SCAF and no SCAF groups.

The Association of Homocysteine and UA

As shown in Figure 2, plasma homocysteine and UA levels were significantly elevated in SCAF patients in comparison with those without SCAF (all $P < 0.001$). No significant difference was observed for the other clinical biomarkers provided in Table S2. Regardless of the SCAF status, men had higher homocysteine and UA levels compared with women (Figure 2A and 2B). We also examined the association between plasma homocysteine and UA levels (Figure 2C and 2D). Given the influence of gender on the metabolism of homocysteine and UA, the cutoff points for homocysteine and UA were analyzed separately for women and men. For

men, a positive association was significantly found between homocysteine and UA levels ($r = 0.118$; $P = 0.001$). Similar findings were found for women ($r = 0.254$; $P < 0.001$).

Quantitative Approach to Markers and SCAF

After we observed that the proportional hazards assumption was satisfied, we further explored the association between quantitative measures of plasma homocysteine and UA levels and SCAF using restricted cubic spline analysis to stratify participants into low- and high-risk groups based on the new reference values of homocysteine and UA (Figure 3). The HR value served as the threshold for further analysis.^{20,21} The cut point for UA was 420 $\mu\text{mol/L}$ in men, and 320 $\mu\text{mol/L}$ in women, respectively, whereas the cutoff point for homocysteine was 14 $\mu\text{mol/L}$ in men and 12 $\mu\text{mol/L}$ in women, respectively.

Relationship Between Homocysteine, UA, and SCAF

The association between plasma concentrations of homocysteine and UA levels and the risk of SCAF is presented in Table S3. In the fully adjusted model, each 1-SD increase in UA was associated with a 69% increase in the risk for SCAF in men (HR, 1.69; 95% CI, 1.37–2.08; $P < 0.001$). Interestingly, our result shows that women demonstrated a higher risk than men (HR, 1.93; 95% CI, 1.57–2.36; $P < 0.001$). Each 1-SD increase in homocysteine was associated with a 39% increase in SCAF among women (HR, 1.39; 95% CI, 1.25–1.54; $P < 0.001$). Also, a 27% increase was observed with a 1-SD increase in homocysteine in men (HR, 1.27; 95% CI, 1.11–1.44; $P < 0.001$). A similar result was observed when plasma homocysteine and UA levels were included in regression models as categorical variables. There was a positive association between the high level of homocysteine/UA and the presence of SCAF after adjusting for potential confounders (Table S3). As shown in Figure 4, there were significant differences in the incidence of SCAF between the cutoff value of homocysteine and UA mentioned above, suggesting that the cutoff points can successfully discriminate between the lower-risk group and the higher-risk group.

The Interaction Effect Between Homocysteine and UA in SCAF

The forest plot illustrates the odds ratios associated with an increase in 1 SD (5.7 $\mu\text{mol/L}$) in homocysteine among participants grouped by UA status and the OR associated with an increase in 1 SD (91 $\mu\text{mol/L}$) in UA among participants grouped by homocysteine status (Figure 5). With an increase in 1 SD of homocysteine,

Table 1. Baseline Characteristics of Entire Patient Group

Variable	Male				Female			
	Total (n=605)	Non-SCAF (n=482)	SCAF (n=123)	P value	Total (n=619)	Non-SCAF (n=517)	SCAF (n=102)	P value
Age, y	70 (62–79)	70 (62–78)	71 (63–79)	0.749	69 (62–77)	69 (61–77)	70 (63–76)	0.704
History								
Hypertension	341 (56)	269 (56)	72 (59)	0.586	370 (60)	309 (60)	61 (60)	0.995
Systolic blood pressure, mm Hg	140 (120–152)	140 (120–150)	141 (120–159)	0.114	140 (126–154)	140 (125–153)	140 (126–156)	0.592
Diastolic blood pressure, mm Hg	76 (68–81)	76 (67–80)	78 (70–85)	0.194	73 (64–80)	73 (64–80)	72 (61–80)	0.517
Congestive heart failure	123 (20)	91 (19)	32 (26)	0.079	112 (18)	95 (18)	17 (17)	0.682
Diabetes	134 (22)	113 (23)	21 (17)	0.129	124 (20)	105 (20)	19 (19)	0.698
Stroke/TIA	52 (9)	45 (9)	7 (6)	0.198	47 (8)	36 (7)	11 (11)	0.183
Coronary artery disease	175 (29)	140 (29)	35 (28)	0.897	109 (18)	89 (17)	20 (20)	0.562
COPD	11 (2)	10 (2)	1 (1)	0.35	9 (1)	9 (2)	0 (0)	0.179
eGFR, mL/min-per 1.73 m ²	84 (72–94)	85 (72–94)	83 (71–93)	0.374	87 (74–95)	87 (74–95)	85 (71–94)	0.217
Excessive alcohol drinking	116 (19)	95 (20)	21 (17)	0.507	2 (0)	2 (0)	0 (0)	0.529
Current smoking	179 (30)	138 (29)	41 (33)	0.308	5 (1)	5 (1)	0 (0)	0.319
CHA ₂ DS ₂ -VASc score	2 (1–4)	2 (1–3)	2.4±1.5	2 (1–4)	3 (2–4)	3 (2–4)	3 (2–4)	0.839
Pacing indication								
Sinus-node disease	234 (39)	192 (40)	42 (34)	0.248	340 (55)	282 (55)	58 (57)	0.667
Atrioventricular node disease	265 (44)	215 (45)	50 (41)	0.430	214 (35)	176 (34)	38 (37)	0.533
Both sinus and atrioventricular node disease	31 (5)	23 (5)	8 (7)	0.437	13 (2)	12 (2)	1 (1)	0.388
Others	75 (12)	52 (11)	23 (19)	0.017	52 (8)	47 (9)	5 (5)	0.163
Device implanted								
PPM	528 (87)	428 (89)	100 (81)	0.026	567 (92)	472 (91)	95 (93)	0.540
ICD	43 (7)	33 (7)	10 (8)	0.621	17 (3)	16 (3)	1 (1)	0.232
CRT-P	10 (2)	7 (1)	3 (2)	0.444	17 (3)	15 (3)	2 (2)	0.664
CRT-D	24 (4)	14 (3)	10 (8)	0.008	18 (3)	14 (3)	4 (4)	0.505
Medications								
Aspirin	269 (44)	202 (42)	67 (54)	0.012	217 (35)	173 (33)	44 (43)	0.061
ACE inhibitor/ARB	268 (44)	210 (44)	58 (47)	0.475	253 (41)	200 (39)	53 (52)	0.013
CCB	305 (50)	238 (49)	67 (54)	0.313	320 (52)	258 (50)	62 (61)	0.044
Statin	359 (59)	273 (57)	86 (70)	0.007	338 (55)	273 (53)	65 (64)	0.043
Echocardiogram parameters								
LAD, mm	38 (35–41)	38 (35–40)	38 (36–42)	0.010	37 (35–39)	37 (34–39)	37 (35–40)	0.032
LVEF, %	58 (57–59)	58 (57–59)	58 (54–59)	0.001	58 (58–59)	58 (58–59)	58 (58–59)	0.466
Biomarkers								
Homocysteine, μmol/L	14.8 (12.7–18.1)	14.4 (12.4–17.5)	16.2 (13.9–19.4)	<0.001	12.2 (10.1–14.3)	11.8 (9.9–13.9)	14.2 (11.7–17.1)	<0.001
UA, μmol/L	366 (310–425)	361 (299–417)	396 (337–448)	<0.001	309 (258–366)	303 (252–352)	364 (296–436)	<0.001

Values are reported as median values (interquartile range) or n (%). ACE indicates angiotensin-converting enzyme; ARB, angiotensin receptor blocker; CCB, calcium channel blocker; COPD, chronic obstructive pulmonary disease; CRT-D/P, cardiac resynchronization therapy with/without defibrillation therapy; eGFR, estimated glomerular filtration rate; ICD, implantable defibrillator; LAD, left atrial diameter; LVEF, left ventricular ejection fraction; PPM, permanent pacemaker; SCAF, subclinical atrial fibrillation; TIA, transient ischemic attack; and UA, uric acid.

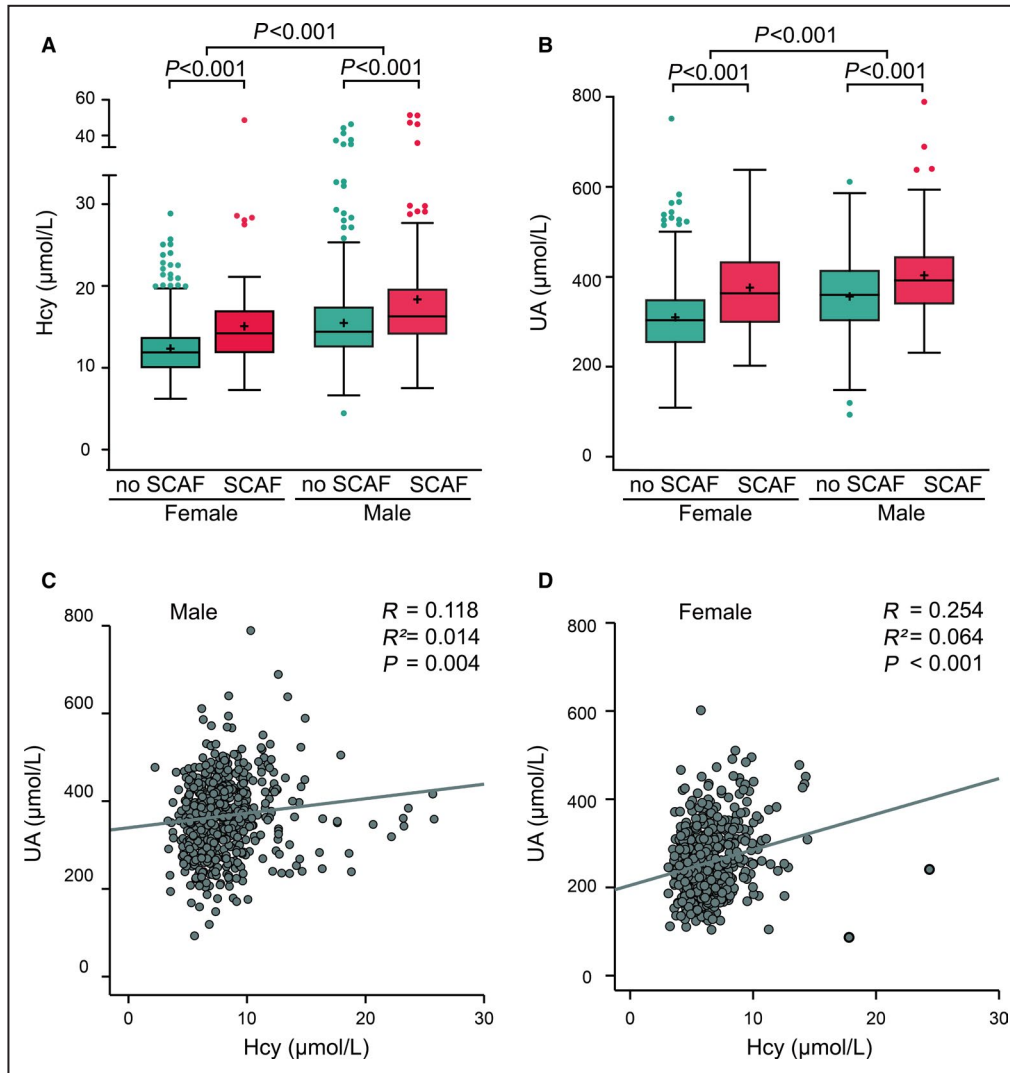


Figure 2. Serum homocysteine (A) and uric acid (B) in patients with or without SCAF and their correlation in males (C) and females (D).

Hcy indicates homocysteine; SCAF, subclinical atrial fibrillation; and UA, uric acid.

the fully adjusted Cox regression model confirmed that patients with high-level UA had a higher risk for SCAF compared with the low-level group in both men and women (both P -interaction < 0.05). When we treated these 2 biomarkers independently, our result showed a significant interaction between homocysteine and dichotomous UA classes for SCAF in women but not in men (P -interaction = 0.048; P -interaction = 0.179, respectively).

Rates of SCAF according to different levels of homocysteine and UA are shown in Figure 6. Based on the low- and high-risk subset defined by the spline curve of homocysteine/UA, we divided patients into 4 groups, which represented different combinations of the 2 biomarkers: group 1: patients with both low-level homocysteine and UA; group 2: patients with high-level UA and low-level homocysteine; group 3: patients with high-level homocysteine and low-level of UA; group

4: patients with high levels of both homocysteine and UA. Patients with high levels of both homocysteine and UA had an increased incidence of SCAF (log-rank test, $P < 0.001$). After the full adjustment, the HRs for the patients in groups 2, 3 and 4 were 1.57 (95% CI, 0.96–2.56), 1.72 (95% CI, 1.16–2.55), and 3.50 (95% CI, 2.39–5.13), respectively. Schoenfeld residual tests revealed that there was no linear correlation between Schoenfeld residuals and time (Figure S1), indicating the proportional hazard assumption was satisfied.

The Additive Effect of Homocysteine and UA Levels on the ESC Model

We performed receiver-operating characteristic analysis to determine the diagnostic utility of the biomarkers (homocysteine and UA) and ESC model to identify patients with SCAF (Figure 7A). The area under the curve

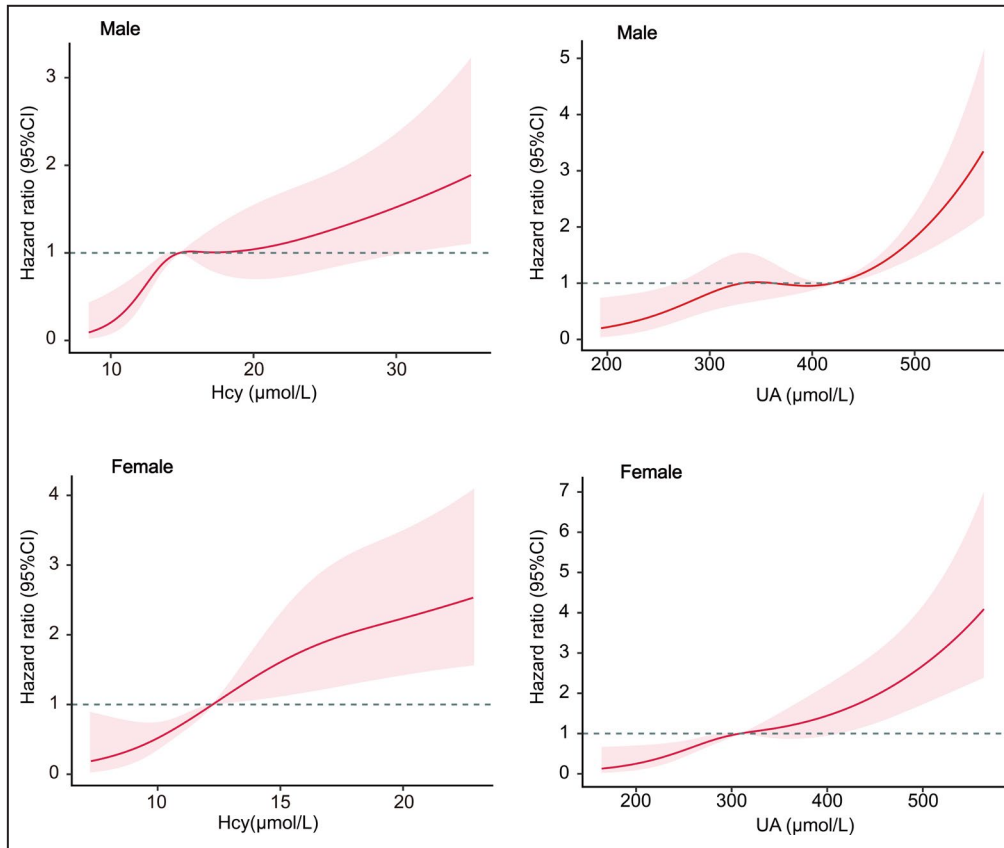


Figure 3. Multivariable adjusted hazard ratios for SCAF incidence according to levels of homocysteine and uric acid on a continuous scale.

Red lines are multivariable-adjusted hazard ratios, with pink areas showing 95% CIs derived from restricted cubic spline regressions with four knots located at the 5th, 35th, 65th, and 95th percentiles. Reference lines for no association are indicated by the dashed gray lines at a hazard ratio of 1.0. Analyses were adjusted for age, smoking, drinking, body mass index, systolic blood pressure, diastolic blood pressure, estimating glomerular filtration rate, coronary heart disease, diabetes, congestive heart failure, chronic obstructive pulmonary disease, left atrial diameter, and left ventricular ejection fraction. Hcy indicates homocysteine; SCAF, subclinical atrial fibrillation; and UA, uric acid.

was 0.607 (95% CI, 0.579–0.634) for the ESC model. We tested the models to improve the identification of patients from the SCAF group by combining both homocysteine and UA biomarkers to the ESC model. The combination of ESC model, homocysteine and UA (final model) reached the highest AUC (0.717; 95% CI, 0.691–0.742) followed by the models of risk factors and UA (0.688; 95% CI, 0.662–0.714) and risk factors and homocysteine (0.688; 95% CI, 0.662–0.714). The AUC results for the biomarkers and ESC risk factors are shown in Figure 7B.

Though the combination in the ESC model and homocysteine increase the AUC by 8.39%, their combination did not result in significant increment in IDI (2.6%; $P=0.050$). Adding UA to the ESC model, improved SCAF detection models by the score, with an IDI of 8.8% ($P<0.001$) (Figure 7B). Moreover, the ESC model combined with homocysteine and UA biomarkers yielded an increase of 11.3% in IDI ($P<0.001$). The aforementioned data suggested that plasma homocysteine and UA increased the

discrimination ability of the clinical model, indicating the addition of the plasma markers not only improves the prediction of the events but also reduces the potential for false-positive rates.

The calibration plot in Figure 7C shows an adequate calibration of the predicted probabilities against the observed proportions of SCAF (Figure 7C). In addition, the decision curve analyses based on the ESC model and final model are shown in Figure 8. The analysis revealed that the combined final model had a higher net benefit than at a threshold probability of 0.025 to 0.25. Hence, it is compelling to choose the combined final model in clinical utility.

DISCUSSION

In this study, we investigated whether SCAF is associated with homocysteine and UA biomarkers in patients with continuous rhythm monitoring. We found that elevated homocysteine and UA were significantly associated with

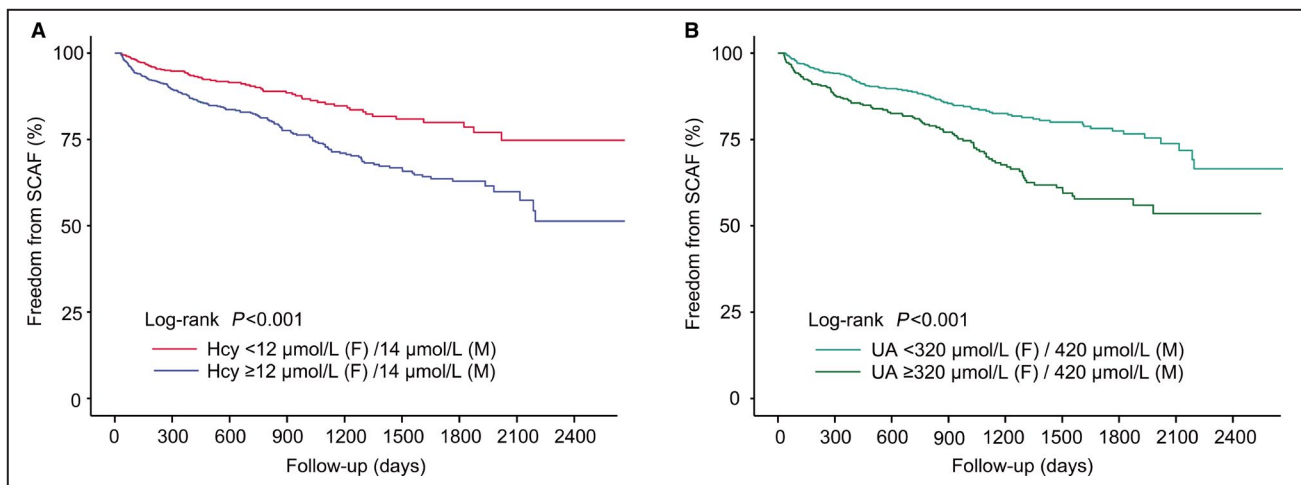


Figure 4. Kaplan-Meier curves showing the incidence of SCAF for different levels of homocysteine and UA.

A, Kaplan-Meier survival curves for homocysteine, the cutoff points for homocysteine 14 $\mu\text{mol/L}$ in men, and 12 $\mu\text{mol/L}$ in women, respectively. **B**, Kaplan-Meier survival curves for UA; the cut points for UA were 420 $\mu\text{mol/L}$ in men and 320 $\mu\text{mol/L}$ in women, respectively. Hcy indicates homocysteine; SCAF, subclinical atrial fibrillation; and UA, uric acid.

SCAF and added to the discriminatory performance of using the clinical risk factors (ESC model) to identify patients with increased incidence of SCAF.

We established the regulation of homocysteine and UA biomarkers in high-risk patients of SCAF based on the clinical history and electrogram recordings. In this study, we found a positive and significant correlation between the homocysteine and UA biomarkers in both men and women categories, consistent with previous findings from a large cross-sectional study.²² Though prior studies using implanted cardiac devices suggest that up to 85% of AF is not clinically recognized,^{3,23} follow-up studies consistently reported SCAF incidence >20% in patients with CIEDs.^{3,4,23} However, the result of the current study found a low SCAF incidence rate compared with the previous studies. This discrepancy could be attributed to the following reasons: First, our study included a younger population (mean age, 69.1 \pm 11.8 years) with fewer comorbid diseases. Second, all patients were assessed at baseline on the basis of self-reported history, 12-lead ECG, and 24-hour Holter to exclude potential unreported AF. Third, a considerable proportion of the participants were receiving statins (56.9%), angiotensin-converting enzyme inhibitors/angiotensin receptor blockers (42.6%), which might mitigate the potential risk for AF.

We established the estimated risk of SCAF in the subgroup of patients with low and high levels of homocysteine and UA. We observed differences not only in the biomarker regulation between the SCAF and no SCAF groups, but also the combination of the high levels of homocysteine and UA amplifies the risk of SCAF. The prognostic relevance of homocysteine-UA has not been prospectively studied, and biomarkers investigated should be interpreted in this context. The current

study aimed to evaluate the use of homocysteine/UA along with the established risk factors in clinical practice to identify patients with increased risk for SCAF. Therefore, it may be reasonable to consider CIED patients with increased homocysteine and UA as candidates for more intensive rhythm monitoring.

Homocysteine, a highly reactive, sulfur-containing amino acid, is an intermediate by-product during dietary methionine metabolism. Previous research has mainly focused on the relationship between the plasma homocysteine and hypertension, left atrium thrombus formation, ischemic events, and recurrence after catheter ablation of the individual diagnosed AF, but only a few clinical studies tried to determine the associations between homocysteine and onset of AF.²⁴ In the present study, we demonstrated that plasma homocysteine levels were significantly higher in patients with new-onset SCAF than those without it, and elevated plasma homocysteine was independently associated with SCAF. Although the exact mechanism linking elevated homocysteine levels and SCAF remains unclear, different pathways including oxidative stress, production, and bioavailability of nitric oxide, and inflammatory response²⁵ are speculated as plausible mechanisms. These mechanisms could further result in atrial structural and electrical remodeling.

UA is a heterocyclic organic compound and an end product of purine metabolism in humans and has been known as an antioxidant and pro-oxidant at its normal and high concentration, respectively.²⁶ Recently, the significance of UA widely emerged in AF and has been broadly investigated in various types of the population with or without chronic disease. According to a community-based study, the elderly population with high UA (UA >416 $\mu\text{mol/L}$ in men and >357 $\mu\text{mol/L}$ in

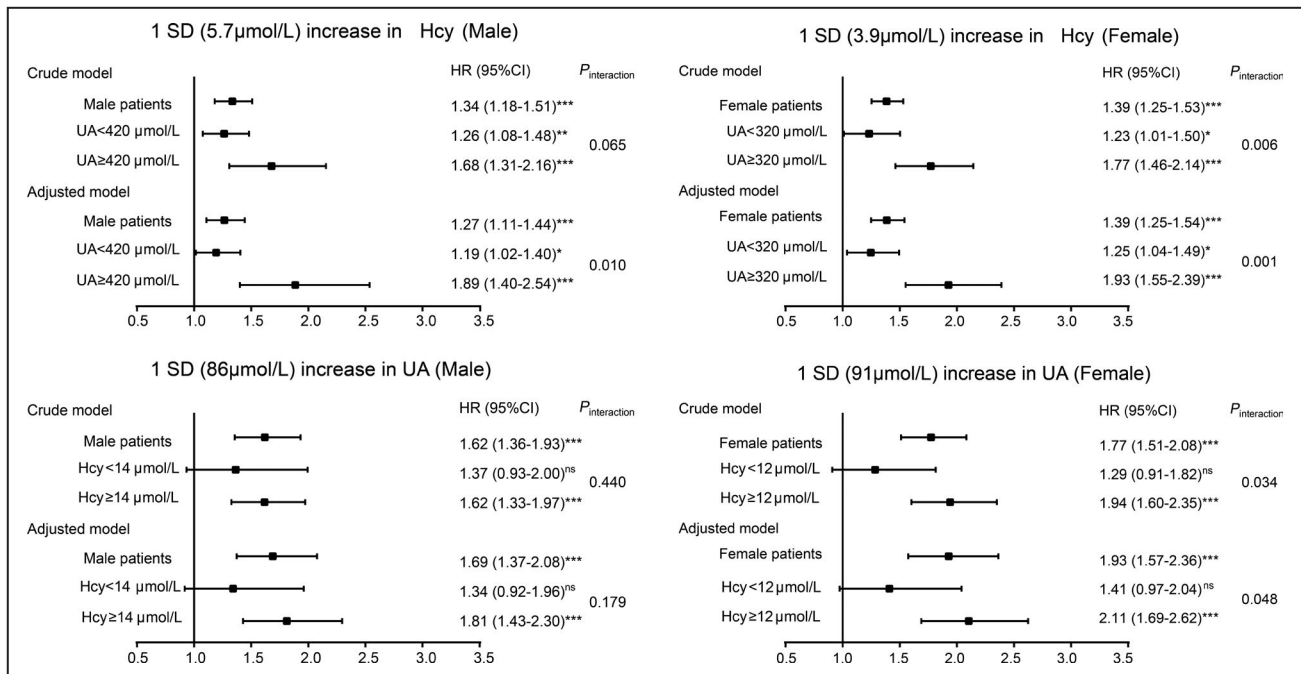


Figure 5. Hazard ratios with 95% CIs for 1 SD increase in Homocysteine/UA of SCAF.

Hcy indicates homocysteine; HR, hazard ratio; SCAF, subclinical atrial fibrillation; and UA, uric acid. **P*<0.05, ***P*<0.01, ****P*<0.001, ns: not significant.

women) had a higher risk of AF.²⁷ Chang-Fu Kuo et al reported that gout is independently associated with a persistently higher risk of AF at diagnosis and 5 to 10 years after diagnosis.²⁸ Another study also showed a positive and independent association between UA and AF in patients with type 2 diabetes with the cutoff

point of 300 μmol/L.²⁹ Akin to homocysteine, UA-induced AF are closely related to electrophysiological and structural alterations via various mechanisms, including oxidative stress,¹¹ inflammation,¹² and fibrosis.¹³

In our follow-up study, we observed differences in the biomarker regulation between the SCAF and no SCAF groups. Also, we confirmed the relationship between high homocysteine and UA levels and the presence of SCAF. The incidence of SCAF in patients with a CIED depends on the clinical profile of the beneficiary and device-specific detection algorithm.³⁰ Many CIED-implemented patients own significantly high ventricular pacing and/or sinus node diseases, consequently, these populations intrinsically have a high incidence of AF.^{31,32} Therefore, we redefined the cutoff points of the low and high risk for SCAF using the spline curve. An increase in homocysteine and UA values was markedly associated with an increased risk for SCAF in both men and women, suggesting that a substantial SCAF risk was present among those patients classified at the high homocysteine (>14 μmol/L in men and >12 μmol/L in women) and high UA (>420 μmol/L in men and >320 μmol/L in women) levels. Therefore, it may be reasonable to interpret in a way that CIED-implemented patients with increased homocysteine and UA as candidates for more intensive rhythm monitoring. In addition, longer SCAF episodes (≥6 minutes) are more relevant to clinical AF³³ and carry a higher risk of systemic embolism/ischemic stroke.³⁴ The pre-existing stroke risk factors aggravate the SCAF-related

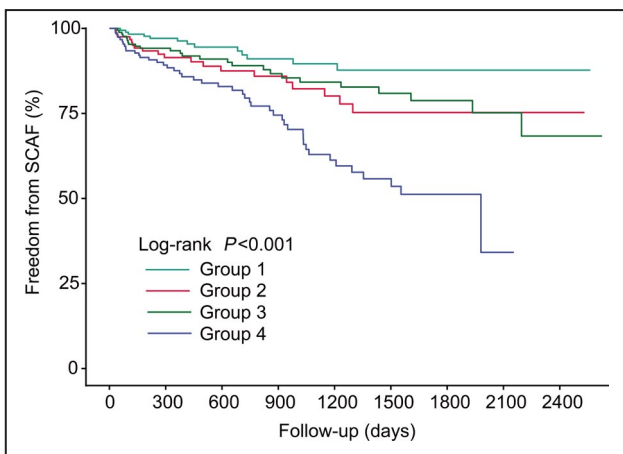


Figure 6. Kaplan-Meier curve showing the incidence of SCAF.

All patients were divided into 4 categories. From top to bottom, the lines represent the following: group 1: patients with both low-level homocysteine and UA (turquoise); group 2: patients with high-level UA and low-level homocysteine (red); group 3: patients with high-level homocysteine and low-level of UA (green); group 4: patients with high levels of both Hcy and UA (blue). Hcy indicates homocysteine; SCAF, subclinical atrial fibrillation; and UA, uric acid.

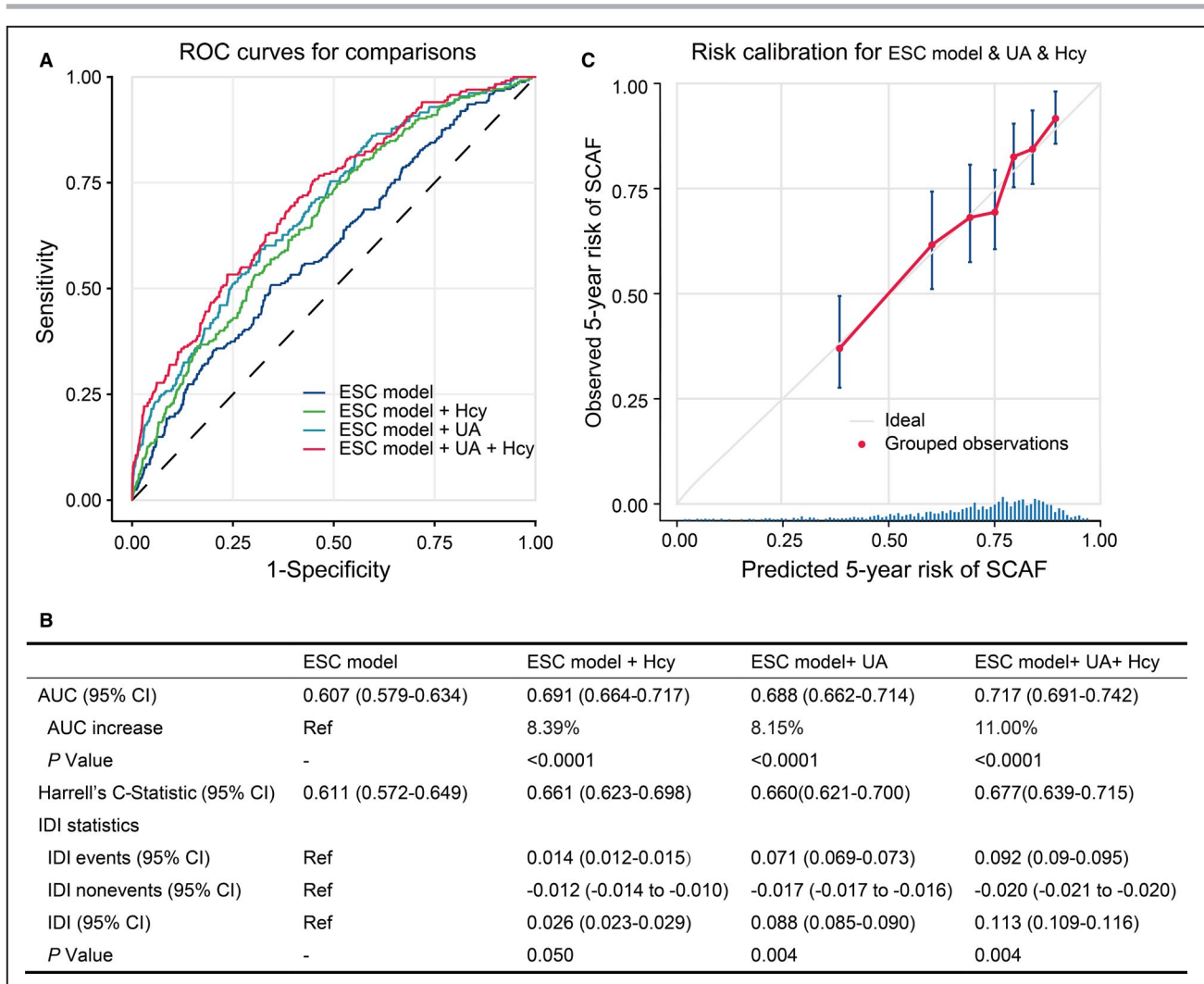


Figure 7. Model-comparison results of predicting the 5-year incidence of SCAF.

A, Receiver operating characteristics (ROC) curves of freedom from SCAF at 5 years for the different risk prediction models. **B**, Comparison of the area under the curve (AUC), Harrel's C-statistic, the integrated discrimination improvement (IDI) statistics, and 95% CIs in the different risk prediction models. **C**, Validation of the final model (ESC model+UA+homocysteine) showing observed incidences of SCAF within 5 years. The diagonal gray line represents a situation of perfect prediction when the observed incidences would be identical to the predicted baseline risks. Points are drawn to represent the averages in 7 discretized bins, and error bars are 95% CIs for the proportion of events in each group. The rug under the plot illustrates the distribution of predictions. ESC indicates European Society of Cardiology; Hcy, homocysteine; SCAF, subclinical atrial fibrillation; and UA, uric acid.

thromboembolic risk.³⁵ Whereas the thromboembolic risk in patients with CIEDs is primarily attributed to the burden of comorbidity (such as the components of CHA₂DS₂-VASc score).³⁵ Adding SCAF duration to the clinical risk scores has significantly improved discrimination for thromboembolism and may be useful in guiding anticoagulation therapy.³⁶ Thus, early identification of SCAF is important to reduce the risk of clinical AF/stroke. The unified model that combined homocysteine and UA levels with the ESC model improves the SCAF risk prediction, indicating patients with SCAF with CIEDs, particularly patients classified as a high-risk group, requires an intensive and strict follow-up to prevent the risk of clinical AF, stroke, and death.

The present study demonstrated that homocysteine and UA biomarkers own a good prognostic performance for SCAF independently. Also, the use of clinical risk factors, particularly those listed in the 2020 ESC guidelines definition for risk factors, has shown a good performance to discriminate SCAF in our study. In this study, compared with the ESC model alone, the unified model that combined the UA or/and homocysteine biomarkers with the ESC model offered an increase in the AUC and IDI statistics in SCAF detection. Therefore, simultaneous application of both homocysteine and UA biomarkers facilitates the prediction of the risk of SCAF more accurately in those with CIEDs in clinical practice. These circulating biomarkers may commonly regulate

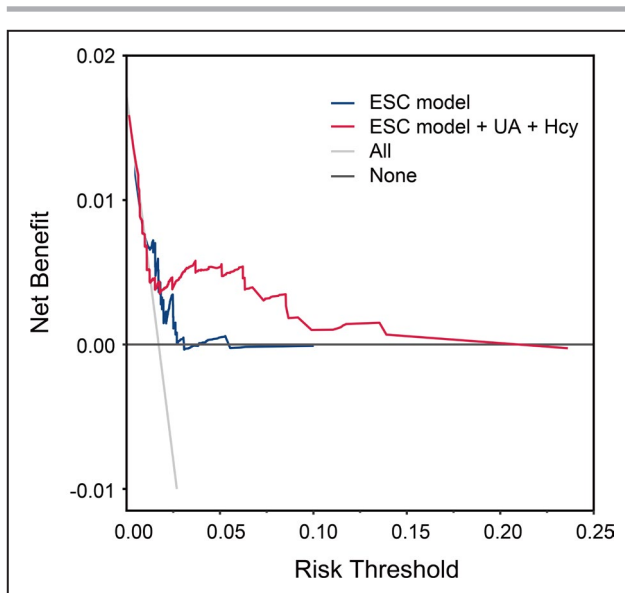


Figure 8. Decision curve analyses (DCA) of the ESC model and final model for 5-year SCAF incidence.

The *x* axis indicates the threshold incidence for SCAF at 5 years, and the *y* axis indicates the net benefit. The horizontal dark gray line: to assume no patients will experience the event; the light gray line: to assume all patients will experience the event. The ESC final model had enhanced net benefit compared with the ESC model at risk threshold >2.5%. ESC indicates European Society of Cardiology; Hcy, homocysteine; SCAF, subclinical atrial fibrillation; and UA, uric acid.

AF-associated mechanisms such as inflammation and oxidative stress activation process. Additionally, homocysteine and UA are involved in the methionine homocysteine cycle. Methionine is converted to S-adenosyl-L-homocysteine, split into homocysteine and adenosine.³⁷ Further, adenosine is metabolized to UA (Figure 9). In this case, it is reasonable to hypothesize that the relationship between the adenosine molecule and UA may contribute to the observed synergy. Bearing in mind that methionine metabolism influences the homocysteine and adenosine levels, the concentration of homocysteine may determine the amount of adenosine, which in turn increase UA levels. Further study is required to investigate the metabolic relationship between homocysteine and UA to solve the route of their synergistic effect in SCAF.

Study Limitations

Considering the high rate of silent AF and the limited sensitivity of routine ECGs, continuous rhythm monitoring provides a strong basis to evaluate the role of plasma homocysteine and UA biomarkers to discriminate for SCAF. The associations of biomarkers with SCAF in our study are strengthened by the continuous monitoring of heart rhythm with months- to years-long observation time, allowing detection of SCAF that might be otherwise overlooked with standard or 24-hour ECG

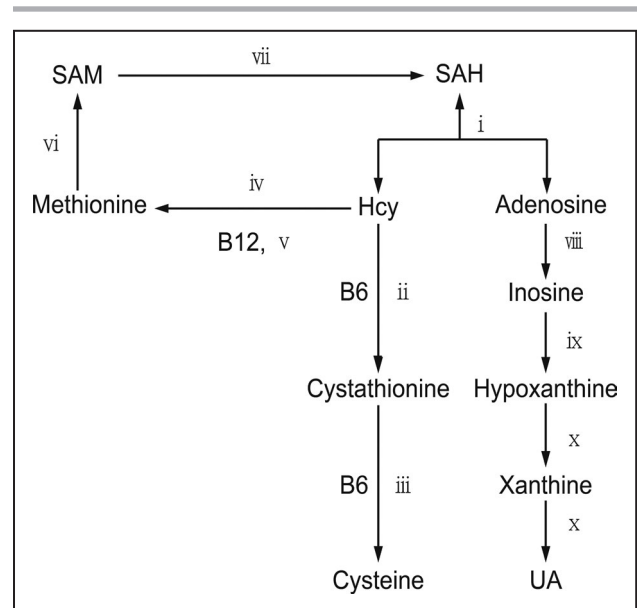


Figure 9. Homocysteine and UA metabolism.

The circled numbers refer to the following enzymes: i, SAH hydrolase; ii, cystathionine- β -synthase; iii, cystathionine- γ -lyase; iv, betaine-homocysteine methyltransferase; v, methionine synthase; vi, methionine adenosyltransferase; vii, methyltransferases; viii adenosine deaminase; ix, phosphorylase; x, xanthine oxidase. SAH indicates S-adenosyl-L-homocysteine; and SAM indicates S-adenosyl-methionine.

recording. However, the present study has several limitations. First, this was a single-center, nonrandomized, retrospective observational study, and was subject to inherent limitations associated with retrospective analyses. Second, some of our patients may have had silent AF before CIED implantation. However, there is no reliable method to identify such patients precisely. Third, CIED-implanted patients are known for their high risk of increased ventricular pacing or sinus node disease that makes them special cases for AF risk, which may limit the generalization of our results in the general population. Finally, there was a slight configuration difference in SCAF detection because of the different permanent pacemaker manufacturers.

CONCLUSIONS

In conclusion, we found strong independent associations between homocysteine and UA concentrations and the presence of SCAF in patients with implanted CIEDs. Also, our study identified that elevated homocysteine and UA were independent prognostic markers for SCAF in patients with CIEDs. The combination of homocysteine and UA with ESC definition of AF risk factors significantly improved the prediction of SCAF in patients with CIEDs, indicating that simultaneous application of this unified model can significantly improve discrimination for SCAF in CIED-implanted patients.

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Disclosures

None.

Supplemental Material

Tables S1–S3

Figure S1

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SUPPLEMENTAL MATERIAL

Table S1. Risk factors for incident AF recommended by the 2020 ESC guideline for AF.

Demographic factors	Age	Health factors and other risk factors	Hypertension
	Male sex		Systolic blood pressure
Health behaviors	Smoking/tobacco use		Diastolic blood pressure
	Alcohol intake		Diabetes mellitus
Cardiovascular conditions/diseases	HF		Renal dysfunction/CKD
	Valvular disease		Obesity
	CAD		Body mass index
Disorders of heart rhythm	PR interval prolongation		Height
	Sick sinus syndrome		Weight
LA remodeling	Left atrial dimension		COPD

AF indicates atrial fibrillation; CAD, coronary artery disease; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; HF, heart failure; LA, left atrium.

Table S2. Baseline characteristics of entire patient groups for other biomarkers.

Variable	Male				Female			
	Total (n=605)	non-SCAF (n=482)	SCAF (n=123)	P- value	Total (n=619)	non-SCAF (n=517)	SCAF (n=102)	P- value
WBC, $\times 10^9/L$	6.4 (5.3-7.4)	6.4 (5.3-7.5)	6.3 (5.2-7.4)	0.903	6.0 (5.0-7.3)	6.0 (5.0-7.4)	5.8 (4.9-7.2)	0.29
Neutrophil, $\times 10^9/L$	3.8 (3.0-4.9)	3.8 (3.0-4.9)	3.9 (3.1-5.0)	0.839	3.5 (2.7-4.6)	3.5 (2.7-4.6)	3.3 (2.5-4.6)	0.202
NLR	2.2 (1.7-3.1)	2.2 (1.7-3.1)	2.2 (1.7-3.0)	0.779	1.9 (1.4-2.6)	1.9 (1.4-2.6)	1.8 (1.3-2.7)	0.723
TG, mmol/L	1.2 (0.8-1.5)	1.2 (0.8-1.5)	1.2 (0.9-1.7)	0.646	1.3 (0.9-1.7)	1.3 (0.9-1.7)	1.2 (0.9-1.5)	0.064
TC, mmol/L	4.4 (3.7-5.0)	4.4 (3.7-5.0)	4.4 (3.5-5.1)	0.961	4.9 (4.2-5.6)	4.9 (4.2-5.6)	4.6 (4.0-5.4)	0.085
LDL, mmol/L	2.4 (1.9-2.9)	2.4 (1.9-2.9)	2.4 (1.9-2.9)	0.824	2.7 (2.2-3.2)	2.7 (2.2-3.2)	2.7 (2.2-3.2)	0.328
HDL, mmol/L	1.1 (0.9-1.3)	1.1 (0.9-1.3)	1.1 (1.0-1.3)	0.442	1.2 (1.0-1.4)	1.2 (1.0-1.5)	1.2 (1.0-1.4)	0.195
LP(a), mg/L	5.3 (4.8-6.3)	5.3 (4.8-6.3)	5.3 (4.7-6.3)	0.996	5.2 (4.7-6.0)	5.2 (4.7-6.1)	5.0 (4.7-5.7)	0.571
FBG, mmol/L	5.3 (4.8-6.3)	5.3 (4.8-6.3)	5.3 (4.7-6.3)	0.459	5.2 (4.7-6.0)	5.2 (4.7-6.1)	5.0 (4.7-5.7)	0.206
BNP, $\mu\text{mol/L}$	116 (56-326)	115 (54-304)	116 (65-416)	0.325	118 (58-301)	116 (58-288)	133 (63-309)	0.518

Values are reported as median values (interquartile range).

BNP indicates brain natriuretic peptide; FPG, fasting plasma glucose; HDL, high-density lipoprotein;

LDL, low-density lipoprotein; LP(a), lipoprotein (a); NLR, neutrophil/lymphocyte ratio; TC, total cholesterol and, TG, triglyceride; WBC, white cell count.

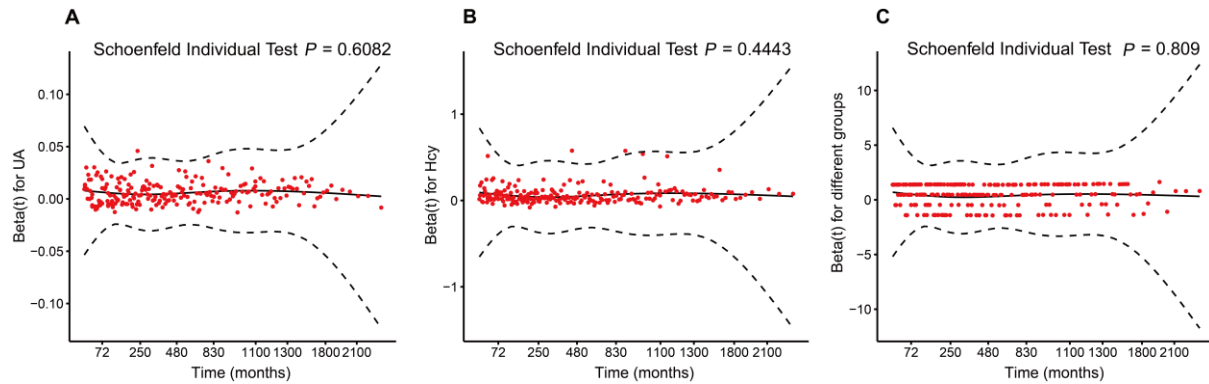
Table S3. Hazard ratios for the association between Homocysteine and Uric acid with SCAF.

		per 1-SD increase			High level group vs. Low level		
		Model 1	Model 2	Model 3	Model 1	Model 2	Model 3
Male	UA	1.62 (1.36, 1.93)***	1.64 (1.37, 1.97)***	1.69 (1.37, 2.08)***	1.93 (1.35, 2.77)***	1.99 (1.39, 2.85)***	2.00 (1.36, 2.95)***
	Hcy	1.34 (1.18, 1.51)***	1.38 (1.18, 1.51)***	1.27 (1.11, 1.44)***	1.98 (1.33, 2.94)***	1.98 (1.32, 2.96)**	1.92 (1.26, 2.92)**
Female	UA	1.77 (1.51, 2.08)***	1.79 (1.52, 2.11)***	1.93 (1.57, 2.36)***	2.30 (1.54, 3.43)***	2.29 (1.53, 3.44)***	2.22 (1.44, 3.43)***
	Hcy	1.39 (1.25, 1.53)***	1.38 (1.25, 1.53)***	1.39 (1.25, 1.54)***	2.00 (1.31, 3.04)**	1.99 (1.30, 3.04)**	1.92 (1.25, 2.95)**

*: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$.

Model 1, crude; model 2, adjustment for age; model 3, full-adjustment model (adjustment for age, BMI, systolic blood pressure, diastolic blood pressure, estimated glomerular filtration rate, congestive heart failure, diabetes, coronary artery disease, chronic obstructive pulmonary disease, smoking, drinking, left atrial diameter, left ventricular ejection fraction).

Figure S1. Analysis of the residuals of Schoenfeld residuals to assess the proportionality assumption.



Figures represent plots of beta-coefficient estimates (log hazard ratios) for uric acid(A), homocysteine(B) and four categories of these two variables(C) against follow-up (time) in months. The black solid line represents a smoothed curve of scaled Schoenfeld residuals with 95% confidence intervals (black dotted lines). (A) uric acid; (B) homocysteine; (C) four categories of predictor: Group 1: patients with both low-level Hcy and UA; Group 2: patients with high-level UA and low-level Hcy; Group 3: patients with high-level Hcy and low-level of UA; Group 4: patients with both high-level of Hcy and UA.