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Endocrine and Mechanical Cardiacfunction Four Months after Radiofrequency Ablation of Atrialfibrillation

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Abstract

Background: Radiofrequency ablation (RFA)is an important treatment option for patients with atrial fibrillation (AF). During RFA, a significant amount of energy is delivered into the left atrium (LA), resulting in considerable LA-injury. The impact of this damage on mechanical and endocrine LA-function, however, is often disregarded.We therefore aimed to evaluate the endocrine- and mechanical function of the heart 4-months after RFA of AF.

Methods: In total 189 patients eligible for RFA of AF were studied. The levels of the N-terminal pro-B-natriuretic peptide (NT-proBNP) and the mid-regional fragment of the N-terminal pro-atrial natriuretic peptide (MR-proANP)were measured. The maximum LAvolume (LAVmax),the LAejection fraction (LAEF) and the LA peak longitudinal strain (PALS), were measured usingtransthoracic echocardiography. The measurements were performed before and 4-months after the intervention.

Results: 87 patients had a recurrence during a mean follow-up of 143±36 days.NT-proBNPand MR-proANPdecreased significantly at follow-up. This reduction was greater in patients who did not suffer any recurrence after RFA.
The LAVmax decreased significantly, whereasthe PALS only improved in patients who did not suffer from any recurrence. On the other hand, LAEF did not change significantly after RFA of AF.

Conclusions: Despite extensiveablation during RFA of AF, the endocrine function of the heart improved 4-months after the index procedure. Patients with no arrhythmia recurrence showed a more pronounced improvement in their endocrinal function. Mechanically, the LAVmax was reduced, and the LA strain improved significantly.

Introduction

Atrial fibrillation (AF) is the most common arrhythmia [1], with an increased riskof embolic stroke and mortality [1, 2]. During atrial fibrillation, thecomplex endocrine functions of the heartare activated, including cardiacnatriuretic peptides. These peptides are synthesized and secreted from atrial and ventricular myocardium [3, 4]. The mid-regional fragment of the prodromal molecule of atrial natriuretic peptide(MR-proANP) and N-terminal pro B-type natriuretic peptide (NT-proBNP)are elevated in patients with AF, even so in patients without overt HF [5, 6]. The key stimuli of production and secretion of natriuretic peptides is the increase of myocardial wall tension [5]. However, data support that local inflammation due to high-frequency contraction of atrial myocytes could constitute a stimulus for synthesis in patients with persistent AF [7].

There are also extra-cardiac neurohormonal systems that play a role in cardiovascular endocrine metabolism. The c-terminal pro-vasopressin (copeptin) and arginine vasopressin (AVP) are both produced in the hypothalamus and released from the neurohypophysis in response to hypervolemia and changes in plasma osmolality [8]. The plasma

Key Words

Atrial Fibrillation, Radiofrequency Ablation, Natriuretic Peptides, Left Atrial Ejection Fraction, Left Atrial Strain.

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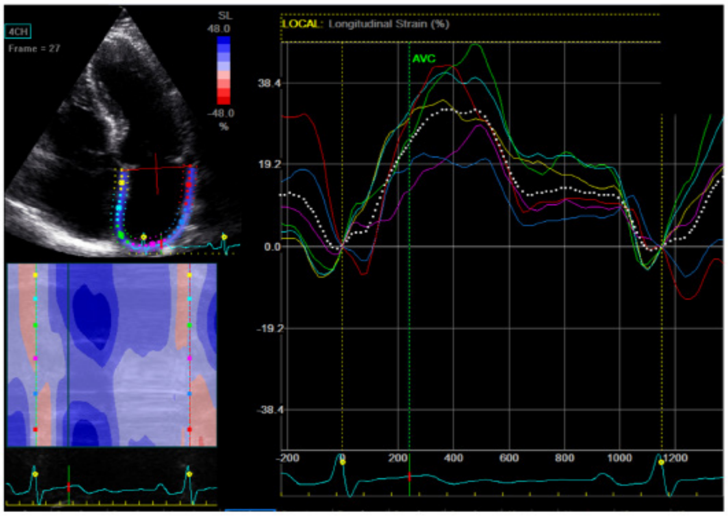

Figure 1: Measuring peak longitudinal atrial strain using speckle tracking echocardiography from apical four chambers view. The dotted curves represent global longitudinal left atrial strain.

concentration of copeptin and AVP increases in patients with HF attributed to inadequate cardiac output, low blood pressure, or increased vascular resistance [9]. Another example of extra-cardiac peptide is the mid-regional portion of pro-adrenomedullin (MR-proADM). MR-proADM is a product of the parental molecule of adrenomedullin. Adrenomedullin is a peptide with vasoactive and natriuretic properties. It is secreted mainly from the adrenal medulla and endothelial cells in response to several hormonal agents and physical stimulants, such as shear stressin blood vessels [10]. Studies have also shown that ADM exists and is actively secreted by cardiac cells [11,12], a finding that makes ADM an interesting biomarker for cardiac diseases such as AF.

From a mechanical standpoint, left atrial (LA) remodelling and enlargement is a powerful predictor of several cardiovascular events, including stroke and death. The maximum LA volume (LAVmax) and the LA emptying fraction (LAEF) are usually measured to assess LA remodelling. Two-dimensional speckle tracking echocardiography is a method for quantifying myocardial wall deformation using tracking of acoustic speckles [13], and as suchprovides a more comprehensive assessment of LA function [14].

For patients with symptomatic AF, radiofrequency ablation (RFA) is an important treatment option [1,15]. There is, however, a large variation regarding reportedsuccess rates after a single RFA procedure [16,17]. During the RFA procedure, a significant amount of energy is delivered into the LA, resulting in considerable LA-injury [18]. Nevertheless, the impact of this damage to endocrine and mechanical atrial function has not been widely studied.

Therefore, the aim of this study wasto evaluate the endocrine- and mechanical function of the heart 4-months after RFA of AF.

Methods

Study design and population

The present study is an observational longitudinal study based on the SMURF-study cohort (Symptom burden, metabolic profile, ultrasound findings, rhythm, neurohormonal activation, hemodynamics, and health-related quality of life in patients with AF). The SMURF-study wasconducted between January 2012 and April 2014 [19].

Patients with AF referred for RFA atthe University Hospital in Linköping, Sweden, were screened. The selection criteria for candidates were: 1) Patients≥ 18 years old with paroxysmal or persistent AF, 2) Referred for first time RFA, and 3) With sufficient knowledge of the Swedish language.

Exclusion criteria were: 1) Previous catheter or surgical AF ablation, 2) Previous or planned heart surgery, 3) Severe heart failure (HF) with left ventricular ejection fraction (LVEF) <35%, or 4) Acute coronary syndrome during the past three months.

The full study protocol has been published previously [19].

Subject measurements

The subject measurements and the ablation procedure have been described previously [19,20].

Table 1: Baseline characteristics of the total population and follow up population. The follow up population is also divided in patients with and without AF recurrence after 4 months of follow up

	All patients	All patients with follow up	AF recurrence	No AF recurrence	p-value
Number of pts	189	119	53 (44%)	66 (56%)	
Female	55 (29.1%)	41 (35%)	21 (40%)	20 (30%)	NS
Age	60.5±10.3	61±10.4	63±8	58.7±11.7	NS
Hypertension	80 (42.3%)	51(43%)	24 (45%)	42 (41.2%)	NS
Diabetes	16 (8.5%)	9 (8%)	3 (6 %)	6 (9%)	NS
BMI kg/m²	27.4 (22.6, 34.2)	26.7 (24.7, 29.4)	27.5 (24.6, 29.1)	27.7 (24.9, 29.9)	NS
CKD (GFR < 60mL/ min/1.73 m²)	40 (21.2%)	21(18%)	9(17%)	12 (18%)	NS
Stroke/TIA	11 (5.8%)	6 (5%)	3 (6%)	3 (5%)	NS
IHD	16 (8.5%)	12 (10 %)	4 (8 %)	8 (12 %)	NS
CHA_2DS_2VASc	2 (0, 5)	2 (0, 3)	2 (0, 3)	1 (0, 2.25)	NS
Beta blocker	139 (73.5%)	86 (72%)	40 (76%)	46 (70%)	NS
AAD	98 (51.9%)	64 (54%)	29 (55%)	35 (53%)	NS
AAD follow up	36 (19 %)	21 (18%)	16 (30%)	5 (8%)	0.001
LVEF < 50%	49 (25.9%)	29 (24%)	13 (25%)	16 (24%)	NS
Paroxysmal AF	71 (37.6%)	42(35%)	20 (37%)	22 (33%)	NS
Atrial fibrillation in admission	53 (28%)	34 (29%)	16 (30%)	18(27%)	NS
Procedural time (min)	188±50	187±49.4	189±47	185±52	NS
Complications	7 (4%)	4 (3%)	3 (6%)	1(2%)	NS
Extra ablation lines	28(15%)	18 (15%)	5 (10%)	13 (21%)	NS

Note 1: Normally distributed continuous data are presented as means with standard deviation. Differences between patients who experienced recurrences and those without recurrences were examined with t-test. Non-parametric data are presented as median values with 25th and 75th percentiles within brackets and tested with Mann-Whitney U test. Categorical data are presented as counts with percent values within brackets and tested with chi-square test.
Abbreviations: AAD: antiarrhythmic drugs; AF: atrial fibrillation; BMI: body mass index; CHA_2DS_2VASc: congestive heart failure, hypertension, age ≥ 75, diabetes, stroke, vascular disease, gender; CKD: chronic kidney failure; LVEF: left ventricular ejection fraction; GFR: glomerular filtration rate; IHD: ischemic heart disease.

Table 2: Lines performed during radiofrequency ablation, in addition to pulmonary vein isolation

Additional lines	Total
RA isthmus	11 (5.7%)
LA isthmus	1 (0.5%)
LA roof	15 (7.8%)
CS line	1 (0.5%)
No additional line	164 (85.4)

Note: Data are presented as counts with percent values within brackets.
Abbreviations: RA: right atrium, LA: left atrium, CS: coronary sinu

In short, all patients underwent a full baseline evaluation the day before the procedure, including medical history andtransthoracic echocardiographic examination (TTE).

The day of the procedure the patients were catheterized, and blood samples were retrieved from the femoral vein for biomarker analysis [19,20].

Follow-up and definition of recurrence

Patients underwent a follow upfour months after RFA and whenever otherwise required due to symptomswith 12-lead electrocardiograms and 24-h Holter ambulatory electrocardiograms. Of note, there was no blanking period after the RFA procedure. Episodesof atrial tachyarrhythmia or AFlasting >30 seconds during the follow up period on ECG, 24-h Holter ambulatory monitoring or on pacemaker/ implantable defibrillator interrogation were registered as clinical recurrences [21]. At the follow-up, blood samples were retrieved from a peripheral veinfor biomarker evaluation,and TTE was performed in patients who were residents of the County of Östergötland [19,22]. The period of four months was chosen on the assumption that the changes made by the RFA would be mainly healed, and that a stable biomarker situation was acquired.

Echocardiography

TTE measurements

All participants underwent TTE prior to RFA and a subgroup of patients underwent acontrol TTE at 4-months follow up [20].

The Simpson's biplane method was used to calculate LVEF. TheLAVmax and the minimum LA volume (LAVmin) were measured according tothe biplane area-length methodand were corrected for body surface area (BSA) [23]. LAEF was calculated according to the following equation ((Vmax - Vmin)/ Vmax) x100 [22-24].

The measurements and evaluations were performed according to the guidelines of the European Society of Echocardiography [25].

Speckle tracking echocardiography

Two-dimensional images of the LA were obtained from the apical 4 chamber (C)(figure 1) and 2C view for speckle tracking evaluation.The frame rate was between 55 and 100 frames/s. The endocardial border of the LA wastraced, and a region of interest (ROI) was generated (Echo PAC version BT 12,GE Healthcare, Horten, Norway). Manual adjustments were performed when necessary. The ROI was divided into 6 segments and if these were of good quality further analysis was performed. Segments of poor quality wererejected from furtherprocessing. Finally, strain curves were generated.The LA peak longitudinal strain (PALS) during the entire cardiac cycle lengthwas calculated from all LA segments obtained from 4C and 2C views.If some segments were excluded from the analysis, the calculation was made by averaging the remaining segments [19,26].

Laboratory tests

The concentrations of NT-proBNP were analyzed on the Elecsys 2010 platform (Roche Diagnostics, Mannheim, Germany). The total coefficient of variation (CV)was 4.6% at 426.5 pg/ml, (n=487) and 3.2% at 2308 pg/ml (n=495). The Kryptor platform (Brahms AG, Hennigsdorf Germany) was utilized for the analysis of the MR-proADM, the MR-proANPand copeptin. The intra assay CV for MR-proADM, was ≤ 10% for concentrations between 0.2 and 0.5 nmol/l, < 4% for concentrations between 0.5 nmol/l and 2 nmol/l, <2% for concentrations between 2 nmol/l and 6 nmol/l, and < 3.5% for concentrations over 6 nmol/l according to the manufacturer. The intra assay CV for MR-proANP was ≤ 5% for concentrations between 10 pmol/l and 20 pmol/l, < 3.5% for concentrations between 20 pmol/l and 1000 pmol/l, and < 3.5% for concentrations over 1000 pmol/l. Copeptin's CV was 4% at a concentration of 15 pmol/l (n=18) and 3.5% at concentrations of 100 pmol/l (n=18)[20,27].

Endpoint

The primary endpoint of the study was changes in MR-proANP, NT-proBNP, MR-proADM, and copeptin concentrations, as well as LAVmax, LAEF and PALS measurements following RFA of AF depending on possible recurrences and after adjustment for covariates such as age, gender, type of AF, rhythm and LVEF (baseline vs 4 months follow-up).

Statistical methods

For baseline data, categorical data were presentedas counts with

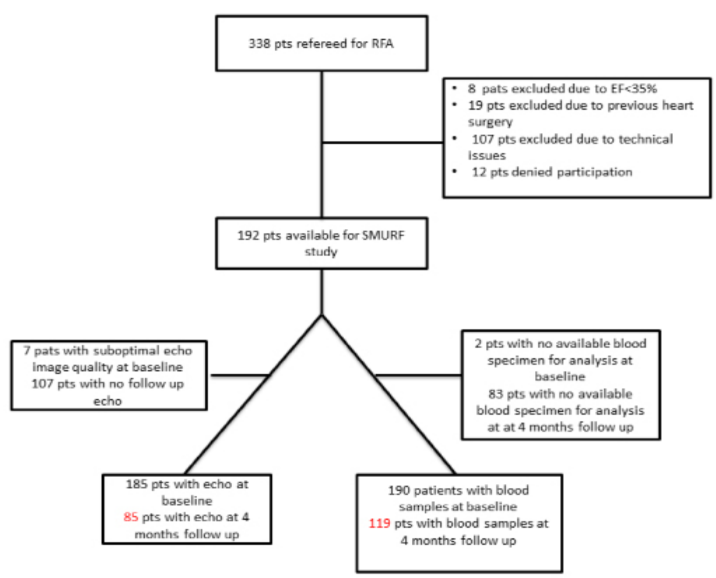

Figure 2: Flow chart of the study participation inclusion

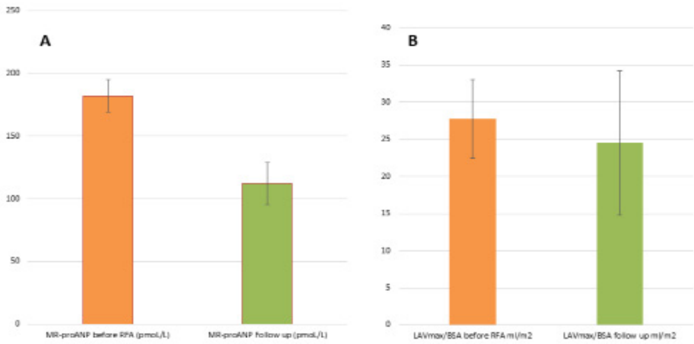

Figure 3: Effect of radiofrequency ablation on; A. MR-proANP, B. LAVmax/BSA Note: $P_{MR\text{-}proANP}$<0.001; $P_{LAVmax/BSA}$ <0.001.

percentages within brackets, continuous variables were expressed as means ± standard deviation (SD) andvariables not normally distributed were presented as medians with 25th and 75th percentiles within brackets. The normality of the samples was checked by the Kolmogorov-Smirnov test. The two-sample Student t-test, Mann-Whitney Utest, and x^2 test were used for comparison of normalcontinuous, not normally distributed, and categoricaldata, respectively.

As there was a substantial number of missing data in the primary study end points, statistical evaluation using mixed linear model was used.MR-proANP, NT-proBNP, copeptin, MR-proADM, LAEF, LAVmax and PALS were used as dependent variables in different mixed models' analyses. Time (baseline and at 4 months follow-up) was used as repeated variable. Unstructured repeated covariance type was chosen (in order to avoid the need for normally distributed data),and the presence of AF or any other atrial tachyarrhythmia during the follow-upperiod was used as fixed factorwith patient indicatoras a random intercept. The analyses were adjusted for covariates: age>65 years, gender, BMI > 30 kg/m2, type of AF (paroxysmal or persistent), rhythm at the time of blood sample retrieval or TTE(sinus rhythm (SR) or AF) and at the time of follow up, LVEF < 50 % and glomerular filtration rate of <60 ml/min/1.73 m2 (calculated by using a previously described cystatin-C formula) [28],hypertension, diabetes,additional ablation lesionsand cumulative delivered energy. Moreover, a sensitivity analysis was performed for the main outcome excluding patients with no follow up data.

The models were fit by an enter method, where all variables were entered into the original model stepwise. Variables with p values of >0.1 were thereafter removed.

Analysis of residuals and multicollinearity diagnostics were performedin order to validate the mixed model analyses.

All reported p values were two-sided and a p-value <0.05 was considered statistically significant. The analyses were performed using the SPSS 24.0 (SPSS, Chicago, IL, USA).

Results

In total, 192 patients with AF were included in the SMURF study (56 women and 136 men), whilethreewere lost from follow up. Blood samplesfrom119 patients were available for biomarker analysis and 85 patients underwent TTE at the 4-month follow-up(figure 2). The baseline characteristics are presented in detail in table 1.

The RFA procedural time was 188±49 min, the total RFA time was 40±13 min and the median cumulative energy delivery was 67280J (38088J, 96472J). Additional ablation lines were performed in 27 participants (table 2).

The complication rate was 3.7%. In total, three patients suffered from pericardial effusion, two of them requiring pericardiocentesis. Moreover, fourpatients developed peripheral vessel complication three patients suffered frompseudoaneurysm, and another patient developed a larger than normal hematoma of the groin [22].

A total of 87 (45 %)patients had a recurrence after a single RFA procedure during a follow up period of 143±36 days. At 4 months follow up,4 patients (3%) were in AF at the time of blood sampling and TTE, whereas 19 % of patients remained on antiarrhythmic drugs and75% onbetablockers.

RFA effect on biomarkers

The main finding was that a highly statistically significant reduction of all four biomarkers (MR-proANP, NT-proBNP as well as copeptin and MR-proADM) was demonstrated four months after RFA compared to baseline concentrations (MR-proANP: p<0.001(figure3); NT-proBNP p=0.019; copeptin p<0.001; MR-proADM p=0.008; table 3, table 4).

RFA effect on biomarkers depending on recurrences

It is interesting to note that patients who did not suffer any recurrence after RFA had lower concentrations of three of the biomarkers (MR-proANP, NT-proBNP and MR-proADM) at 4 months follow up compared to those who had recurrences (MR-proANPp<0.001 (figure

Table 3: Effect of RFA on the endocrine and structural function four months after RFA compared to baseline for the whole population; and for patients without recurrences compared to those who suffered recurrences 4 months after RFA, with their combined loading vectors

	Whole population	Patients without recurrences compared to patients with recurrences 4months after RFA
MR-proANPpg/mL	↓	↓
NT-proBNPpmol/L	↓	↓
Copeptinpmol/L	↓	N
MR-proADMpmol/L	↓	↓
LAVmax/BSA ml/m²	↓	N
LAEF %	N	N
PALS %	N	↑

Arrows pointing down are biomarkers or echocardiographic markers that lower their levels 4 months after RFA compared to baseline (or lower levels in patients without recurrences compared to patients with recurrences 4 months after RFA in the last column). Arrows pointing up are biomarkers or echocardiographic markers that showed higher levels 4 months after RFA compared to baseline (or higher levels in patients without recurrences compared to patients with recurrences 4 months after RFA in the last column). 'N' stands for no change after the RFA.
Abbreviations: AF: atrial fibrillation; BSA: body mass index; LAEF: left atrial ejection fraction; LAVmax: maximum left atrial volume; LVEF: left ventricular ejection fraction; PALS: peak atrial longitudinal strain; MR-proADM: mid-regional portion of pro-adrenomedullin; MR-proANP: mid-regional fragment of the N-terminal precursor of atrial natriuretic peptide; NT-proBNP: N-terminal pro B-type natriuretic peptide; RFA: radiofrequency ablation; SR: sinus rhythm.

Table 4: RFA effect on the endocrine and structural cardiac function

	N	Mean baseline (95% CI)	N	Mean follow up (95% CI)	Mean difference (95% CI)	P value
MR-proANPpg/mL	189	182.2 (169.5-194.8)	119	129.1 (112.2-154.7)	40.2 (29.3-51.1)	<0.001
NT-proBNPpmol/L	189	390.7 (330.4-451.1)	119	294 (221.5-366.6)	96.7 (16.2-177.1)	0.019
Copeptinpmol/L	189	17.04 (12.6-21.5)	119	9.02 (7.82-10.21)	8.02 (3.59-12.45)	<0.001
MR-proADMpmol/L	189	0.798 (0.77-0.826)	119	0.766 (0.734-0.798)	0.032 (0.009-0.056)	0.008
LAVmax/BSA ml/m²	180	27.7 (22.4-33.1)	81	24.5 (14.8-34.2)	3.23 (1.9-4.5)	<0.001
LAEF %	180	0.29 (0.25-0.34)	81	0.3 (0.25-0-35)	-0.007 (-0.03-0.02)	0.651
PALS %	185	16.9 (14-19.8)	85	15.5 (12.4-18.7)	1.4 (-0.2- 2.9)	0.08

Note 1: Data are presented in estimated means with 95% CI
Note 2: Analyses were performed via a mixed linear model method. Time (baseline and at 4 months follow up) was used as repeated variable. Unstructured repeated covariance type was chosen and the presence of any recurrence during the follow up was used as fixed factor with patient indicator as a random intercept. The analyses were adjusted for various covariates including age>65 years, BMI > 30 kg/m², type of AF, gender, rhythm at the time of blood sample retrieval or TTE(SR or AF), in respect to the dependent variable, LVEF < 50 % and glomerular filtration rate of <60 ml/min/1.73 m² (calculated by using a previously described cystatin-C formula). The potential statistical significances of those analyses are presented with P-values. Statistical significance in bold font
Abbreviations: AF: atrial fibrillation; BMI: body mass index; BSA: body mass index; CI: confidence interval; LAEF: left atrial ejection fraction; LAVmax: maximum left atrial volume; LVEF: left ventricular ejection fraction; PALS: peak atrial longitudinal strain; MR-proADM: mid-regional portion of pro-adrenomedullin; MR-proANP: mid-regional fragment of the N-terminal precursor of atrial natriuretic peptide; NT-proBNP: N-terminal pro B-type natriuretic peptide; RFA: radiofrequency ablation; SR: sinus rhythm; TTE: transthoracic echocardiogram.

4); NT-proBNP: p=0.01; MR-proADM: p=0.03; table 3, table 5))

However,the copeptin concentration at 4 months follow up did not differ between patients who suffered a recurrence and those who did not(copeptin: p=0.116; table 4).

RFA effect on echocardiographic parameters

LAVmaxcorrected for BSA was significantly lower at the 4 months follow up visit after RFA compared to baseline (p<0.001; table 3, table 4, figure 3).However, in the total group, LAEF and PALS showed no significant change 4 months after the RFA compared to baseline(LAEF: p=0.651; PALS: p=0.08; table 3, table 4).

Patients undergoing only pulmonary vein isolation (PVI) had higher LAEF and PALS, compared to those with PVI and additional lesions (LAEF p= 0.043, PALS p=0.044).

The interobserver variation for PALS using speckle tracking echocardiography was not statistically significant (Supplementary figure 1).

RFA effect on echocardiographic parameters depending on recurrences

Interestingly, PALS in patients with no documented recurrences was higher compared to those who had episodes of AF or AT (p=0.02; table 3, table 5, figure 4). However, no differences were observed in LAEF or LAVmaxcorrected for BSA between patients who suffered a recurrence and those who did not (LAEF: p=0.867; LAVmax /BSA: p=0.222; table 3, table 5).

Sensitivity Analysis

The sensitivity analysis excluding patients with no follow-up data did not result in any noteworthy change compared to the main analysis. The results of this analysis are presented in the supplement (Sensitivity analysis in the supplement).

Discussion

Given the amount of radiofrequency energy that is delivered during RFAof AF, it is important to studythe endocrine and mechanical cardiac function, due to the tissue injury that is produced through the procedure, and to evaluate the response to the healing process in the heart. We report the endocrine and mechanical consequences of the RFA procedure after four months of follow-up, which is a time window when it could be expected that the tissue damage has healed.

We assessed the cardiac endocrine function with two known ¨cardiac peptides¨MR-proANPand NT-proBNP. Furthermore, we analyzed copeptin and MR-proADM, which have intimate relation to both volume changes, but also to cardiac function. After adjusting for various well-known clinical covariates that might influence the level of the biomarkers evaluated, we found an improved endocrine function 4 months after RFA as appraised from changes in the concentration of the four biomarkers evaluated.

The atrial mechanical function was determined with LAVmax/BSA, LAEF and PALS by use of specle tracking methodology. The evaluation demonstrated a decrease of the LAVmax, andan improved LA strain in the patients without AF recurrences, a method more sensitive compared to volumetric measures [29]. As expected, we could not demonstrate any improvement of the LAEF after a follow-up period of four months, in concurrence with results from the literature [30].

Given previous reported results in the literature, and the size of thestudy population, this study providesa more detailed information about the effect of RFA on the endocrine and mechanical functions of the heart.

Effect of RFA on natriuretic peptides

Restoration of sinus rhythm leads to a sustained decrease in natriuretic peptides, a fact observed following electrical cardioversion [30, 31]. The

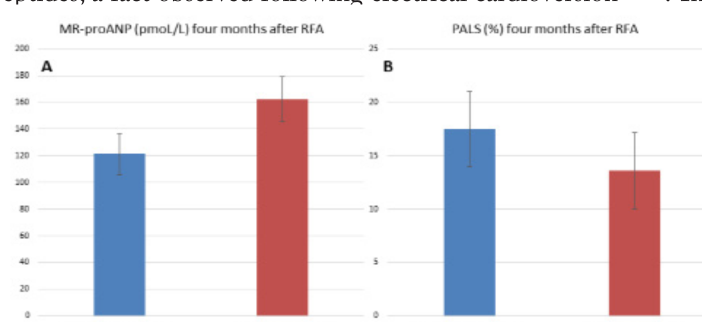

Figure 4: Effect of radiofrequency ablation on; A. MR-proANP, B. PALS depending on recurrences
Note: $P_{MR\text{-}proANP}$<0.001; PPALS=0.02

Table 5: **RFA effect on the endocrine and structural cardiac function depending on possible recurrences**

	N	Mean follow up (patients without recurrences) (95% CI)	N	Mean follow up (95% CI)	Mean difference (95% CI)	P value
(patients with recurrences)	Mean difference (95% CI)	P value	119	129.1 (112.2-154.7)	40.2 (29.3-51.1)	<0.001
MR-proANPpg/mL	66	121.4 (105.8-137)	53	162.5 (145.7-179.2)	-41.1 (-60.7 - -21.5)	<0.001
NT-proBNPpmol/L	66	197.1 (99.8-294.3)	53	391 (282.7-499.4)	-193.9 (-340 - -47.9)	0.01
Copeptinpmol/L	66	9.68 (8.28-11.1)	53	8.36 (6.86-9.87)	1.31 (-0.32 – 2.96)	0.116
MR-proADMpmol/L	66	0.738 (0.7-0.777)	53	0.793 (0.751-0.835)	-0.06 (-0.10 - -0.006)	0.03
LAVmax/BSA ml/m2	46	24.3 (5.3-43.4)	35	24.6 (3.7-45.5)	-0.25 (-3.18 – 2.68)	0.867
LAEF %	46	0.32 (0.26-0.38)	35	0.28 (0.23-0.34)	0.04 (-0.02-0.09)	0.222
PALS %	49	17.5 (14-21.1)	36	13.6 (10-17.2)	3.96 (0.645-7.29)	0.02

Note 1: Data are presented in estimated means with 95% CI
Note 2: Analyses were performed via a mixed linear model method. Time (baseline and at 4 months follow up) was used as repeated variable. Unstructured repeated covariance type was chosen and the presence of AF or any other atrial tachyarrhythmia during the follow-up was used as fixed factor with patient indicator as a random intercept, presence of any recurrence during the follow up period was used as fixed factor with patient indicator as a random intercept. The analyses were adjusted for various covariates including: age>65 years, BMI > 30 kg/m^2, type of AF gender, rhythm at the time of blood sample retrieval or TTE(SR or AF), in respect to the dependent variable, LVEF < 50 % and glomerular filtration rate of <60 ml/min/1.73 m^2 (calculated by using a previously described cystatin-C formula). The potential statistical significances of those analyses are presented with P-values. Statistical significance in bold font
Abbreviations: AF: atrial fibrillation; BMI: body mass index; BSA: body mass index; CI: confidence interval; LAEF: left atrial ejection fraction; LAVmax: maximum left atrial volume; LVEF: left ventricular ejection fraction; PALS: peak atrial longitudinal strain; MR-proADM: mid-regional portion of pro-adrenomedullin; MR-proANP: mid-regional fragment of the N-terminal precursor of atrial natriuretic peptide; NT-proBNP: N-terminal pro B-type natriuretic peptide; RFA: radiofrequency ablation; SR: sinus rhythm; TTE: transthoracic echocardiogram.

decrease in the concentration of the natriuretic peptides four months after RFA is most prominent in those with SR. However, regardless of arrhythmia recurrence, a decrease in biomarker concentration can be demonstrated, which is in accordance with previouspublished data. This can be attributed to the decrease of total arrhythmia burden in patients with recurrences [32]. Furthermore,improvement of the diastolic heart function [33] and LA reverse remodelling post AF ablation [34] can contribute to the improvement of the endocrine function of the heart due toa decrease of atrial myocardial wall tension.

RFA effect on copeptin and MR-proADM

Copeptin reveals information regarding volume, but also in cardiac wall tension. This has also been reported in a recent study from our group showing immediately increased levels of copeptin after RFA due to myocardial damage, fluid volume administration and endocrinestress response during RFA [20]. The decrease in copeptin concentration could possibly beattributed to the improvement of both systolic and diastolic heart function [33] after restoration of sinus rhythm, even though copeptin is not produced in the myocardium as far as we know today [20].

MR-proADM is a product of the parental molecule of adrenomedullin. In patients with HF,a ventricular-derived production of adrenomedullin is observed [35]. MR-proADMis also found to predict arrhythmia recurrence after RFA [36].

An increase in adrenomedullin usually reflects an overflow from local sites of production besides hemodynamic alterations and volume overload [12].Thus, increased levels of MR-proADM are found in patients with more severe cardiac disease [37]. Hence, we assume that decreased levels of MR-proADMafter RFA of AF found in this study reflect structural atrial reversed remodeling and atrial hemodynamic improvement. Finally, both copeptin and MR-proADM are sensitive biomarkers for volume changes in the body, something positively influenced by the restoration of SR.

RFA effect on mechanical function of LA

An important finding in our study was an improvement of PALS, and a reduction in LAV max/BSA, four months after RFA.

It has been reported that RFA can cause LA scarring that can influence LA structure and function negatively [18]. At the same time, RFA is a method designed to remove the underlying electrophysiological mechanism of AF, which leads to structural and functional LA remodeling, including changes in LA size, strain and LAEF [38].Previous studies have shown that enlargement of LA can be reversed after a successful catheter ablation. Thus, LA reverse remodeling after RFA may be an indicator of successful RFA[21, 39].

Studies have reported that atrial scarring and loss of myocardial mass caused by ablation prevent further LA dilatation.[40] However, LA systolic function can be impairedunder such circumstances [30]. Furthermore, Kim et al. reported that during a follow-upperiod of 12 months,40-70% of patients who underwent ablation for AF showed a decrease in LAEF [41], whereas one third of patients with electrocardiographic p wave restoration was not accompanied by hemodynamic evidence of effective atrial systole [30].These findings concur with the reduction of LAV with no concomitant increase of LAEF found in our study.

However, PALS might represent an earlier index of atrial function, and was improved in this evaluation.

Patients with AF have decreased global longitudinalstrain,possibly because of theatrial remodeling process [42]. During peak positive deformation (when PALS is measured), the LA is stretched, mainly due to venous return from the pulmonary veins, and functions as a reservoir. Therefore,higher PALS suggests better LA reservoir function [43]. Furthermore, LA strain has been shown to correlate with the progression of LA wall fibrosis in patients with AF. Hence, the improvement of PALS in patients without arrhythmia recurrence after RFA can be a marker of reverse remodeling of LA after ablation, even though the LAEF does not improve [44]. The pre-RFA fibrosis is a potentially important factor that is discussed by Packer as a possible variable to prevent an increased LAEF function post-RFA[30].

Additionally, PALS depends on the longitudinal movement of the LA wall while LAEF is more dependent on the LA contraction (i.e., radial movement). This can explain theobserved discrepancy between PALS and LAEF changes after RFA.

In conclusion, the mechanical parameters improved, and the tissue injury caused by the RFA process seems to be healed after four months of follow-up, as can be noted from the results above.

Limitations

This is a single-centre cohort study with a moderate sample size. Our cohort consists of patients with both paroxysmal and persistent AF, presenting in both SR and AFas well as normal and reduced EF. Additionally, weneitherrandomizedthe patients nor used a control groupwhere no ablation was carried out, thus our study can show association but not causality. Nevertheless, using a control group in such an occasion (invasive procedure)cannot constitute an alternative from an ethical point of view. Furthermore, we were not able to follow up all patients with TTE and blood sampling due to geographical and logistical difficulties as the patients were recruited from a large geographical area. This issue can raise some concerns regarding therisk of selection bias and hence, the robustness of our results.In order to address this issue, a mixed model analysis was chosen to deal with missing values, as all the data obtained were included in the analysis. Furthermore, no differences in baseline characteristics were observed between the group that we have followed up and those with no follow up data (supplementary table 1) and the sensitivity analysis excluding patients with no follow-up data did not result in any significant change compared to the main analysis (Sensitivity analysis in the supplementary)

In total, 87 patients (48 %) suffered a recurrence during the 4-months follow up. This constitutes a high percentage of recurrence in our population. This finding can be attributed to 1) our patients were subjected to a thorough follow up which,in clinical routine, is not as rigorous, 2)in similar studies, the first 3 months after the ablation procedure are considered 'blanking period' and the recurrences are not counted during this period. We choose four months as an appropriate follow-up time since the healing process to a large extent is completed at that time point.Thus, we regard the obtained results as relevant and informative.

Perspectives

In order to provide an optimal treatment, the effects of the treatment to target organs and the resulting mechanisms due to the treatment is fundamental. This study provides additional knowledge of both endocrine and mechanical changes after RFA of AF. The combination of data from cardiac, and, for the first time, extra-cardiac endocrine systems as well as the presentation of the mechanical function of the LA with a reproducible technique provide a clearer picture of the effect of RFA. Furthermore, the size of the population of this study and the broader inclusion criteria compared to previous studies further strengthens the results.[32, 34]

Taken together, we argue that the endocrine and mechanical function as assessed by natriuretic peptides, MR-proADM, LAVmax and strain before and after the ablation could be valid instruments to assess the impact of RFA of AF on the LA function.

Conclusions

In the present study, we demonstrate that despiteextensive atrial ablation during RFA of AF, the endocrine function of the heart, assessed by different biomarkers, is significantly improved four months after the index procedure. Patients with no arrhythmia recurrence showed a more pronounced improvement in their endocrinal function.

As a result of the RFA procedure a reduction of LAV could be demonstrated, as well as an improvement of LA strain four months after the index procedure. However, the LAEF did not increase during the follow-up period.

Statements

Informed consent and ethical considerations

The protocol of this study has been approved by the Regional Ethical Review Board of the Faculty of Health Sciences, Linköping, Sweden, (Dnr 2011/40-31, 2012/226-32). Written informed consent was obtained from all patients. The study complies with the Declaration of Helsinki[45].

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

EC, LK, UA and HW designed the study. EC analysed the data and drafted the manuscript. EC, LK, C-JC, IL, AHJ, HW and UA interpreted the results and edited the manuscript critically. All the co-authors have read and accepted this version of the manuscript.

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