SUPPLEMENTAL MATERIAL

SUPPLEMENTAL METHODS

Assessment of Effective Refractory Period & Sinus Node Function

Effective refractory period (ERP) at the proximal and distal coronary sinus was performed. Briefly, ERPs were measured at two cycle lengths (600ms/400ms) using a pacing drive train of 8 beats followed by a single extrastimulus commencing at a coupling interval of 150ms and incrementing by 10ms until local capture. The ERP was defined as the longest extrastimulus that failed to capture. Sinus node recovery time (SNRT), evaluated by 30 second burst pacing (600ms/500ms/400ms), was determined as the longest time from the stimulus artefact to the earliest atrial activity. The corrected SNRT was determined by correcting for the underlying sinus cycle length.

Ablation Procedure

Wide antral circumferential pulmonary vein isolation (PVI) was achieved utilizing an irrigated-tip radiofrequency ablation catheter (power: 25W [posteriorly] – 30W; contact force: 10-40g anteriorly, 10-25g posteriorly). The primary procedural endpoint was demonstration of entrance and exit block of all pulmonary veins following adenosine and 30 minute waiting period.

Electrogram Analysis

Bipolar intracardiac electrograms and 12-lead surface electrocardiography (ECG) were recorded simultaneously on a digital amplifier system (EPMed Systems, NJ). Intracardiac electrograms were filtered from 30 to 500 Hz.

For activation time, points was analysed at 200 mm/s sweep speed and annotated at the maximum negative dV/dt for unipolar signals or the peak sharp of the bipolar electrogram. Although data was collected using the Confidense algorithm, an internal point filter to within

5mm of the chamber surface geometry was applied and all acquired points were manually annotated. Only points demonstrating near-field characteristics of at least 2 sharp peaks and consistent with anatomically adjacent signals in terms of signal quality and electrogram timing were included. Points that did not fit these criteria, ectopic beats and artefact were excluded. Following extensive annotation, signal processing was also performed offline (MATLAB 9.1, Mathworks, MA, USA).

Conduction Velocity Analysis

To determine conduction velocity, the polynomial algorithm assigns a fitting 'window' per region with a minimum of 20 points required. Each region is assigned subsets of Cartesian coordinates in space and activation time. These are fitted to a smooth polynomial surface in three-dimensional space, using a standard least squares algorithm, which provides robustness against outliers. The gradient is then calculated from the fit and used to calculate velocity components, with final velocities calculated from the weighted average of velocity components from every fit including that point.

Follow-up and Arrhythmia Detection

Antiarrhythmic medications and anticoagulation were managed according to physician preference and current guidelines. In general, antiarrhythmics were stopped in the absence of arrhythmia at 4-6 weeks. Recurrence was defined as documented AF or atrial tachycardia (AT) >30s following a 3-month blanking period. Time to recurrence was determined using either cardiac rhythm management devices (pre-existing pacemaker or insertable cardiac monitor [ICM – Medtronic Linq™, Minneapolis, MN, USA]) or Holter monitoring at 3, 6, and 12 months post-ablation followed by 6-monthly thereafter until study completion. Repeat ablation procedures were performed in patients with refractory symptomatic recurrent atrial

arrhythmia with adjunctive lesion sets (cavotricuspid isthmus and posterior wall isolation) performed according to operator preference.

Statistical Analysis

Data analysis was performed using SPSS software (Version 23, IBM, Armonk, New York). Normality of all quantitative variables was assessed using the Shapiro-Wilk test.

A mixed random effects model was used for comparison between multiple regional measures within each patient (ie. CV, voltage). To investigate regional variation, region (6 LA segments) and group (male and female) were modelled as fixed effects with a group x region interaction term.

Conventional two-group comparisons were made using unpaired t test for continuous variables, or the chi-squared test for categorical variables for data (ie. Sex, LA size).

General linear model analysis was also performed separately on each parameter to determine univariable predictors of voltage, CV and complex signals. Following univariable analysis, all predictors with $p<0.1$ were included in a multivariable model. Age and AF duration adjustment was also performed given non-significant differences between groups. Survival curves for freedom from arrhythmia (AF/atrial tachycardia [AT]) were determined using the Kaplan-Meier method and compared using the log-rank test. Cox proportional hazards regression was used to determine univariable ($p<0.1$), age-adjusted, AF-duration adjusted and multivariable predictors of arrhythmia-free survival following single and multiple procedures. Of the electrophysiologic variables which denote substrate with $p<0.1$, voltage was included in the multivariable model given its reproducible and robust association with atrial fibrosis[29]. Subgroup analysis was also performed between males and females for each variable (ie. AF phenotype).

SUPPLEMENTAL TABLES

Supplemental Table I. Electrophysiological substrate analysis stratified by atrial fibrillation phenotype and sex.

Substrate Parameter	Male	Female	p-value
Bipolar Voltage (mV)			
Paroxysmal AF	1.92 ± 0.22	1.62 ± 0.24	p(group)<0.001
Persistent AF	1.48 ± 0.25	1.10 ± 0.17	p(interactive*)=0.252
Low Voltage Points (%)			
Paroxysmal AF	18.4 ± 4.3	24.3 ± 6.5	p(group)=0.021
Persistent AF	26.9 ± 5.3	34.2 ± 5.8	p(interactive*)=0.124
Conduction Velocity (cm/s)			
Paroxysmal AF	46.6 ± 6.0	37.8 ± 7.2	p(group)=0.001
Persistent AF	40.2 ± 6.3	28.5 ± 3.6	p(interactive*)=0.775
Complex Signals			
Paroxysmal AF	4.2 ± 2.3	8.7 ± 1.7	p(groups)<0.001
Persistent AF	7.4 ± 1.8	13.0 ± 1.9	p(interactive*)=0.643

Values are mean±SD. *interactive: group x atrial fibrillation phenotype.

Supplemental Table II. Sinus Node Function and Atrial Refractory Periods

	AF Patients		
	Male	**Female**	**p-value**
CSNRT 600ms (ms)	326 ± 250	283 ± 169	0.583
CSNRT 500ms (ms)	403 ± 163	391 ± 343	0.919
CSNRT 400ms (ms)	365 ± 118	453 ± 212	0.382
CSd ERP 600ms (ms)	257 ± 45	265 ± 69	0.699
CSd ERP 400ms (ms)	229 ± 29	232 ± 43	0.809
CSp ERP 600ms (ms)	236 ± 38	232 ± 43	0.892
CSp ERP 400ms (ms)	224 ± 28	234 ± 24	0.431

CSNRT = Corrected Sinus Node Recovery Time; CSd = Distal Coronary Sinus; CSp = Proximal Coronary Sinus, ERP = Effective Refractory Period

Supplemental Table III. Univariable and multivariable analysis of voltage, conduction velocity and complex signals

	Univariate		Multivariate	
Bipolar Voltage	**B coefficient (95% CI)**	**p-value**	**B coefficient (95% CI)**	**p-value**
Age	-1.86 (-2.39 to -1.32)	<0.001	-0.03 (-0.05 to -0.01)	0.001
Female Gender	-0.46 (-0.76 to -0.16)	<0.001	-0.28 (-0.67 to -0.08)	0.028
Persistent AF	-0.32 (-0.61 to -0.02)	0.005	-0.38 (-0.86 to -0.04)	0.019
AF Duration (months)	-0.003 (-0.006 to -0.001)	0.038	-0.001 (-0.005 to 0.002)	0.256
Body Mass Index	-0.01 (-0.05 to 0.02)	0.266		
Obesity (BMI>30)	-0.26 (-0.57 to 0.06)	0.046	0.16 (-0.73 to 1.06)	0.513
Presenting Lab Rhythm AF	-0.16 (-0.47 to 0.16)	0.102		
Hypertension	-0.19 (-0.51 to 0.12)	0.141		
Dyslipidemia	-0.05 (-0.37 to 0.27)	0.654		
Diabetes Mellitus	-0.19 (-0.59 to 0.98)	0.728		
Ischaemic Heart Disease	-0.03 (-0.48 to 0.55)	0.933		
Stroke/TIA	-0.10 (-0.80 to 1.01)	0.783		
CHA_2DS_2VA	-0.15 (-0.28 to -0.02)	0.016	-0.04 (-0.22 to 0.14)	0.477
OSA	-0.06 (-0.39 to 0.28)	0.710		
Regular Alcohol Intake	-0.17 (-0.47 to 0.13)	0.157		
Smoker	-0.39 (-1.16 to 0.38)	0.216		
ERT	0.42 (-0.22 to 1.05)	0.111		
LVEF	0.01 (-0.02 to 0.01)	0.093	0.01 (-0.03 to 0.01)	0.058
LA Size	-0.01 (-0.02 to 0.00)	0.016	-0.01 (-0.02 to 0.01)	0.382
LA Area	-0.01 (-0.02 to 0.00)	0.125		

Valvular pathology (mild-moderate)	-0.03 (-0.31 to 0.47)	0.587		
Conduction Velocity				
Age	-0.27 (-0.76 to 0.21)	0.134		
Female Gender	-10.52 (-19.43 to -1.62)	0.002	-9.42 (-17.02 to -1.39)	0.012
Persistent AF	-6.52 (-15.31 to 2.24)	0.037	-8.66 (-19.94 to -1.19)	0.047
AF Duration (months)	-0.002 (0.04 to -0.01)	0.562		
Body Mass Index	-0.19 (-1.16 to 0.78)	0.593		
Obesity (BMI>30)	-0.80 (-9.92 to 8.33)	0.858		
Presenting Lab Rhythm AF	-3.71 (-12.94 to 5.60)	0.463		
Hypertension	-4.20 (-13.42 to 5.02)	0.129		
Dyslipidemia	-0.27 (-9.46 to 9.10)	0.908		
Diabetes Mellitus	-3.17 (-13.12 to 14.46)	0.843		
Ischaemic Heart Disease	-0.68 (-15.71 to 14.36)	0.585		
Stroke/TIA	-7.45 (-33.99 to 19.11)	0.470		
CHA_2DS_2VA	-3.56 (-7.38 to -0.20)	0.013	-2.45 (-6.78 to 1.87)	
OSA	-1.54 (-11.46 to 8.38)	0.793		
Regular Alcohol Intake	-0.04 (-8.87 to 8.79)	0.896		
Smoker	-0.01 (-12.91 to 10.22)	0.994		
ERT	0.89 (-7.41 to 7.20)	0.892		
LVEF	0.34 (0.11 to 0.79)	0.125		
LA Size	-0.35 (-0.69 to -0.01)	0.006	-0.23 (-0.58 to 0.13)	0.075
LA Area	-0.62 (-1.59 to 0.34)	0.078		
Valvular pathology (mild-moderate)	-1.87 (-5.06 to 2.26)	0.132		

Complex Signals				
Age	0.11 (0.03 to 0.30)	0.078	0.36 (-0.05 to 0.77)	0.428
Female Gender	4.88 (1.41 to 8.34)	<0.001	5.32 (3.34 to 9.41)	0.007
Persistent AF	3.22 (0.14 to 6.42)	0.004	6.41 (3.05 to 8.88)	0.572
AF Duration (months)	0.01 (-0.03 to 0.05)	0.495		
Body Mass Index	0.04 (-0.41 to 0.34)	0.740		
Obesity (BMI>30)	0.62 (-2.88 to 4.12)	0.707		
Presenting Lab Rhythm AF	1.80 (-5.43 to 1.82)	0.451		
Hypertension	1.33 (-2.28 to 4.95)	0.937		
Dyslipidemia	1.78 (-5.43 to 1.88)	0.163		
Diabetes Mellitus	1.71 (1.56 to 5.81)	0.750		
Ischaemic Heart Disease	2.41 (-8.28 to 3.46)	0.721		
Stroke/TIA	2.68 (-7.76 to 13.12)	0.500		
CHA_2DS_2VA	0.69 (-0.86 to 2.19)	0.256		
OSA	0.24 (-3.64 to 4.13)	0.715		
Regular Alcohol Intake	0.84 (-2.61 to 4.31)	0.384		
Smoker	0.91 (-9.76 to 7.93)	0.778		
ERT	-1.03 (-5.69 to 2.62)	0.382		
LVEF	-0.09 (-0.09 to 0.27)	0.959		
LA Size	0.07 (-0.22 to 0.07)	0.166		
LA Area	0.15 (-0.21 to 0.51)	0.253		
Valvular pathology (mild-moderate)	0.85 (-2.72 to 3.01)	0.791		

Abbreviations: AF – Atrial Fibrillation; CI – Confidence Interval; CHA_2DS_2-VA – Heart Failure, Hypertension, Age, Diabetes, Stroke, Vascular Disease; ERT - Estrogen Replacement

Therapy; IHD – Ischaemic Heart Disease; OSA – Obstructive Sleep Apnea; LA – Left Atrial; LV – Left Ventricular; TIA – Transient Ischemic Attack

Supplemental Table IV. Electrophysiological substrate analysis of sex differences adjusted by age and AF duration, respectively.

Substrate Parameter	**Male**	**Female**	**Age-adjusted**		**AF-duration adjusted**	
			B Coefficient (95% CI)	**P-value**	**B Coefficient (95% CI)**	**P-value**
Bipolar Voltage (mv)	1.84 ± 0.15	1.45 ± 0.17	-0.16 (-0.53 to -0.05)	0.037	-0.24 (-0.77 to 0.10)	<0.001
Low Voltage Points (%)	23.9 ± 15.4	31.7 ± 17.5	27.0 (2.60 to 65.84)	0.033	20.63 (5.40 to 35.86)	<0.001
Conduction Velocity (cms/s)	44.1 ± 6.9	34.9 ± 6.1	-6.59 (-10.81 to -0.13)	0.012	-11.03 (-30.41 to -2.09)	0.029
Complex Signals (%)	6.0 ± 1.7	9.9 ± 1.7	5.79 (0.74 to 13.77)	0.041	6.41 (0.12 to 12.95)	0.007

Supplemental Table V. Procedural characteristics

	Male (n=74)	**Female (n=42)**	**p-value**
Procedure time (mins)	141 ± 26	138 ± 41	0.645
Fluoroscopy time (mins)	10.2 ± 5.3	8.5 ± 3.1	0.098
Radiation dose (mGy x cm^2)	15 927 ± 9 125	15 152 ± 8 457	0.315
Total RF time (mins)	37 ± 8	35 ± 9	0.235
Peri-procedural DCR (%)	27 (36)	16 (38)	0.923
PV isolation (%)	74 (100)	42 (100)	1.000
Cavotricuspid isthmus ablation (%)	12 (16)	5 (12)	0.586

Abbreviations: DCR – Direct Current Cardioversion; PV – Pulmonary Vein

Supplemental Table VI. Follow-up data

	Male (n=72)*	**Female (n=41)***	**p-value**
Continuous Monitoring (%)	40 (56)	21 (51)	0.455
Mean surveillance time (months)	22.9 ± 7.5	21.8 ± 8.7	0.425
Holter Monitoring (%)	32 (44)	20 (49)	0.539
Mean surveillance time (hours)	174 ± 29	167 ± 34	0.310
Mean follow-up (months)	23 ± 8	21 ± 9	0.144
Post-ablation antiarrhythmic therapy			
- Any antiarrhythmic (%)	12 (17)	14 (34)	0.037
- Flecainide (%)	2 (3)	7 (17)	0.010
- Sotalol (%)	5 (7)	2 (5)	0.658
- Amiodarone (%)	5 (7)	5 (12)	0.374

*3 patients lost to study follow-up (2 males, 1 female)

Supplemental Table VII. Subgroup analyses of single-procedure arrhythmia-free survival stratified by sex.

Subgroup	**Male**	**Female**	**Hazard Ratio (95% CI)**	**P value for interaction**
	no. of events/no. of patients			
Type of Atrial Fibrillation				0.540
Paroxysmal AF	4/33	8/21	3.27 (1.02-10.85)	
Persistent AF	14/39	11/20	1.77 (0.95-3.91)	
Age				0.712
<65 years	11/45	9/22	1.66 (0.67-4.13)	
≥65 years	7/27	10/19	2.12 (0.79-5.70)	
Body Mass Index				0.167
<30 kg/m^2	11/49	13/24	2.41 (1.44-7.29)	
≥30 kg/m^2	7/23	6/17	1.16 (0.43-2.02)	
Hypertension				0.361
No	10/46	13/26	2.12 (1.04-5.19)	
Yes	8/26	6/15	1.20 (0.38-3.78)	
CHADSVA Score				0.910
<2	13/48	12/25	1.86 (0.83-4.14)	
≥2	5/24	7/16	1.69 (0.49-5.84)	
Obstructive Sleep Apnoea				0.380
No	9/48	12/29	2.58 (1.09-6.14)	
Yes	9/24	7/12	1.45 (0.54-3.92)	
Alcohol Intake				0.221
No	7/35	11/26	1.83 (1.16-7.30)	
Yes	11/37	8/15	1.68 (0.87-3.56)	

Flecainide				0.407
No	14/57	9/24	1.32 (0.53-3.32)	
Yes	4/15	10/17	2.52 (0.77-8.26)	
Sotalol				0.308
No	13/48	14/31	1.43 (0.65-3.13)	
Yes	5/24	5/10	2.64 (0.81-7.45)	
LVEF				0.136
<50%	3/17	6/11	4.14 (1.37-9.44)	
≥50%	15/55	13/30	1.39 (0.66-2.93)	
Valvular pathology				0.649
No	15/57	15/32	1.823 (0.88-3.78)	
Yes	3/15	4/9	2.69 (0.60-8.42)	

Abbreviations: AF – Atrial Fibrillation; CI – Confidence Interval; CHA_2DS_2-VA – Heart Failure, Hypertension, Age, Diabetes, Stroke, Vascular Disease; OSA – Obstructive Sleep Apnea; LVEF – Left Ventricular Ejection Fraction

Supplemental Table VIII: Age-adjusted and AF duration-adjusted analysis of single-procedure arrhythmia-free survival

Variable	**Age-adjusted**		**AF-duration adjusted**	
	Hazard ratio (95% CI)	**p-value**	**Hazard ratio (95% CI)**	**p-value**
Age	1.02 (0.94-1.09)	0.294	1.01 (0.99-1.01)	0.481
Female Gender	2.16 (1.21-4.18)	0.021	2.11 (1.10-4.08)	0.026
Persistent AF	2.85 (1.33-6.14)	0.007	2.25 (1.11-4.55)	0.024
AF Duration (months)	1.01 (0.99-1.01)	0.481	1.00 (0.99-1.01)	0.685
Body Mass Index	1.02 (0.93-1.08)	0.656	1.21 (0.94-1.08)	0.847
Obesity (BMI>30)	1.65 (0.70-4.04)	0.244	1.15 (0.42-1.74)	0.657
Presenting Lab Rhythm AF	1.34 (0.67-2.67)	0.408	1.44 (0.74-2.79)	0.286
Hypertension	1.18 (0.39-1.70)	0.585	1.14 (0.43-1.73)	0.678
Dyslipidemia	1.21 (0.59-2.46)	0.605	1.37 (0.70-2.66)	0.360
Diabetes Mellitus	1.43 (0.39-4.99)	0.702	1.11 (0.27-4.62)	0.888
Ischaemic Heart Disease	1.10 (0.27-2.98)	0.862	1.13 (0.27-2.90)	0.846
Stroke/TIA	1.33 (0.49-5.02)	0.676	1.42 (0.32-4.14)	0.728
CHA_2DS_2VA	1.05 (0.71-1.29)	0.749	1.04 (0.73-1.28)	0.811
OSA	1.64 (0.82-3.27)	0.159	1.77 (0.92-3.42)	0.087
Regular Alcohol Intake	1.25 (0.91-1.68)	0.647	1.09 (0.48-1.75)	0.783
Estrogen Replacement Therapy	0.72 (0.38-1.57)	0.438	0.79 (0.24-1.83)	0.355
LVEF (%)	0.97 (0.99-1.09)	0.070	0.96 (0.99-1.07)	0.153
LA Size	1.01 (0.97-1.02)	0.684	1.01 (0.97-1.02)	0.630
LA Area	1.03 (0.93-1.07)	0.894	1.00 (0.93-1.07)	0.862
Valvular Pathology (Mild-moderate)	1.47 (0.68-3.25)	0.208	1.74 (0.77-3.84)	0.314

Electrophysiologic Parameters Bipolar Voltage (per -1mV)	1.93 (1.03-3.28)	0.040	1.76 (1.01-3.07)	0.046
Low voltage points (%)	1.01 (1.00-1.05)	0.012	1.02 (1.01-1.06)	0.038
Conduction Velocity (per -1cm/s)	1.02 (0.99-1.04)	0.212	1.02 (0.98-1.03)	0.251
Complex Signals (%)	1.04 (1.00-1.09)	0.036	1.05 (1.01-1.09)	0.021

Supplemental Table IX: Univariable and multivariable analysis of multi-procedure arrhythmia-free survival

	Univariable		**Multivariable**	
Variable	**Hazard ratio (95% CI)**	**p-value**	**Hazard ratio (95% CI)**	**p-value**
Age	1.03 (0.98-1.12)	0.329		
Female Gender	2.79 (1.36-5.88)	0.007	2.26 (1.01-5.06)	0.049
Persistent AF	2.56 (1.15-5.82)	0.021	2.73 (1.16-6.62)	0.031
AF Duration (months)	1.01 (0.99-1.01)	0.119		
Body Mass Index	1.01 (0.93-1.10)	0.875		
Obesity (BMI>30)	1.65 (0.70-4.04)	0.244		
Presenting Lab Rhythm AF	1.28 (0.61-2.70)	0.520		
Hypertension	1.19 (0.38-1.87)	0.674		
Dyslipidemia	1.38 (0.63-2.86)	0.442		
Diabetes Mellitus	1.52 (0.35-6.25)	0.569		
Ischaemic Heart Disease	1.32 (0.45-5.56)	0.708		
Stroke/TIA	1.28 (0.46-3.57)	0.637		
CHA_2DS_2VA	1.08 (0.74-1.43)	0.857		
OSA	1.54 (0.77-3.23)	0.245		
Regular Alcohol Intake	1.96 (0.91-4.17)	0.128		
Estrogen Replacement Therapy	0.53 (0.16-1.74)	0.291		
LVEF (%)	0.96 (0.92-1.02)	0.097	0.96 (0.94-1.02)	0.064
LA Size	1.01 (0.99-1.04)	0.259		
LA Area	1.02 (0.93-1.10)	0.717		
Valvular Pathology (Mild-moderate)	1.61 (0.56-4.67)	0.379		

Electrophysiologic Parameters Bipolar Voltage (per -1mV)	2.63 (1.39-4.76)	0.003	1.75 (0.87-3.57)	0.116
Low voltage points (%)	1.03 (1.01-1.05)	0.004		
Conduction Velocity (per -1cm/s)	1.02 (0.99-1.04)	0.294		
Complex Signals (%)	1.05 (1.01-1.09)	0.035		

Abbreviations: AF – Atrial Fibrillation; CI – Confidence Interval; CHA_2DS_2-VA – Heart Failure, Hypertension, Age, Diabetes, Stroke, Vascular Disease; IHD – Ischaemic Heart Disease; OSA – Obstructive Sleep Apnea; LA – Left Atrial; LV – Left Ventricular; TIA – Transient Ischemic Attack