Methods

Paroxysmal and persistent AF

Paroxysmal atrial fibrillation (AF) is defined by a spontaneous onset and offset of rapid irregular atrial activation with irregular ventricular responses. We included for analyses the paroxysmal AF episodes that lasted for at least 10 min. Persistent AF includes patients with continuous AF throughout the recording period. We recorded a total of 56 hospitalized patients at IU Health Methodist Hospital, Indianapolis, Indiana, USA between April 2015 and December 2017. These patients included 39 with a history of paroxysmal AF and 17 with persistent AF. Among the patients with paroxysmal AF, 26 had no AF episodes during recording and 5 had the AF lasting less than 10 min. Five patients with persistent AF were excluded because of pacing artifacts during recording. The remaining 8 patients with paroxysmal AF and 12 patients with persistent AF were included in this study. The data from 5 of the 8 patients with paroxysmal AF were used in previous reports to study the relationship between SKNA and arrhythmia onset/termination.[1, 2]

Average SKNA and VR during AF

We used a modified portable ME6000 Biomonitor (Mega Electronics Ltd, Finland) for data acquisition. The sampling rate was 10,000 /s. All patients had the first pair of bipolar electrodes placed in the right and left subclavian areas (ECG Lead I) and a second bipolar pair placed on the left arm.[1] We analyzed recordings from both channels using custom-written software. The neuECG signals were amplified and band-pass filtered between 0.05 Hz and 150 Hz to display ECG and between 500 Hz and 1000 Hz to show the skin sympathetic nerve activity (SKNA). We full-wave rectified and integrated all digitized SKNA signals every 100 ms and displayed the results over time to simulate the display methods of microneurography (iSKNA, μV.s).[3] Furthermore, we divided the total voltage by the number of digitized samples in the same window to obtain the average of SKNA (aSKNA, μV).[4] We manually detected the AF episodes

during entire recording period in each patient and excluded the time periods with artifacts and noise. The aSKNA of lead I and VR in each 60-s window were determined and used for analyses.

Burst analyses

We plotted the proportion of amplitude distribution of all aSKNA recorded from each patient to visualize two groups of nerve activities: the baseline (non-burst) nerve activity and the high amplitude bursting activity. These two groups of nerve activity are then fitted with expectation-maximization method to identify two Gaussian distributions. From the Gaussian representing the lower amplitude activity, the mean plus 3 times standard deviation was used as the threshold amplitude for burst determination.[2] All 1-min aSKNA values above threshold were detected and the area between aSKNA and baseline of every 1-min segment was calculated and added together as burst area (μV*min). The SKNA burst parameters were calculated as below: Burst frequency (bursts/hr) = Burst episodes / Total duration of the data window analyzed (hr); Burst duration (%) = Total burst duration (hr) / Total duration of the data window analyzed (hr) × 100; Total burst area (μV*min) = Sum of burst area in the data window analyzed. Each burst was classified by quartiles of the burst area as below: quartile 1 = below 25 percentile; quartile 2 = between 25 – 50 percentile; quartile 3 = between 50 – 75 percentile; quartile 4 = above 75 percentile. We also detected the maximal aSKNA and VR during each SKNA burst.

Statistical analysis

Continuous variables were summarized by median (Interquartile range). Categorical variables were summarized by frequency and percentage. The Mann-Whitney U test was used to compare continuous measures and the chi-square test was used for dichotomous variables.

The Wilcoxon signed rank test was used within group comparisons. Pearson's or Spearman's correlation coefficient (r value) was used to measure linear correlation between two variables. Correlation coefficients were transformed into Fisher's z score for generating overall estimates and were transformed to its origin format with the meta R-package (version 4.9-4). Box-and-whiskers plots show medians and 25th and 75th percentiles, and the whiskers show the minimum and maximum excluding outlying values. Black dot indicates outlying value. In forest plots of correlation coefficient, each patient is represented by a line. Patient ID of paroxysmal and persistent AF are indicated by A-H and I-T, respectively. Second column from left (Study duration or Burst episodes) shows study duration (min) of each patient or the number of SKNA bursts in each patient and sum of all data. The mid-point of the gray box and box area indicate the point effect estimate and the weight given for each patient, respectively. The gray diamond shows the overall effect estimates of correlation coefficient with 95% CI. The overall correlation coefficient of random effect model was used to test the difference between two correlations. Heterogeneity was examined using Higgin's I-squared and Tau-squared. Multiple regression analysis with standard least squares were performed to determine the contribution of clinical variables to VR during AF. We averaged the repeated measures for each person, which was then used to fit the model for multiple regression analysis. Generalized additive mixed-effects models were used to characterize the trajectory over 24-hr. Two-sided p values ≤ 0.05 were considered statistically significant. The statistics were analyzed using JMP Pro (version 14, SAS Institute Inc, Cary, NC).

References

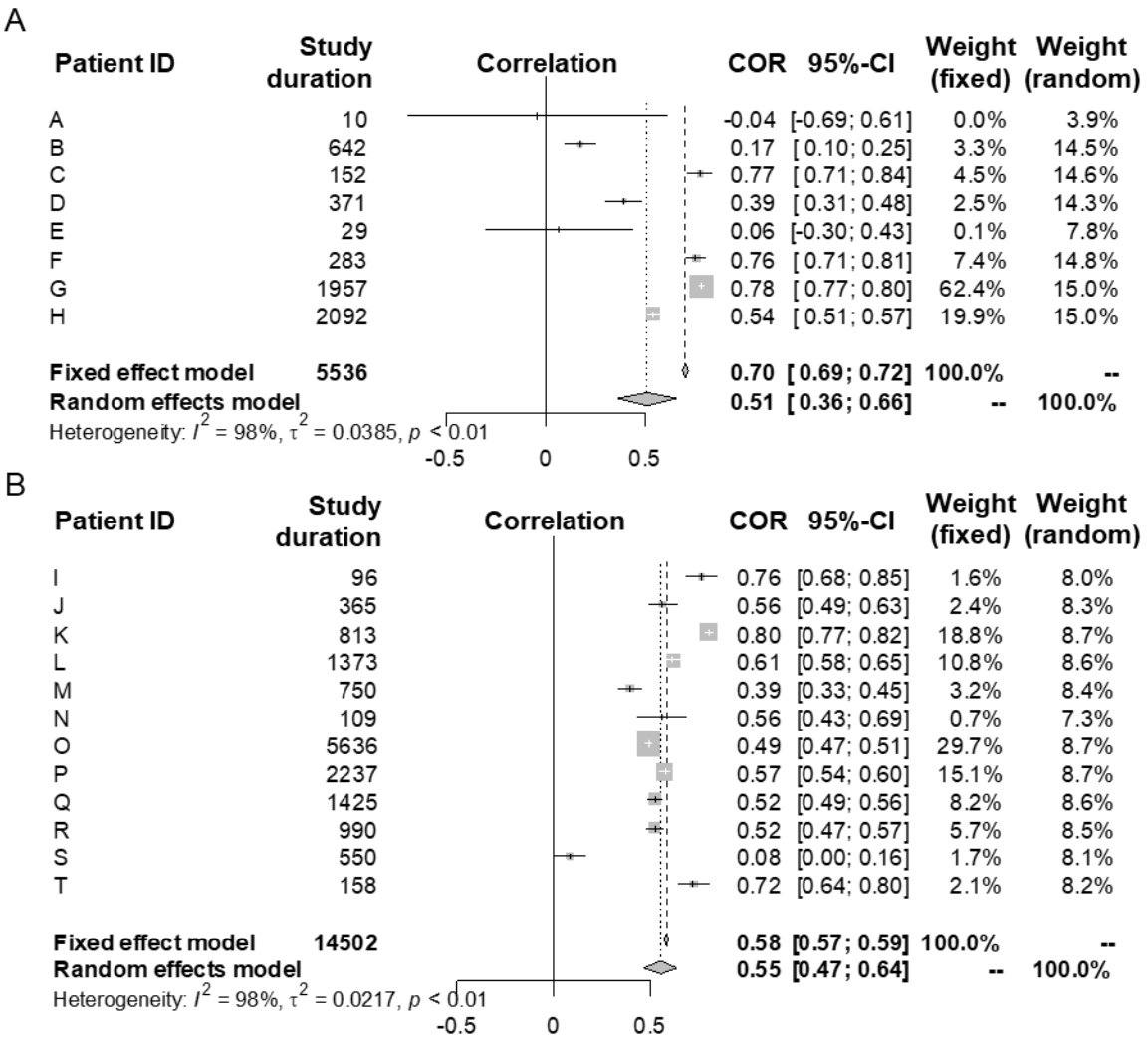
1. Uradu A, Wan J, Doytchinova A, et al. Skin sympathetic nerve activity precedes the onset and termination of paroxysmal atrial tachycardia and fibrillation. Heart rhythm 2017;14:964-971.

2. Kusayama T, Wan J, Doytchinova A, et al. Skin sympathetic nerve activity and the temporal clustering of cardiac arrhythmias. JCI insight 2019;4:e125853.

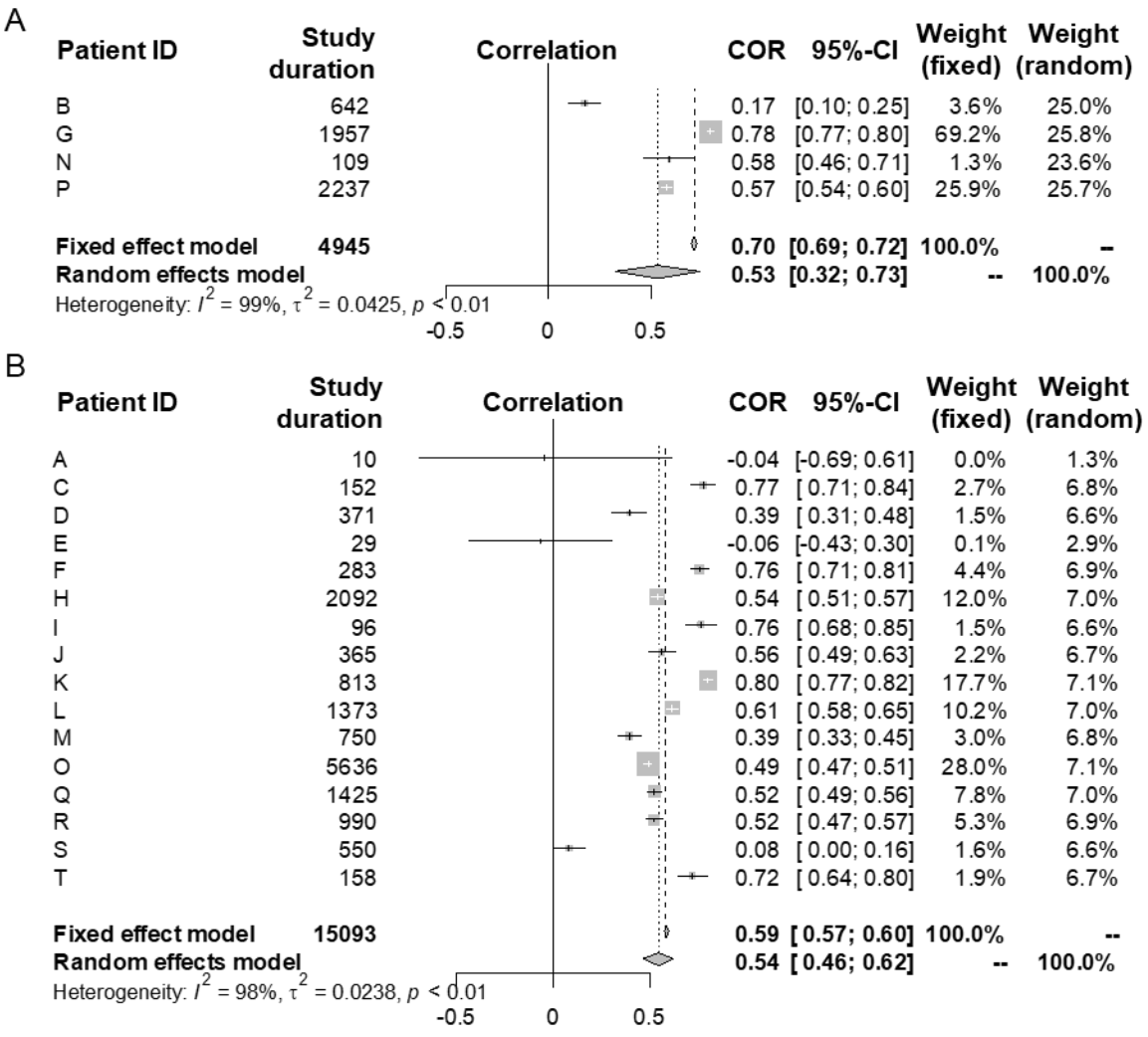
3. White DW, Shoemaker JK, Raven PB. Methods and considerations for the analysis and standardization of assessing muscle sympathetic nerve activity in humans. Autonomic neuroscience : basic & clinical 2015;193:12-21.

4. Doytchinova A, Hassel JL, Yuan Y, et al. Simultaneous noninvasive recording of skin sympathetic nerve activity and electrocardiogram. Heart rhythm 2017;14:25-33.

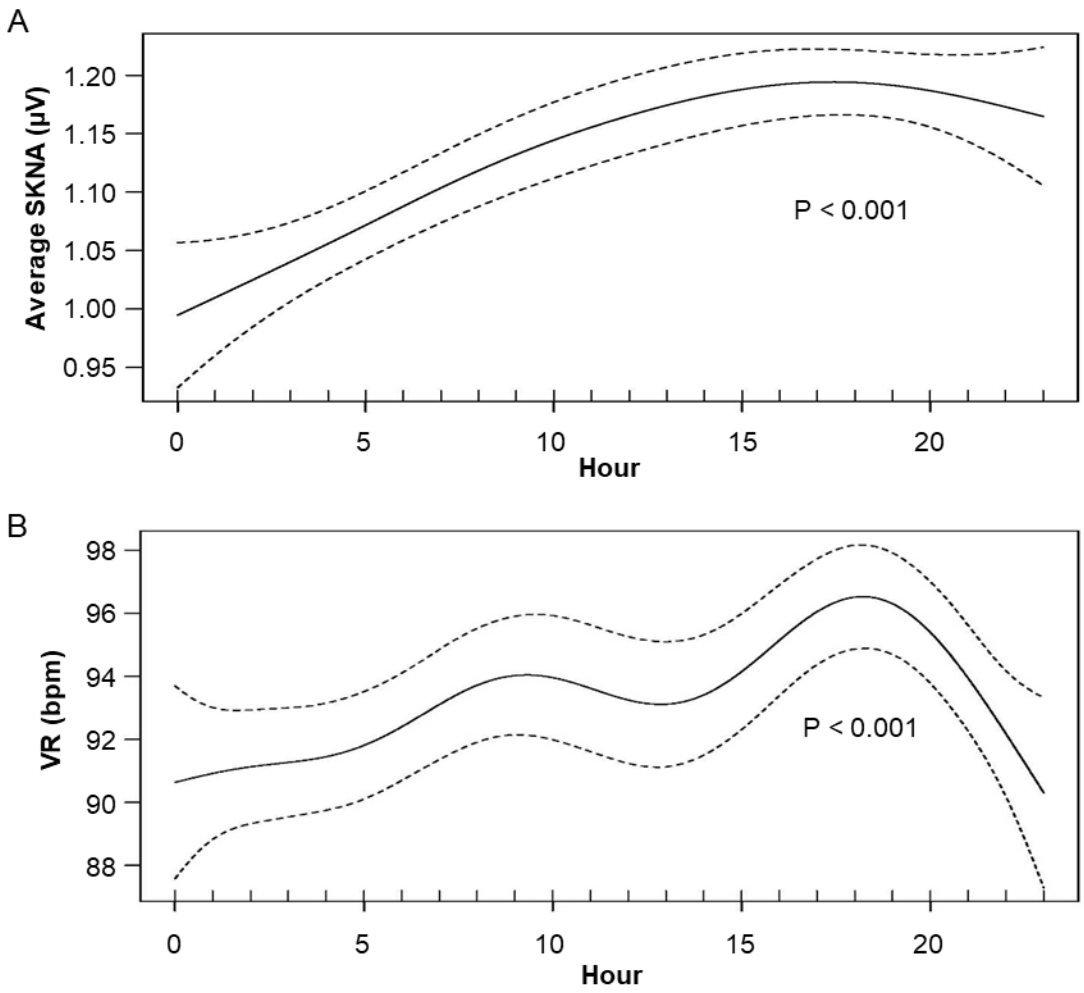
5. Lip GY, Nieuwlaat R, Pisters R, Lane DA, Crijns HJ. Refining clinical risk stratification for predicting stroke and thromboembolism in atrial fibrillation using a novel risk factor-based approach: the euro heart survey on atrial fibrillation. Chest 2010;137:263-272.



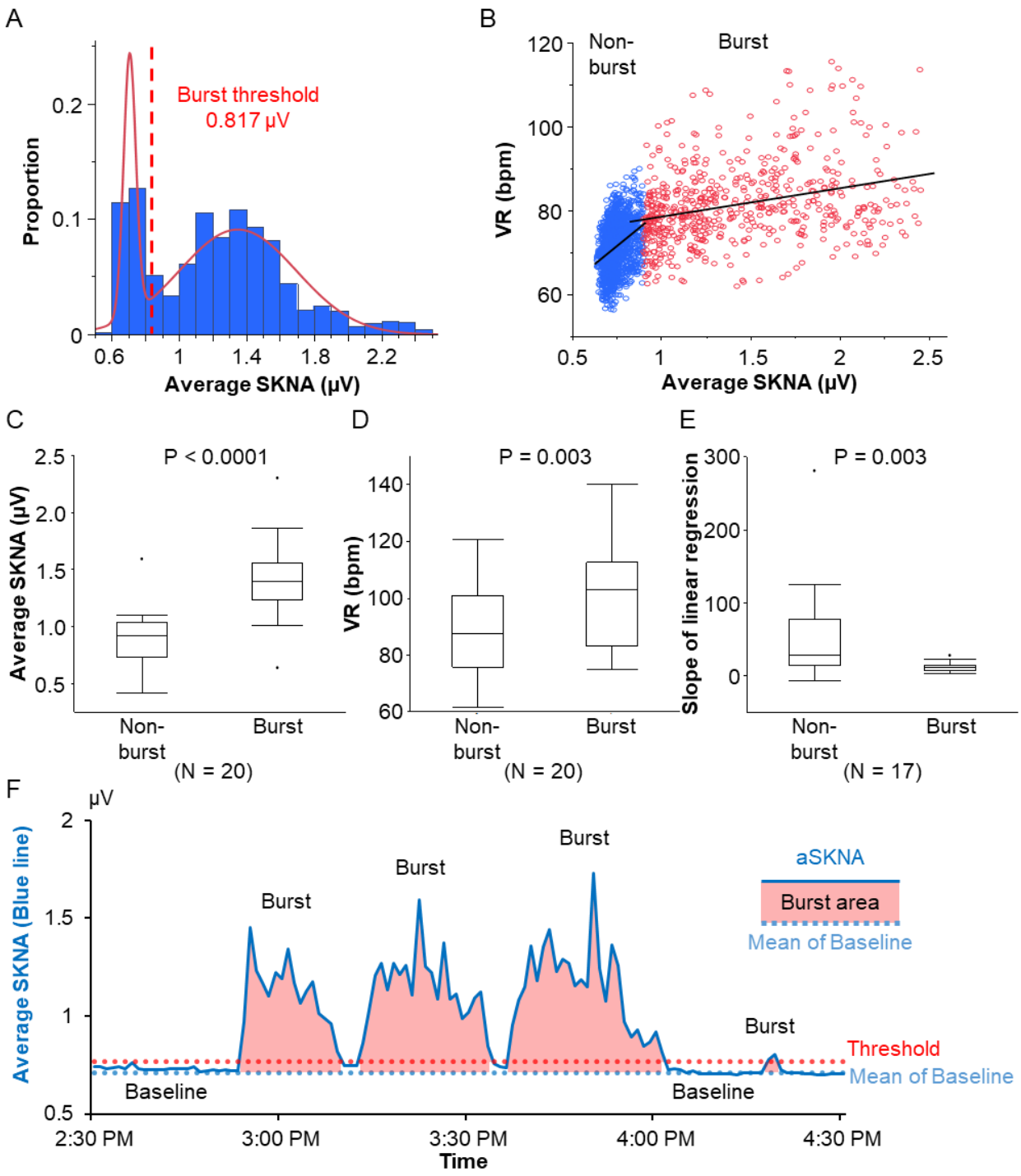
Supplemental Figure 1. Forest plots of correlation coefficient between aSKNA and VR during paroxysmal (A) and persistent (B) AF. Each patient is represented by a line. In random effects model, there were no differences in overall estimate of the correlation coefficient between paroxysmal AF and persistent AF (0.51 [95% CI 0.36 – 0.66] vs. 0.55 [95% CI 0.47 – 0.64] respectively, p = 0.609). COR = correlation; CI = confidence interval; I^2 = I-squared; τ^2 = Tau-squared; SKNA = skin sympathetic nerve activity; AF = atrial fibrillation; VR = ventricular rate.



Supplemental Figure 2. Forest plots of the correlation coefficient between aSKNA and VR during AF with (A) and without (B) beta blocker therapy. In random effects model, there were no differences in overall estimate of correlation coefficients between aSKNA and VR during AF with or without beta-blocker therapy (0.54 [95% CI 0.46 – 0.62] vs. 0.53 [95% CI 0.32 – 0.73], p = 0.999). Abbreviation as Supplemental Figure 1.

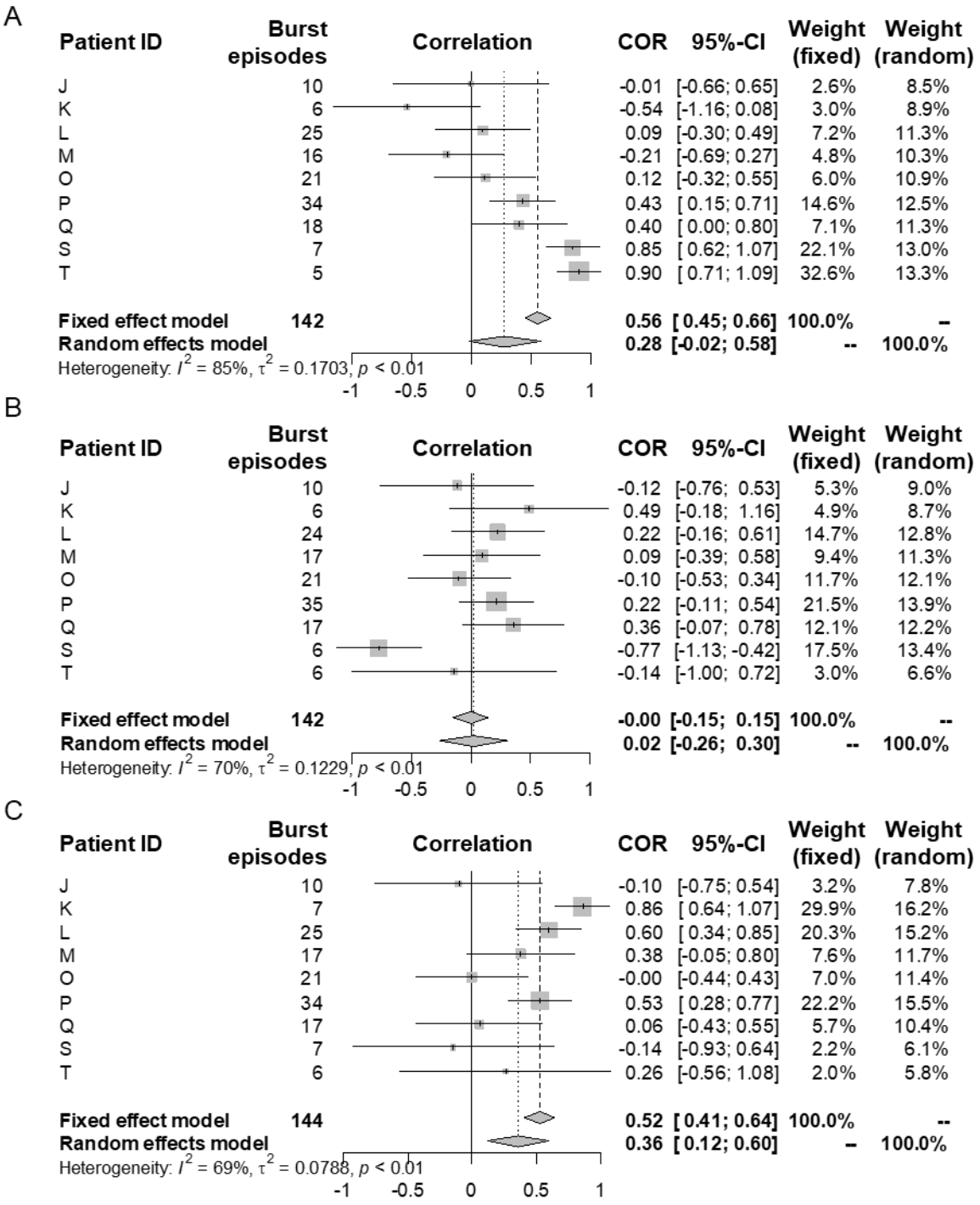


Supplemental Figure 3. Trajectory of aSKNA and VR. We included all AF episodes from 19 patients for these analyses. The data from one patient (patient A) were excluded because none of the episodes lasted > 10 min. Generalized additive mixed-effects models were used to characterize the trajectory over 24-hr. A) The aSKNA was low during midnight and increased gradually during the day, reaching the peak in the early evening. B) The VR during AF had two peaks, one in the morning and the other in early evening. There was a drop of VR but not aSKNA around noon time. Abbreviations as Supplemental Figure 1.



Supplemental Figure 4. Relationship between VR and SKNA bursts. A) The distribution of aSKNA amplitudes in patient K (Figure 3) can be fitted into two Gaussian distributions. We used the red dotted line to mark the mean + 3 SD of the Gaussian distribution on the left. All SKNA to

the right of the red line are deemed to be bursts. B) Representative correlation between the VR and aSKNA during SKNA burst and non-burst from patient P from Figure 3. The black line segments indicate the linear regression. The box-and-whiskers plots in (C) and (D) show aSKNA and VR during SKNA bursts were significantly higher than during non-burst period. E) The slope of linear regression during SKNA non-burst period was steeper than during SKNA bursts. P values of (C), (D) and (E) were determined by the Wilcoxon signed rank test. F) Enlarged view of aSKNA from 2:30 PM to 4:30 PM in Figure 6A. Red and right blue dotted lines indicate threshold and mean of baseline, respectively. Pink region is the aSKNA area over baseline, or burst area. Abbreviation as Supplemental Figure 1.



Supplemental Figure 5. Forest plots of correlation coefficient between maximal VR and aSKNA during SKNA bursts across quartile 1 (A), 2 (B), and 3 (C) of the burst area. In random effects model, there were no strong relationships in overall estimate of correlation coefficients

between maximal aSKNA and VR during SKNA bursts (0.28 [95% CI -0.02 – 0.58], p = 0.068, 0.02 [95% CI -0.26 – 0.30], p = 0.887; 0.36 [95% CI 0.12 – 0.60], p 0.003, respectively).

Abbreviation as Supplemental Figure 1.

Supplemental Table 1. Patient characteristics

Characteristics	Paroxysmal (N = 8)	Persistent (N = 12)	Overall (N = 20)
Age	66.0 (59.0 – 77.0)	73.0 (60.5 – 80.0)	70.5 (59.8 – 77.0)
Male	4 (50)	6 (50)	10 (50)
Body-mass index †	31.4 (25.5 – 51.0)	32.2 (27.4 – 40.1)	31.4 (26.8 – 40.1)
LVEF, %	51.5 (42.0 – 63.5)	47.5 (26.5 – 67.0)	49.0 (38.0 – 63.5)
Left atrial diameter, mm	45.5 (38.8 – 51.0)	46.0 (40.5 – 51.3)	45.5 (40.5 – 51.3)
	(N = 8)	(N = 10)	(N = 18)
CHA_2DS_2-VASc score ‡	3.0 (2.3 – 5.5)	4.0 (2.3 – 4.8)	3.5 (2.3 – 4.8)
0 or 1	1 (12.5)	0	1 (5)
2	1 (12.5)	3 (25)	4 (20)
3 - 9	6 (75)	9 (75)	15 (75)
Hypertension	6 (75)	11 (91.7)	17 (85)
Diabetes mellitus	4 (50)	5 (41.7)	9 (45)
Chronic Kidney Disease	4 (50)	4 (33.3)	8 (40)
Coronary Artery Disease	4 (50)	5 (41.7)	9 (45)
Valvular Heart Disease	3 (37.5)	4 (33.3)	7 (35)
Beta-blockers	6 (75)	10 (83.3)	16 (80)
Metoprolol Succinate 25 mg, once daily	0	3 (25.0)	3 (15)

Metoprolol Tartrate 25 mg, twice daily	3 (37.5)	1 (8.3)	4 (20)
Metoprolol Succinate 50 mg, once daily	0	2 (16.7)	2 (10)
Metoprolol Tartrate 100 mg, twice daily	2 (25)	1 (8.3)	3 (15)
Metoprolol Succinate 150 mg, once daily	0	1 (8.3)	1 (5)
Carvedilol 3.125 mg, twice daily	1 (12.5)	1 (8.3)	2 (10)
Carvedilol 25 mg, twice daily	0	1 (8.3)	1 (5)
Antiarrhythmic drug	3 (37.5)	2 (16.7)	5 (25)
Amiodarone 400 mg, once daily	2 (25)	0	2 (10)
Amiodarone intravenous infusion	1 (12.5)	0	1 (5)
Amiodarone 200 mg, once daily, and Dofetilide 125 µg, twice daily	0	1 (8.3)	1 (5)
Digoxin 125 µg, once daily	0	1 (8.3)	1 (5)
Calcium channel blocker	3 (37.5)	4 (33.3)	7 (35)
Verapamil 120 mg, three times daily	1 (12.5)	0	1 (5)
Amlodipine 5 mg, once daily	1 (12.5)	0	1 (5)
Amlodipine 5 mg, once daily, and Diltiazem 240 mg, once daily	1 (12.5)	0	1 (5)
Diltiazem 180 mg, once daily	0	2 (16.7)	2 (10)
Felodipine 10 mg, once daily	0	1 (8.3)	1 (5)
Nifedipine 60 mg, once daily	0	1 (8.3)	1 (5)

All values are presented as median (interquartile range) or n (%). There were no significant differences in clinical characteristics or demographics between patients with paroxysmal and persistent AF. † The body-mass index is the weight in kilograms divided by the square of the height in meters. ‡ CHA_2DS_2-VASc score is a measure of the risk of stroke in patients with AF, with scores ranging from 0 to 9, allotting 1 point each for congestive heart failure, hypertension, age ≥ 65 years, diabetes mellitus and vascular disease (history of myocardial infarction,

peripheral artery disease, or aortic plaque), or female sex and 2 points each for age ≥ 75 years or prior stroke, transient ischemic attack, or systemic embolism.[5] AF = atrial fibrillation; LVEF = Left ventricular ejection fraction.