Title: FLOW Mapping Electrogram VALidation in Patients with Persistent Atrial Fibrillation (FLOW EVAL-AF)

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Sponsored By

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1.0 PROTOCOL SUMMARY

Sponsor	Ablacon, Inc.
Study Name	FLOW EVAL-AF
Study Title	Electrographic flow mapping electrogram validation in patients with persistent atrial fibrillation
Clinical Document No/Revision	Version 1.0
Clintrials.Gov Identification	NCT05093868
Objectives	To correlate electrogram morphology of atrial fibrillation measured by a high-density regional mapping to low density global electrographic flow mapping generated by the Ablamap® software
Procedure	10 subjects with persistent atrial fibrillation
Test Devices	Ablamap® Software v.9.0.2 (With Abbott FIRMap Catheter)
Study Design	Prospective, observational, single center pilot trial
Clinical Site	Herz-und Diabeteszentrum NRW, Ruhr-Universitat Bochum, Bad Oeynhausen, Germany
Study Endpoint	Successful acquisition of global EGF maps from each of the right and left atrial chambers, and simultaneous high density electrogram acquisitions from at least 3 sites in each chamber.
Primary Outcome	Descriptive correlation of electrogram patterns using traditional high density regional mapping versus low density global EGF mapping using a novel mapping software.

2.0 BACKGROUND

2.1 Mapping and ablation of persistent atrial fibrillation

It is hypothesized that initiation and propagation of atrial fibrillation (AF) is dependent, at least in part, on rapid atrial stimulation from focal sources. It is debated whether the mechanism of arrhythmogenesis at these foci is abnormal automaticity, triggered activity, or reentry. However, after activation emerges from these "driver" sites, fibrillatory conduction ensues resulting in the disorganized conduction pattern of AF.[1,2]

Conventional mapping systems can either achieve high spatial resolution by sequential tachycardia beats following a fixed intra-atrial activation pattern (as in macroreentrant atrial flutter), or high temporal resolution with very low spatial resolution achieved through multielectrode basket catheters. Activation mapping in AF with commercially available mapping systems has been unsuccessful in identifying driver sites because atrial activation patterns change on a beat-to-beat basis precluding the use of sequential mapping approaches, and real-time mapping with basket electrodes lacks sufficient resolution to delineate the complex patterns of conduction.[2,3]

2.2 Ablamap® mapping system

Electrographic Flow (EGF) mapping (Ablamap®, Ablacon, Inc, Wheat Ridge, CO) is a unique method to assess dominant patterns of intra-atrial conduction during ongoing atrial fibrillation and has been previously described.[4-6] EGF mapping reconstructs global atrial wavefront propagation in near real-time. Recordings from a multielectrode basket catheter are analyzed for electrical wavefront activation over sequential 2-second segments during a 60 second acquisition period. Patterns of reproducible wavefront activation are used to identify driver sources for AF. Multiple sources are often identified in patients with persistent AF.[7] It is anticipated that substrate modification of these source regions will eliminate the AF drivers and result in a favorable response to catheter ablation as shown in a previously published retrospective analysis.[8]

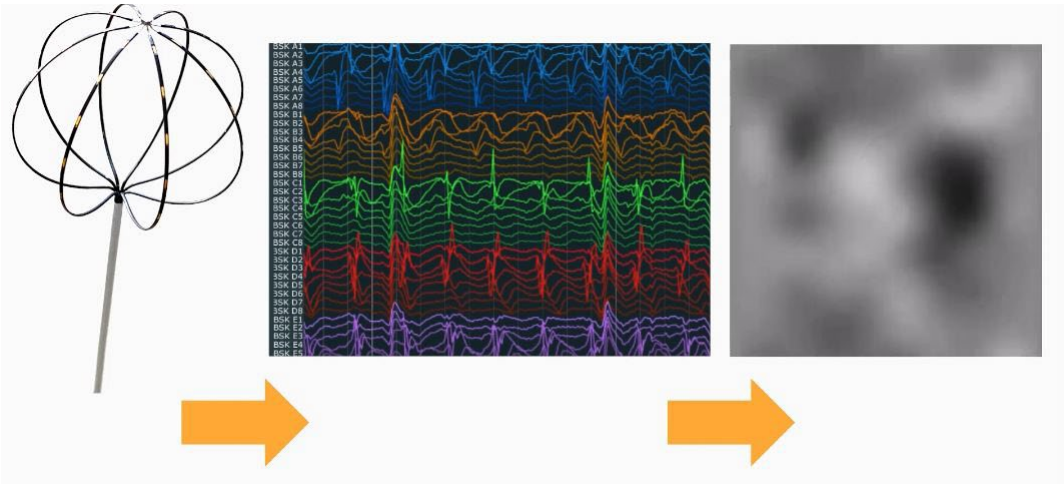

Figure 1: FIRMap basket mapping catheter (left) captures 64 unipolar electrograms (center). After undergoing biharmonic spline interpolation based on Green's function, the moving image is produced (right).

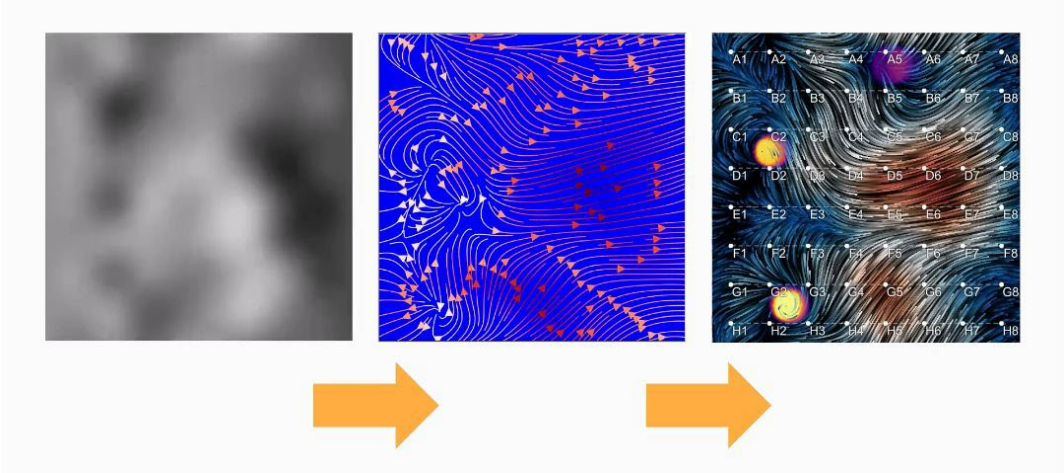

Figure 2: The transformed data are divided into 2 second segments and activation vectors are produced (center). Multiple segments are compared, and dominant activation wavefronts (vector flows) are identified (right).

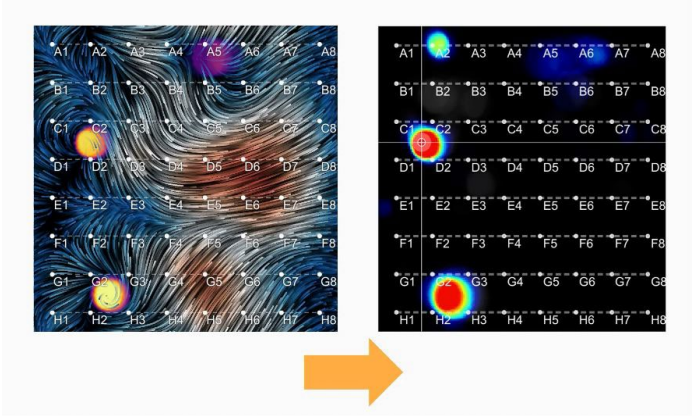

Figure 3: The EGF maps are summated over multiple segments and a composite map identifying reproducible sources (red dots, right) are identified. It is hypothesized that these source areas are the locations of microreentry, triggered or rotational activity that account for the persisting activation and re-activation of atrial fibrillation wavefronts. Flow consistency of the wavefront activation is weighted on a color scale of blue (low consistency) to maroon (high consistency).

2.3 Electrographic Flow Consistency

In addition to the identification of AF driving sources, EGF mapping allows for the quantification of local and global electrographic flow consistency (EGFC), which measures the overall magnitude and temporal consistency of wavefront patterns.[9] Regions of low EGFC are therefore associated with areas of chaotic or slowed conduction and conduction block.

In the context of AF, a well-established correlation exists between bipolar low voltage areas detected on high density electroanatomic mapping and the presence of scar tissue as revealed by late gadolinium-enhanced cardiac magnetic resonance imaging, a surrogate marker for atrial fibrosis. Areas of atrial scar, as identified from bipolar voltage maps, have also been associated with worse outcomes, and larger low voltage areas obtained during SR mapping have been associated with the presence and progression of AF.[10,11] This study aims to extend this understanding by investigating the correlation between bipolar voltage maps and EGFC in multiple rhythms to correlate areas of delayed versus healthy conduction.

2.4 Preliminary Clinical Data of Ablamap®

The FLOW-AF study (NCT 04473963) was a prospective, randomized controlled multi-center study to evaluate the reliability of the CE-marked EGF algorithm technology (Ablamap®, Ablacon, Inc, Wheat Ridge, CO), which identified AF sources and guided ablation therapy in patients with persistent atrial fibrillation. In total, 85 patients with persistent or long-standing persistent AF indicated for redo ablation were enrolled in the FLOW-AF study. In this population, EGF

mapping identified AF sources for ablation in 60% of the patients. Source elimination by means of radiofrequency ablation was achieved in 95.0% (19/20 patients). AF-free survival at 12-months was improved among patients randomized to PVI+EGF-guided source ablation (68%) versus PVI-alone (17%; p = 0.042); freedom from AF/AT/AFL at 12-months was 51% versus 14% (p=0.103), respectively. Freedom from adverse safety events was 97.2%. Two adverse safety events occurred (decompensated heart failure and arteriovenous fistula), both of which were adjudicated by an independent Clinical Events Committee as related to the procedure but not related to EGF mapping/ablation. EGF-guided ablation of AF sources seems to be an efficacious adjunct to PVI in non-paroxysmal AF patients undergoing redo-ablation. Publication of the FLOW-AF trial is expected in the first quarter of 2024.

In addition, several sites in the EU have been and continue to perform commercial cases using the CE-marked Ablamap® software, and over 172 additional patients have been treated with ablation using EGF-guidance outside of the FLOW-AF randomized clinical trial.

3.0 STUDY DESIGN

3.1 Objectives

To identify driver sources in subjects with ongoing AF using EGF mapping and describe the activation patterns observed from concomitant high density (HD) mapping of those regions.

3.2 Study Endpoint

Successful acquisition of global EGF maps from each of the right and left atrial chambers and simultaneous high density electrogram acquisitions from at least 3 sites in each chamber.

3.3 Design

Prospective, observational, single center pilot trial.

3.4 Study Population

Subjects with persistent or longstanding persistent AF that are scheduled for elective catheter ablation of AF (De Novo and Redo).

3.5 Subject Selection

3.5.1 Subject Identification

All subjects who are scheduled for catheter ablation of AF, including those with prior history of AF catheter ablation, were considered. Screening was performed by physician staff at the time of procedure scheduling.

3.5.2 Inclusion Criteria

Subjects will be eligible to participate if the following criteria are met:

- Scheduled to undergo elective catheter ablation of AF
- History of persistent or longstanding persistent AF
- Able to provide written informed consent prior to the procedure
- Age ≥18 years

3.5.3 Exclusion Criteria

Subjects will not be eligible to participate if any of the following criteria are met:

- Presence of a permanent pacemaker or other transvenous pacing/defibrillation leads
- Presence of a prosthetic mitral heart valve
- Known reversible causes of AF
- Any cerebral ischemic event (strokes or transient ischemic attacks) which occurred during the 6-month interval preceding the consent date
- History of thromboembolic event within the past 6 months or evidence of intracardiac thrombus at the time of the procedure
- Unable to provide own informed consent

3.5.4 Informed Consent

- Written informed consent was obtained from the subject by the research coordinator or investigator within 30 days of the procedure.

4.0 STUDY METHODS

4.1 Baseline Data

Subjects will undergo baseline evaluation in accordance with hospital's standards.

4.2 Study Procedure

4.2.1 Catheter Ablation Procedure

- During the study, subjects with PeAF or long-standing PeAF are prospectively enrolled at the Heart and Diabetes Center NRW in Bad Oeynhausen, Germany.
- All subjects should be able to provide written informed consent and should be at least 18 years of age.
- All procedures are performed under sedation after obtaining informed consent.
- All subjects included in this analysis will undergo 3-dimensional electroanatomic mapping with an Ensite mapping system (Abbott, Abbott Park, IL) and Ablamap®.

- Pulmonary vein isolation (PVI) ablation or PVI touch-up should be performed according to standard hospital ablation procedures, using an irrigated tip radiofrequency ablation catheter.
- Ablation catheter power and temperature settings are at the operator's discretion.
- Intravenous heparin should be administered for systemic anticoagulation to maintain an activated clotting time (ACT) > 300 seconds prior to insertion of the 64-pole basket mapping catheter.

4.2.2 High-Density Voltage Mapping and EGF Mapping

- All subjects should first undergo high-density voltage mapping using the 16-pole grid mapping catheter (HD-Grid, Abbott, Abbott Park, IL) followed by baseline EGF mapping in their presenting rhythm: AF or SR.
- PVI is then performed in standard fashion at the physician's discretion.
- For any ablation of PVs, confirmation of PVI (entrance or exit block) should be performed after a mandatory minimum 20-minute wait after the last RF application. PV reconnections should be ablated when necessary.
- By necessity, the workflow will vary depending on the subject's presenting rhythm (SR or AF).
  - If subjects present in SR, they undergo both EGF mapping and high-density bipolar voltage mapping in SR first, followed by PVI. Post-PVI, AF should be induced and EGF mapping and high-density bipolar voltage mapping in AF is then be performed. Cardioversion back to SR should be performed at the end of the procedure.
  - If subjects present in AF, they undergo EGF mapping and high-density bipolar voltage mapping in AF first, followed by PVI. Post-PVI, if the subject remained in AF, cardioversion should be performed followed by EGF mapping and high-density bipolar voltage mapping in SR.

4.2.3 *EGF Mapping*

- EGF mapping should be performed both pre- and post-PVI using the Ablamap® software (Ablacon, Inc., Wheat Ridge, CO).
- Unipolar electrograms (EGMs) are recorded using a commercially available 64-electrode basket mapping catheter (FIRMap™, Abbott Laboratories, Abbott Park, IL) connected to a proprietary, CE-marked recording system (EP Map, Herdecke, Germany).
- EGF mapping is performed by obtaining 1-minute recordings from the 64-pole basket catheter placed in multiple standardized positions within each atrium.
- In the LA, there are typically 2 standardized basket positions: 1) posterosuperior LA with the dome of the basket aiming toward the left

PVs and LA appendage (LAA); 2) lateral LA with basket dome pointing toward LAA and the mitral valve.

- In the right atrium (RA), there are typically 3 standardized basket positions: 1) superior vena cava (SVC)/RA junction with basket position halfway into the SVC; 2) central RA with basket dome pointing to SVC with vertical catheter shaft orientation and basket expanded to fill RA; 3) anterolateral RA with basket dome pointing to RA appendage and tricuspid valve and catheter deflected anterior and lateral from SVC.
- If additional rhythm changes occurred spontaneously, EGF mapping should be conducted in the new rhythm without requiring accompanying voltage maps.

4.2.4 *Procedure Completion*

- Due to time considerations and other practical considerations, voltage maps and EGF maps can be omitted from the protocol at the physician's discretion.
- Adjunctive ablations can be performed at physician's discretion, after all mapping is completed. This includes EGF-identified source ablations.
- Before concluding the procedure, any subjects who are in AF are cardioverted to SR.
- At the end of the procedure, heparin is discontinued. Catheters and sheaths are removed, and hemostasis is achieved per standard protocol.
- The de-identified raw electrogram signal data should be downloaded onto portable media. These anonymized data should be securely transmitted electronically to the technical staff at Ablacon, Inc. for analysis.

5.0 SIGNAL ANALYSIS

5.1 EGF Maps

- Unipolar electrogram signals from the basket catheter are analyzed using the Ablamap® software for each 1-minute recording to generate an EGF Summary Map. EGFC scores are calculated from all EGF summary maps. EGFC is computed from the Euclidean length of the vector field estimates over time. This provides a measure of the overall magnitude of flow averaged over all basket electrodes in arbitrary units (AUs).
- Additionally, an overall EGFC score is computed for each atrium of each subject, by averaging the positional EGFCs within each atrium.

5.2 Comparison of EGF Maps and HD Grid Maps

- The EGF patterns observed on Ablamap® and their EGFC scores are compared with the unipolar and bipolar voltage maps observed on HD grid maps from those same regions.

5.3 Statistical Analysis

- Continuous variables are reported as mean ± standard deviation and where appropriate as median ± interquartile range. For all statistical tests, the null hypothesis is rejected at the level of $P < 0.05$.
- P-values for group comparisons are computed using two-tailed t-tests for continuous variables and chi-squared tests for proportions, as appropriate.
- T-tests are paired when compared across the same subjects and independent when compared among different subjects.
- Analyses are performed by a biostatistician and data scientist using both SAS software for Windows version 9.3 and SciPy version 1.10.1.

6.0 REFERENCES

1. Iwasaki YK, Nishida K, Kato T, Nattel S. Atrial fibrillation pathophysiology: implications for management. Circulation 2011;124:2264-74.
2. Calkins H, Hindricks G, Cappato R, et al. 2017 HRS/EHRA/ECAS/APHRS/SOLAECE expert consensus statement on catheter and surgical ablation of atrial fibrillation. Heart Rhythm 2017;14:e275-e444.
3. Stiles MK, Sanders P, Lau DH. Targeting the Substrate in Ablation of Persistent Atrial Fibrillation: Recent Lessons and Future Directions. Front Physiol 2018;9:1158.
4. Bellmann B, Lin T, Ruppersberg P, et al. Identification of active atrial fibrillation sources and their discrimination from passive rotors using electrographical flow mapping. Clin Res Cardiol 2018;107:1021-32.
5. Bellmann B, Zettwitz M, Lin T, et al. Velocity characteristics of atrial fibrillation sources determined by electrographic flow mapping before and after catheter ablation. Int J Cardiol 2019;286:56-60.
6. Swerdlow M, Tamboli M, Alhusseini MI, et al. Comparing phase and electrographic flow mapping for persistent atrial fibrillation. Pacing Clin Electrophysiol 2019;42:499-507.
7. Haines DE, Kong MH, Ruppersberg P, et al. Analysis of Behavior of AF Sources with Electrographic Flow Mapping. Heart Rhythm 2021;18:1626-7.
8. Szili-Torok T, Kis Z, Bhagwandien R, et al. Functional Electrographic Flow Patterns in Patients with Persistent Atrial Fibrillation Predict Outcome of Catheter Ablation. J Cardiovasc Electrophysiol 2021.
9. Szili-Torok T, Neuzil P, Langbein A, et al. Electrographic Flow-Guided Ablation in Redo Patients with Persistent Atrial Fibrillation (FLOW-AF): Design and Rationale. Heart Rhythm O2 2023.
10. Teh AW, Kistler PM, Lee G, et al. Electroanatomic remodeling of the left atrium in paroxysmal and persistent atrial fibrillation patients without structural heart disease. J Cardiovasc Electrophysiol 2012;23:232-8.
11. Lin Y, Yang B, Garcia FC, et al. Comparison of left atrial electrophysiologic abnormalities during sinus rhythm in patients with different type of atrial fibrillation. J Interv Card Electrophysiol 2014;39:57-67.

7.0 STUDY DEFINITIONS

Persistent AF	Arrhythmic episodes lasting > 7 days or requiring cessation with pharmacological or direct current cardioversion between 48 hours to 7 days duration.
Longstanding Persistent AF	Persistent AF that has been continuous for greater than 12 months.

8.0 STUDY ABBREVIATIONS

ACT	Activated Clotting Time	LA	Left Atrium
AF	Atrium Fibrillation	LAA	Left Atrial Appendage
AU	Arbitrary Units	PVI	Pulmonary Vein Isolation
EGF	ElectroGraphic Flow	SAC	Source Activity
EGFC	ElectroGraphic Flow Consistency	SR	Sinus Rhythm
EGM	Electrograms	SVC	Superior Vena Cava
HD	High Density	RA	Right Atrium