

RESEARCH PROTOCOL

Revised 6/13/2017

Title of project: TRanscutaneous Electrical vAgus nerve sTimulation to suppress Atrial Fibrillation (TREAT-AF)

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Abstract: Atrial fibrillation (AF) is the most common cardiac arrhythmia. In previous experimental studies from our laboratory, we found that low-level vagus nerve (VN) stimulation (LLVNS), at voltages substantially below that which slowed the sinus rate, significantly suppressed AF inducibility and decreased AF duration. We subsequently developed a non-invasive neuromodulatory therapy, in which LLVNS was delivered to the auricular branch of the VN located at the tragus, the anterior protuberance of the outer canine ear (low level tragus stimulation; LLTS). The anti-arrhythmic effects of LLTS were similar to those of LLVNS delivered to the cervical VN trunk. More recently, in a proof-of-concept study in humans, we showed that in patients with drug-refractory AF undergoing AF ablation, LLTS for just one hour significantly shortened the AF duration and decreased inflammatory cytokines.

The overall objective of this proposal is to translate in ambulatory patients with paroxysmal AF the results of our previous studies showing acute suppression of AF and inflammation in anesthetized canines as well as humans, in order to examine the long-term therapeutic effects of this approach. We hypothesize that intermittent (1 hour daily) LLTS for 6 months may result in long-term decrease of AF burden and suppression inflammatory cytokines in patients with paroxysmal AF. Patients will be randomized to either active or sham LLTS. LLTS will be delivered through a transcutaneous vagus nerve stimulation (tVNS) device for 1 hour daily over a 6-month period. AF burden will be defined as the percent of time spent in AF over a 2-week period, assessed by noninvasive continuous ECG monitoring at baseline, 3 and at 6 months. In addition, blood samples will be collected from patients at baseline, and at 3 and 6 months, for cytokine measurement. These investigations will establish the first evidence of the long-term effects of LLTS on AF suppression in patients with paroxysmal AF and may provide the basis for a potential expansion of the therapeutic targets of this treatment modality beyond AF.

IRB NUMBER: 5631
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A. Specific aims

Specific Aim 1: To examine the effect of intermittent (1 hour daily) LLTS on AF burden in patients with paroxysmal AF over a 6-month period relative to sham LLTS. Patients will be randomized to either active or sham LLTS. LLTS will be delivered through a transcutaneous vagus nerve stimulation (tVNS) device for 1 hour daily over a 6-month period. LLTS will be delivered by the patients themselves at home after individual training. AF burden will be defined as the percent of time spent in AF over a 2-week period, assessed by noninvasive continuous ECG monitoring at baseline, and at 3 and 6 months.

Specific Aim 2: To examine the long-term effect of intermittent (1 hour daily) LLTS on inflammatory cytokines, including tumor necrosis factor (TNF)- α and interleukin (IL)-6, as well as their gene expression, in patients with paroxysmal AF over a 6-month period. The importance of this aim is highlighted by accumulating evidence for a role of inflammatory mechanisms in AF. Blood samples will be collected from patients at baseline, and at 3 and 6 months. These cytokines will be measured using multiplex immunoassays. Gene expression of these inflammatory cytokines will be measured from peripheral blood monocytes, using commercially available microarrays. All the tests in Specific Aim 2 will be conducted at the laboratory of Dr. Humphrey (collaborator).

B. Background and significance

Atrial fibrillation is the most common clinically significant cardiac arrhythmia and is associated with increased cardiovascular morbidity and mortality ^{1,2}. Both the incidence and prevalence of AF increase with age ^{3,4} and based on a recent estimate, the number of patients with AF in the US is expected to reach 15 million by 2050, as the population ages ⁵. The total incremental cost of AF is estimated to be approximately \$8,700 per patient per year, driven primarily by the increased rate of hospitalizations, while treating patients with AF costs an additional \$26 billion per year to the US healthcare bill ⁶. Despite these alarming numbers, the therapeutic success of currently available strategies for treating AF (antiarrhythmic medications and catheter ablation) is far from being optimal ⁷. Specifically, the long-term efficacy of catheter ablation for AF, as reported recently by 2 very experienced centers, has been disappointing, ranging from 29% to 46% at 5 years after a single procedure ^{8,9}. Catheter ablation, with the poor long-term success rate and high cost, is not likely to be the mainstay therapy for a large population of patients with drug-refractory AF. The development of alternative non-pharmacological, non-ablative therapies is essential for the management of patients with AF.

Neuromodulation is a novel therapy that has been successfully used in a variety of diseases. Vagus nerve stimulation delivered through an implantable device is being frequently used for the treatment of drug-refractory epilepsy ^{10,11}. Preliminary studies of the use of VN stimulation through an implantable device in patients with heart failure showed promising results, including

improvement in heart failure functional class, quality of life, left ventricular ejection fraction and left ventricular end-systolic volume ^{12,13}. We ¹⁴⁻¹⁸ and others ¹⁹ have recently shown that low level vagus nerve stimulation (LLVNS), at voltages substantially below the threshold for slowing the sinus rate or AV conduction, suppressed AF inducibility and decreased the duration of AF episodes that were induced by strong cholinergic stimulation. In those experiments, LLVNS applied to both vagal trunks dissected in the neck ¹⁴⁻¹⁶, to the right vagus nerve alone ¹⁸, or to the vagal preganglionic fibers at the posterior wall of the superior vena cava ¹⁷, exerted equally strong anti-arrhythmic effects.

The anti-inflammatory properties of the VN have been recently recognized ^{20,21}. The cholinergic anti-inflammatory pathway can be activated experimentally by electrical VN stimulation to inhibit inflammatory cytokine production (including TNF- α), prevent tissue injury and improve survival in multiple experimental models of systemic inflammation and sepsis ²⁰⁻²⁴. A substantial body of evidence suggests that AF and inflammatory pathways have a bidirectional relationship ²⁵. Several studies suggested that inflammation may confer an increased risk of AF. In a large population-based study, CRP levels predicted both the presence of AF at baseline and the development of AF during follow-up, even after adjustment for other known cardiovascular risk factors, and the risk of AF was progressively higher with increasing CRP quartiles ²⁶. In a recent experimental study, TNF- α overexpressing mice developed spontaneous episodes of AF and were found to have electrophysiological and structural changes known to promote AF, including increased calcium transient and atrial fibrosis ²⁷. Whether AF is the cause or the consequence of inflammation cannot be determined on the basis of the available evidence.

Based on the observation that transcutaneous electrical stimulation of the tragus, the anterior protuberance of the outer ear, where the auricular branch of the vagus nerve is located, elicits evoked potentials in the brainstem in humans ²⁸, we examined the effects of low-level tragus stimulation (LLTS) for inhibiting AF in a canine model of rapid atrial pacing ²⁹. Notably, the anti-arrhythmic effects of LLTS were similar to those of LLVNS delivered to the cervical vagus nerve trunk ²⁹. We recently translated these results into patients with drug-refractory AF undergoing AF ablation, and showed that LLTS for just one hour significantly shortened the AF duration, and suppressed inflammatory cytokines, indicating that neuromodulation by LLTS is a very promising, non-invasive therapy to treat AF and AF-related inflammation ³⁰. Before these results can be translated into clinical practice, the long-term clinical efficacy of this approach needs to be shown in ambulatory patients with AF. It has been recently shown that transcutaneous VN stimulation applied for just 15 minutes in healthy volunteers increases heart rate variability (HRV) and reduces sympathetic nerve activity ³¹. In this study, we hypothesize that intermittent (1 hour daily) LLTS for 6 months may decrease AF burden and suppress inflammatory cytokines in patients with paroxysmal AF. These investigations will establish the first evidence of the long-term effects of LLTS on AF suppression in patients with paroxysmal AF and may provide the basis for a potential expansion of the therapeutic targets of this treatment modality beyond AF, into other inflammatory conditions, including atherosclerosis ³².

C. Preliminary studies

IRB NUMBER: 5631
IRB APPROVAL DATE: 07/10/2017



LLTS suppresses AF in dogs: We studied the effects of transcutaneous electrical stimulation at the tragus for inhibiting AF. In 10 pentobarbital-anesthetized dogs, LLTS (20 Hz) in the right ear

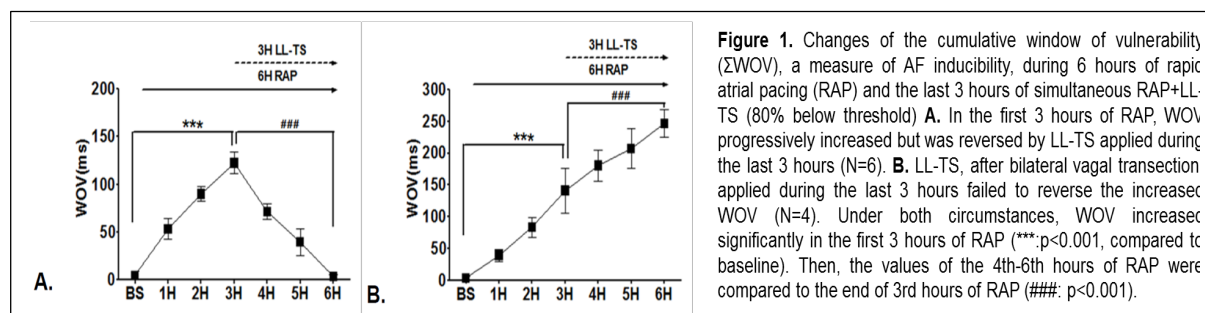


Figure 1. Changes of the cumulative window of vulnerability (ZWVOV), a measure of AF inducibility, during 6 hours of rapid atrial pacing (RAP) and the last 3 hours of simultaneous RAP+LL-TS (80% below threshold) **A.** In the first 3 hours of RAP, WOV progressively increased but was reversed by LL-TS applied during the last 3 hours (N=6). **B.** LL-TS, after bilateral vagal transection applied during the last 3 hours failed to reverse the increased WOV (N=4). Under both circumstances, WOV increased significantly in the first 3 hours of RAP (***) compared to baseline. Then, the values of the 4th-6th hours of RAP were compared to the end of 3rd hours of RAP (###: $p < 0.001$).

was accomplished by attaching two alligator clips onto the tragus. The voltage slowing the sinus rate was used as the threshold for setting the LLTS at 80% below threshold. The antiarrhythmic effects of LLTS were similar to those of LLVNS delivered to the cervical VN trunk (Figure 1A). In contrast, LLTS after transection of both VN failed to show any antiarrhythmic effect, indicating that the efferent VN are essential for its antiarrhythmic effects (Figure 1B). We concluded that LLTS can reverse RAP-induced atrial remodeling and inhibit AF inducibility, suggesting a potential non-invasive treatment for AF²⁹.

Neuromodulation suppresses AF and inflammation in humans: We have recently shown that transcutaneous electrical stimulation of the auricular branch of the vagus nerve at the tragus (low-

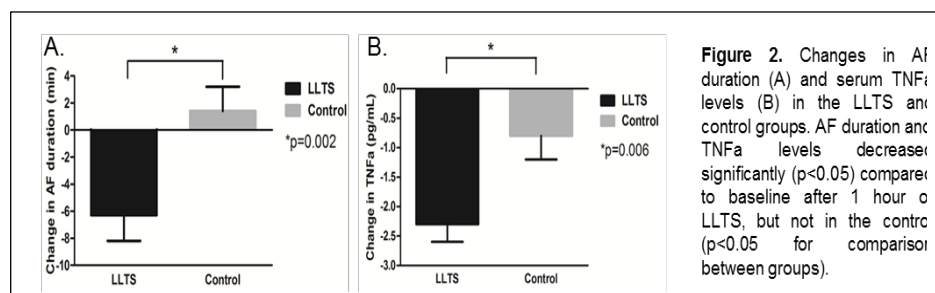


Figure 2. Changes in AF duration (A) and serum TNF α levels (B) in the LLTS and control groups. AF duration and TNF α levels decreased significantly ($p < 0.05$) compared to baseline after 1 hour of LLTS, but not in the control ($p < 0.05$ for comparison between groups).

level tragus stimulation; LLTS) in humans has antiarrhythmic and anti-inflammatory effects³⁰. Forty patients with paroxysmal AF who presented in sinus rhythm for AF ablation, were randomized to either 1 hour of LLTS (n=20) or control (n=20). LLTS in the right ear, 50% lower than the voltage that slowed the sinus rate, was accomplished by attaching a flat metal clip onto the tragus. Under general anesthesia, AF was induced at baseline and after 1 hour of LLTS or sham stimulation and were analyzed for inflammatory cytokines, including TNF α and CRP, using a multiplex immunoassay. Pacing-induced AF duration decreased significantly by 6.3 ± 1.9 min compared to baseline in the LLTS group, but not in the control ($p = 0.002$ for comparison between groups; Figure 2A). AF cycle length increased significantly from baseline by 28.8 ± 6.5 ms in the LLTS group, but not in the control ($p = 0.0002$ for comparison between groups). Systemic (femoral vein) but not coronary sinus TNF α and CRP levels decreased significantly only in the LLTS group (Figure 2B). Importantly, the magnitude of decrease in TNF α levels by LLTS was comparable with the difference between patients with active vs. inactive inflammatory diseases³³. We concluded that LLTS acutely suppresses AF and decreases inflammatory cytokines in patients with paroxysmal AF, supporting the emerging paradigm of

neuromodulation to treat AF³⁰. The results of this study were published in the Journal of the American College of Cardiology³⁰.

D. Research design and Methods

Specific Aim 1: *To examine the effect of intermittent (1 hour daily) LLTS on AF burden in patients with paroxysmal AF over a 6-month period.*

This is a prospective double-blind randomized controlled study. Patients with paroxysmal AF, documented by ECG, pacemaker/implantable cardioverter defibrillator (ICD) electrogram or Holter monitor, within 3 months of randomization on 2 separate occasions, at least 1 day apart, of at least 30 seconds duration, will be eligible for inclusion in the study.

Patients will be excluded if they have any of the following:

1. Left ventricular dysfunction (Left ventricular ejection fraction <40%)
2. Significant valvular disorder (i.e., prosthetic valve or hemodynamically relevant valvular diseases)
3. Recent (<6 months) stroke or myocardial infarction
4. Severe heart failure (NYHA IV)
5. Left atrial dilatation (>55mm)
6. Recurrent vaso-vagal syncopal episodes
7. Unilateral or bilateral vagotomy
8. Sick sinus syndrome, 2nd or 3rd degree AV block, bifascicular block or prolonged (PR>300ms) 1st degree AV block

Patients will be recruited from the inpatient services and outpatient clinics of the University of Oklahoma Health Sciences Center. A meeting with all study personnel to review the study protocol will be held before initiation of the study, as well as every 3-6 months, depending on study progress. Informed consent will be obtained prior to enrollment in the study. The randomization schedule will be implemented with the use of concealed envelopes and it will be stratified by sex. Blocked randomization will be used to decrease the chance of imbalances in the two groups over time.

After informed consent and screening procedures are completed, all patients will undergo noninvasive continuous ECG monitoring for 2 weeks to evaluate their AF burden (the percent of time spent in AF) using an adhesive waterproof continuous monitoring patch (Zio[®] Patch). The Zio[®] Patch (iRhythm Technologies, Inc) is an FDA approved, single-lead, lightweight, 14-day ambulatory ECG adhesive patch monitor. The patch is well tolerated and allows significantly longer continuous monitoring than a regular Holter monitor, leading to an improved AF detection rate³⁴. After the 2-week monitoring period, patients will be randomly assigned (1:1) to active or sham LLTS. Patients will be blinded to the treatment allocation, and every effort will be made to conceal from the study personnel the nature of the intervention in each study arm. Blinding of the patients will be accomplished by having a sham control arm, in which stimulation will be delivered to a different site (the ear lobe), devoid of vagal innervation³⁵. By doing so, all patients will be aware that they are receiving stimulation, but they will not be told which site achieves active VN

stimulation. The patients will be requested to refrain from discussing the details of their treatment with other patients in the clinic and with the physicians, nurses and the rest of clinic staff. The clinical coordinator and a clinic physician who will not participate in any of the other study related assessments will be unblinded to the treatment allocation and will instruct the patients on the proper use of the device. These study personnel will be designated to address the patients' questions and concerns as well as to record any side effects related to the use of the device.

Active LLTS will be performed by use of a transcutaneous vagus nerve stimulation (tVNS) device with electrodes attached to the tragus of the ear, which is innervated by auricular branch of the vagus nerve ³⁵.

The Parasymp targeted transcutaneous vagus nerve stimulation (tVNS) device (Parasymp Ltd) will be used for LLTS or sham stimulation. The device will be connected to a clip electrode that will be attached to the external ear. In the active group, the ear clip electrode will be attached to tragus in the active stimulation group (Figure 3). The ear (tVNS) device has not been previously reviewed by the FDA for experimental studies. It has been however studied in Europe and is available in the European market under the name Parasymp®. The same LLTS protocol will be followed in sham LLTS arm, but the electrode will be placed on the ear lobe, which is devoid of vagus innervation ³⁵. The LLTS unit will be set at a pulse width of 200 μ s

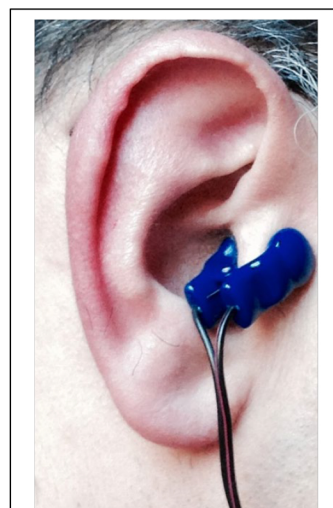


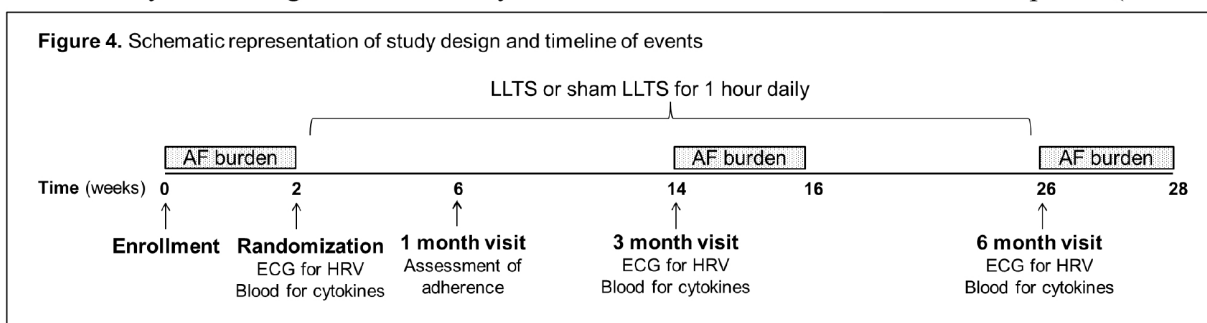
Figure 3. Position of the electrode clip used for active stimulation on the tragus.

and a pulse frequency of 20 Hz. Amplitude will be titrated to the level of sensory threshold, typically in the range of 10–50 mA. The stimulation strength will be gradually increased until the patient experiences mild discomfort, then decreased by 1 mA below that threshold. LLTS will be applied continuously for 1 hour daily. After individual training, participants will apply LLTS by themselves as part of their daily routine. Participants will be requested to keep a daily log with the time and duration of LLTS application, amplitude settings and any comments related to each daily session to monitor adherence. Patients and investigators will be blinded to the treatment allocation. Blinding of the patients will be accomplished by having a sham control arm, in which stimulation will be delivered to a different site (the ear lobe), devoid of vagal innervation ³⁵. Thus, all patients will be aware that they are receiving stimulation, but they will not be told which site achieves active stimulation. The clinical coordinator will be unblinded to treatment allocation and will instruct the patients on the proper use of the device. In patients with pacemakers, the pacemaker will be

tested with the LLTS unit to assess for interaction. In case of interaction (the stimulation artifact of the LLTS unit picked up by the pacemaker), the patients will be excluded from the study.

Patients will be anticoagulated according to the 2014 ACC/AHA guidelines ³⁶. Specifically, patients with a CHADS2-VASc score of 1 or greater will receive anticoagulation, with either one of the new oral anticoagulants or warfarin (target INR 2-3). All other medications, including antiarrhythmic medications will be continued. The choice of antiarrhythmic medications will be left at the discretion of the treating physician. The experimental device will be used in addition to all the other medications; in other words, the device will be used only with individuals continuing drug treatment.

Patients will be enrolled in the study over 17 months and the total duration of the study will be 24 months (each patient will be followed for 7 months). During the baseline visit (after the 2 week monitoring period), a complete history and physical examination will be done by one of the investigators. Laboratory data and echocardiographic data, when available, will also be reviewed. A 5-minute ECG for HRV analysis (see below) will be performed at the same time. After the ECG is completed, 10ml of blood will be drawn for cytokine analysis (see below). Since blood draw is likely to cause an increase in the sympathetic tone, it will be performed after the ECG is completed, to avoid any interfering with HRV analysis. After the ECG and blood draw is completed (to avoid



contamination of the baseline data by stimulation), patients will be instructed on how to use the LLTS and attach the electrode pads and will be asked to repeat the process themselves under direct supervision, to ensure correct use of the device. This process will be done by a clinical coordinator and/or physician who is unblinded to random allocation.

We intend to recruit 52 patients. Participants will be evaluated at 1, 3 and 6 months. At each follow-up visit, patients will have a brief history and physical examination, and any cardiovascular events, including symptomatic AF episodes will be recorded. In addition, the discomfort threshold for tragus stimulation will be obtained to ensure that patients are receiving adequate stimulation. At the 1 month visit, the patients will be interviewed by the unblinded physician or clinical coordinator to address any issues with LLTS administration at home, and to assess their adherence to the stimulation protocol. At the 3 and 6 month visit, patients will have a 5-minute ECG and blood drawn, as during the baseline visit. In addition, patients will undergo noninvasive continuous ECG monitoring for 2 weeks to evaluate their AF burden using an adhesive patch (Zio[®] Patch).

A schematic representation of the study design and timeline of events is shown in Figure 4.

HRV analysis: HRV is a marker of VN activity and can be easily measured by software calculating the distance between consecutive R waves on the ECG³⁷. Importantly, measures of HRV have been inversely correlated with inflammatory cytokines (C-reactive protein, IL-6) in the general population³⁸⁻⁴⁰. At each visit, a 5-minute ECG will be obtained for HRV analysis. ECG will be obtained in the supine position after resting for 15 minutes while heart rate, blood pressure and respiration will be monitored. All participants will be asked to avoid caffeine for 4 hours and alcohol, smoking and exercise for 12 hours prior to the visit to avoid any interfering with the results of their ECG. This test will be done using a PC-Based ECG device. Analysis and interpretation of the HRV data will be performed in a blinded fashion.

Specific Aim 2: *To examine the effect of intermittent (1 hour daily) LLTS on inflammatory cytokines and their gene expression in patients with paroxysmal AF over a 6-month period.*

The same group assignment described in Aim 1 will be used for Aim 2. The importance of this Aim is highlighted by accumulating evidence that inflammation promotes the persistence of AF^{25,41}. Blood samples (10ml) will be collected at baseline and at 3 and 6 months for cytokine measurement. Samples will be centrifuged (4000g for 10 min), and serum will be stored in aliquots at -20°C until assayed. Patients' serum will be saved frozen and processed in batches of 10 to 12. The investigators performing the cytokine assays will be blinded to group assignment. All assays will be run at the Humphrey Lab at OUHSC. Inflammatory cytokines, including TNF- α , and IL-6, will be measured using commercially available immunoassays analyzed on a flow cytometer (multiplex assays). In addition, peripheral blood mononuclear cells (PBMCs) will be isolated using MACS magnetic bead purification. Inflammatory cytokine gene expression profile will be measured at the above time points, using commercially available microarrays. Finally, we will perform exploratory analyses in order to determine relationships between clinical and cytokine responses to LLTS and identify predictors of response based on baseline cytokine levels. It is known that vagus nerve stimulation attenuates inflammation through the cholinergic anti-inflammatory pathway²⁰⁻²⁴. If this pathway is important in AF as well, we expect that the decrease in cytokines will be correlated with the anti-arrhythmic effect.

Duration of participation

Enrolled patients will participate for a maximum of 7 months with 3 outpatient office visits at 1, 3 and 6 months.

Patient enrollment

A maximum of 52 patients meeting the inclusion criteria and not meeting the exclusion criteria will be enrolled in the study. The enrollment period is expected to last for at least 17 months, given that on average 30 patients with paroxysmal AF are seen in our clinics each month and assuming that 1 out of 4 eligible patients would agree to participate in the study.

Data collection and analysis

The final data will be pooled and analyzed by the investigators.

E. Statistical methods

The primary outcome of this study is the percent of time spent in AF over the monitoring period (AF burden) at the 6-month time point. Secondary outcomes include total duration of AF over the 2-week monitoring period, longest daily duration of AF, measures of HRV and serum cytokine levels. Outcomes will be compared between groups using a Generalized Estimating Equations modeling approach, with 3 time points (baseline, 3 months, 6 months) and 4 terms included in the model (group effect, time effect, group by time interaction, adjustment for baseline measure).

Significant interactions will be followed by time trend analyses stratified by intervention group. If the interaction is not significant, the interaction term will be dropped from the model and the between-group comparisons will be made by combining the 3-month and 6-month data. For all pair-wise testing, we will adjust for multiple comparisons using Tukey's method. The modeling assumptions will be evaluated by plotting the residuals by the predicted values (for the constant variance assumption) and comparing the normal QQ plot with the QQ plot of the residuals (for the normality assumption). If the modeling assumptions are not satisfied, we will perform a natural log transformation of the data, to better satisfy these assumptions. For 0 values, a value of 0.01 will be added to each measure before applying the natural log. Analyses will be based on the intention-to-treat principle. In post-hoc exploratory analyses, we will use logistic regression analysis to identify predictors of clinical and cytokine response to LLTS, defined as a decrease of AF burden and a decrease in cytokines at 6-month follow-up, respectively. Those variables that which are associated with response to LLTS in univariate analysis at $p < 0.1$ will be entered into a multivariate logistic regression model, using a backward elimination method to identify the most parsimonious model. In light of the limited sample size, such analyses will be underpowered and will be considered hypothesis-generating only. Statistical significance will be declared at $p < 0.05$.

Sample size and power calculations: For purposes of sample size calculations we have simplified the analysis to focus on the AF burden at the 6-month time point between the 2 groups. Assuming a mean±standard deviation AF burden in the control group of $28.4 \pm 31.2\%$ ³⁴ and a 87% reduction in the LLTS group to 3.7% (assuming a common standard deviation of 31.2%), 26 patients per group would provide 80% power to detect the specified effect sizes at a two-sided significance α level of 0.05. Approximately 30 patients with paroxysmal AF are seen in our clinics each month. Assuming that 1 out of 4 eligible patients would agree to participate in the study, we should be able to enroll the required number of patients within the proposed 17 months, allowing for 6 months of additional follow up and a final month for data analysis.

F. Gender/Minority/Pediatric Inclusion for Research

Participants will be age 21 or older. Race, minority status and gender will not affect enrollment.

G. Human participants

The study population will include 52 male/female patients with paroxysmal AF, aged 21 or older, including all races. Since AF is extremely rare in patients less than 21 years old, this age limit is not expected to affect the overall results.

Inclusion Criteria:

1. Male and female patients older than 21 year old
2. Paroxysmal AF

Exclusion Criteria:

IRB NUMBER: 5631
IRB APPROVAL DATE: 07/10/2017



1. Left ventricular dysfunction (Left ventricular ejection fraction <40%)
2. Significant valvular disorder (i.e., prosthetic valve or hemodynamically relevant valvular diseases)
3. Recent (<6 months) stroke or myocardial infarction
4. Severe heart failure (NYHA IV)
5. Left atrial dilatation (>55mm)
6. Recurrent vaso-vagal syncopal episodes
7. Unilateral or bilateral vagotomy
8. Pregnancy or breast feeding
9. Sick sinus syndrome, 2nd or 3rd degree AV block, bifascicular block or prolonged (PR>300ms) 1st degree AV block.

2. Identify sources of research material in the form of specimens, records or data.

The research information will consist of the AF burden and serum inflammatory cytokines at each time point specified in the protocol (see Figure 4).

3. Describe plans for recruitment and consent procedures to be followed.

- a. Describe the location where consent is most likely to take place (will consent be obtained while an inpatient, in the ER/ICU, will consents be mailed with follow-up meeting to discuss the consent, etc.).

Patients will be recruited from the inpatient services and outpatient clinics of the University of Oklahoma Health Sciences Center. All candidate subjects will have the purpose of the study explained to them, including the benefits and risks and options, prior to scheduled ablation procedure, will be asked to read the consent form, and after questions have been answered, will be asked to participate. Finally, they will be asked to sign the consent form.

- b. Describe provisions for recruiting non-English speaking participants.

Only English-speaking persons will be recruited. Non-English-speaking adults constitute less than 1% of the patients referred for ablation.

- c. Describe measures to decrease coercion of participants (allowing adequate time to review the consent, avoid recruitment of your own private patients – or have a qualified assistant assist with the consent of these individuals, avoid recruiting employees or staff to serve as controls, etc.).

Patients will have adequate time to review the consent. Recruiting of employees or staff by the principal investigator will be avoided.

3. Describe risks and assess likelihood and seriousness.

IRB NUMBER: 5631
IRB APPROVAL DATE: 07/10/2017



Transcutaneous VNS has generally been shown to be safe and well tolerated with only minor side effects, like tingling sensation, dysesthesia, skin redness and pressure marks at the site of stimulation, painful stimulation, dizziness, mild dyspnea and headaches ^{42,43 44}. No significant effects on heart rate, blood pressure, or peripheral microcirculation could be detected during short term tVNS in a sample of 10 patients with tinnitus ⁴⁵ and in 22 healthy volunteers ⁴⁶. In a pilot study of 24 patients with tinnitus treated with tVNS over 3-10 weeks, 2 adverse cardiac events (one classified as a severe adverse event) were registered but considered very unlikely to have been caused by tVNS since other explanations for the symptoms were evident ⁴². One patient had experienced sinus arrhythmic episodes already in the past, and in the other patient comorbid hypertension had caused concentric cardiac hypertrophy which might have contributed to the described temporary left bundle branch block. Retrospective analyses of ECG parameters revealed a trend toward shortening of the QRS complex by tVNS. This was observed after the 2 patients with cardiac adverse events were excluded from the analysis, but not when the whole sample of patients was analyzed. There was definitely no prolongation of the QRS complex which is a known predictor of cardiac morbidity and mortality. In conclusion, in subjects with no known pre-existing cardiac pathology, there has been no indication of arrhythmogenic effects of tVNS ^{45 42 44 43}. This is in line with the low incidence of adverse cardiac reactions during the long-term experience in more than 50,000 patients with implanted left VNS for treatment of epilepsy and depression ⁴⁷.

The risks of the study are considered minimal and acceptable compared to alternative treatments for AF. The potential benefits in knowledge and determining whether there may be potential usefulness of this treatment therefore justify these risks. tVNS is a noninvasive, well-tolerated modality that has been extensively used for treatment of pain in various settings. tVNS has not been associated with side effects more than minimal discomfort at the area of application. Based on the FDA information sheet guidance for IRBs, clinical investigators, and sponsors, it is considered a non-significant risk device: <http://www.fda.gov/downloads/RegulatoryInformation/Guidances/UCM126418.pdf>. Previous studies examining tVNS for vagus nerve stimulation in healthy individuals ³¹ and in patients with tinnitus ⁴⁵ and epilepsy ⁴⁸ showed that this approach was safe and well tolerated. The ECG procedure for recording of HRV is similar to a routine ECG and is not associated with side effects or additional risks. Blood draw will be done using standard sterile techniques is not associated with more than minimal risk. The samples will be de-identified before processing and the technician performing the processing will only have access to de-identified data.

In light of the increasing number of patients with AF and the poor success and potential side effects of the currently available treatment options, an alternative approach such as the one tested in this study (tragus stimulation) may benefit a large number of patients. We believe that our study will establish the first evidence of the long-term effects of tragus stimulation on suppression of inflammation and AF in patients with paroxysmal AF and may provide the basis for a potential expansion of the therapeutic targets of this treatment modality beyond AF, into other inflammatory conditions. If this therapy proves to be effective in suppressing inflammation and AF, both study participants and others may benefit from this non-invasive treatment option with less risk involved.

The results of this study may form the basis for a larger clinical trial to further investigate the utility of LLTS in humans.

5. Describe procedures for protecting against or minimizing potential risks.

We will provide formal training to the patients on how to use the tVNS device, how to place the electrode pads and apply simulation. During the baseline visit all patients will be asked to repeat the process themselves under direct supervision, to ensure correct use of the device. The clinical coordinator will contact the patients once a week to ensure proper use of the device and assist with battery replacement, if needed. Blood draw will be done using standard sterile techniques is not associated with more than minimal risk. The blood samples will be processed and stored in the Immunology Lab at BRC. The samples will be de-identified before processing and the technician performing the processing will only have access to de-identified data.

- a. Address measures instituted to protect the privacy and/or confidentiality of participant PHI (locking cabinets for participant records containing PHI, use of password protected programs, limited access to PHI, etc.).

The patient charts will be obtained after informed consent. A representative data sheet will be constructed and approved for confidentiality. Names will be replaced by numbers and the subject's code and name will be kept in separate repositories.

6. Describe potential benefits and importance to the participants and others.

Catheter ablation, with the poor long-term success rate and high cost, is not likely to be the mainstay therapy for a large population of patients with drug-refractory AF. The development of alternative non-invasive therapies is essential for the management of patients with AF. If this therapy proves to be effective in suppressing AF and AF-related inflammation, both study participants and others may benefit from this non-invasive treatment option with less risk involved. The results of this study may form the basis for a larger clinical trial to further investigate the utility of LLTS in humans.

7. Discuss why risks are reasonable in relation to benefits.

AF is the most common cardiac arrhythmia, affecting more than 10 million Americans, and is associated with significant morbidity and mortality. Current therapies for AF (medications and catheter ablation) are limited by suboptimal efficacy and potential toxicity. In this study, we hypothesize that intermittent (1 h daily) LLTS, an alternative non-invasive approach, for 6 months will suppress inflammatory cytokines and decrease AF burden in patients with paroxysmal AF. These investigations will establish the first evidence of the long-term effects of LLTS on suppression of inflammation and AF in patients with paroxysmal AF and may provide the basis for a potential expansion of the therapeutic targets of this treatment modality beyond AF, into other inflammatory conditions. Importantly, in light of the increasing number of patients with AF and

the poor success and potential side effects of the currently available treatment options, an alternative approach such as the one tested in this study (tragus stimulation) may benefit a large number of patients in Oklahoma.

H. Data and Safety Monitoring Plan

1. Describe the Data and Safety Monitoring Plan (DSMP)

An independent Data Safety Monitoring Board (DSMB) committee consisting of 2 cardiologists and 1 statistician will monitor the study. All adverse events will be reported to the DSMB committee. The committee will review recruitment and retention data, protocol adherence data, data quality, and safety data every 6 months

a. reporting mechanisms for adverse events to the IRB, FDA, and NIH

Clinical staff will track from patient report, physical assessments and documentation of clinical emergencies, adverse events according to federally published grading (0=no adverse event; 1=mild; 2=moderate; 3=severe and undesirable adverse events; 4=life threatening/disabling; or 5=death) and attribution (1=unrelated; 2=unlikely; 3=possible; 4=probable; 5=definite) scales. Clinical staff will report findings to the Investigator, who will then advise the IRB, NIH and FDA as indicated. Given the mild risk nature of the study, composite reports will be reviewed every ten patients to assure that untoward events do not occur systematically, and dropout cases will be reviewed singly to learn whether further inquiry or modifications should be made to improve study implementation.

b. adverse event (AE) grading

0=no AE; 1=mild; 2=moderate; 3=severe and undesirable AE; 4=life threatening/disabling; or 5=death

c. plan for unanticipated AE reporting

Unanticipated AE will be reported to the IRB.

d. plan for annual reporting of AEs

Use of IRB forms for reporting. Our data sheet will have a column detaining any AEs that might occur.

e. interim efficacy analysis where appropriate

Not indicated.

2. Describe the Data and Safety Monitoring Board (DSMB) that will be responsible for monitoring the study.

IRB NUMBER: 5631
IRB APPROVAL DATE: 07/10/2017



- Institutional studies provide:
 - a. Chair, members

Dr. Ralph Lazzara

Dr. Nicole Tran

Dr. Ding Kai
 - b. Frequency of safety reviews

After each study

I. Literature cited

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