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RESEARCH PROTOCOL

Title: Neuromodulation of Inflammation to Treat Heart Failure with Preserved Ejection Fraction (TIN HF)

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Funding: National Institutes of Health

Abstract:

Heart failure with preserved ejection fraction (HFpEF) is a leading cause of mortality in the elderly, Outcomes of patients with HFpEF are poor and so far, no treatment has been shown to decrease morbidity or mortality. Recent animal and human studies suggest that a systemic proinflammatory state, produced by comorbidities, including aging, plays a central role in the development of HFpEF, supporting the notion that attenuating the proinflammatory state is an attractive therapeutic target for HFpEF. We have previously shown that low-level transcutaneous electrical stimulation of the vagus nerve at the tragus (LLTS) suppresses inflammation in patients with atrial fibrillation. The overall objective of this proposal is to examine the effects of LLTS on diastolic dysfunction, exercise capacity and inflammation in patients with HFpEF and determine whether these effects are mediated by suppression of inflammation and fibrosis in a well-established rat model of HFpEF. Our specific aims include: 1. To examine the effect of intermittent (1 hour daily for 3 months) LLTS on diastolic dysfunction and exercise capacity, relative to sham stimulation, in patients with HFpEF and 2. To examine the effect of intermittent (1 hour daily for 3 months) LLTS on inflammatory cytokines, relative to sham stimulation, The proposed proof-of-concept human studies and mechanistic animal studies will provide the basis for the design of further human studies using LLTS among populations with HFpEF. In light of the increasing number of elderly patients with HFpEF and the poor success of the currently available treatment options, an alternative and novel approach such as LLTS has the potential to impact clinical practice and improve health outcomes among a large number of patients.

A. Specific aims:

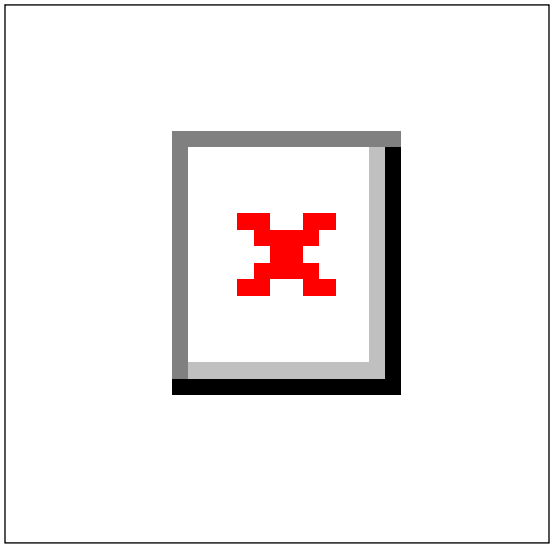
Aim 1: To examine the effect of intermittent (1 hour daily for 3 months) LLTS on diastolic dysfunction and exercise capacity relative to sham stimulation, in patients with HFpEF. We will measure echocardiographic markers of diastolic dysfunction, including early diastolic mitral annulus velocity and strain and 6-minute walk distance in patients with HFpEF over a period of 3 months. Patients will be randomized to sham vs. active LLTS and will undergo echocardiography and 6-minute walk test at baseline and 3 months.

Aim 2: To examine the effect of intermittent (1 hour daily for 3 months) LLTS on inflammatory cytokines relative to sham stimulation, in patients with HFpEF. The same groups as in Aim 1 will be used. Blood samples will be collected from patients at baseline and at

3 months. Inflammatory cytokines will be measured in serum using multiplex immunoassays. Gene expression of inflammatory cytokines will be measured from RNA isolated from whole blood, using commercially available microarrays. All the tests in Aim 2 will be conducted at the laboratory of Dr. Humphrey (collaborator).

B. Background and significance

Heart failure with preserved ejection fraction (HFpEF) is a leading cause of mortality in the elderly[1]. The prevalence of HFpEF increases from 1% at age 40 to about 10% at age 80 [2]. It is the most common cause of hospitalization in the patients ≥65 years of age and the growing elderly population is expected to further worsen these trends[2,3]. Outcomes of patients with HFpEF are poor (5-year mortality rate approximately 50%[4]), and so far, no treatment has been shown to decrease morbidity or mortality[5]. Recent animal and human studies suggest that a systemic proinflammatory state, produced by comorbidities, including diabetes, hypertension and aging, plays a central role in the development of HFpEF[6-10] (Figure 1). This systemic proinflammatory state leads to decreased nitric oxide (NO) bioavailability, left ventricular (LV) fibrosis, increased myocardial stiffness, diastolic dysfunction and heart failure[8]. Therefore, attenuating the proinflammatory state is an attractive therapeutic target for HFpEF. Importantly, reduction of inflammation and fibrosis normalized LV diastolic function and improved survival in a rat model of HFpEF, without attenuation of LV hypertrophy, suggesting that inflammation and fibrosis are not only causative in HFpEF, but also potentially reversible [11].



The anti-inflammatory properties of vagus nerve stimulation are well established[12,13]. Current evidence suggests that the vagus nerve provides the efferent and possibly the afferent limb of the cholinergic anti-inflammatory pathway, by which the brain modulates inflammation [12,13]. The cholinergic anti-inflammatory pathway can be activated experimentally by electrical vagus nerve stimulation to inhibit inflammatory cytokine production, prevent tissue injury and improve survival in multiple experimental models of systemic inflammation and sepsis [12-17]. Vagus nerve stimulation through an implantable device has been used successfully in a preliminary clinical trial in patients with rheumatoid arthritis [18]. Specifically, chronic vagus nerve stimulation at 10Hz for 60 seconds up to 4 times daily inhibited whole blood lipopolysaccharide-induced tumor necrosis factor (TNF)-α production and decreased serum interleukin (IL)-6 levels in patients with rheumatoid arthritis, raising the intriguing possibility that vagal stimulation for only short periods of time may be sufficient to induce a long-lasting anti-inflammatory response [18]. In our recent proof-of-concept randomized study in humans, we showed that in patients with atrial fibrillation (AF), low-level transcutaneous vagus nerve stimulation (LLTS) for just one hour delivered at the tragus of the ear, where the auricular branch of the vagus nerve is located, significantly suppressed AF and decreased systemic inflammatory cytokines, including TNF-α[19]. These results support the

scientific premise of the use of LLTS as a novel non-pharmacological treatment modality for AF and possibly other conditions, where inflammation plays a key role, including HFpEF.

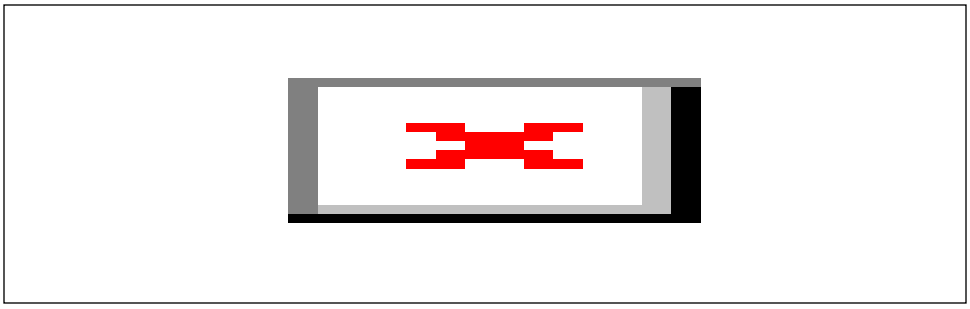
In our ongoing randomized, double-blind, 2x2 cross-over pilot study, including patients (n=10) with normal LV ejection fraction and evidence of echocardiographic diastolic dysfunction, LLTS for 1 hour acutely ameliorated diastolic dysfunction, as assessed by strain echocardiography. Specifically, global longitudinal strain decreased by 2.7±1.4% in the LLTS group compared to sham (-22.4±2.4% vs. -19.7±2.4%, respectively; p=0.05), representing a favorable and clinically significant response. The preliminary data suggest that such a treatment may be used chronically to improve diastolic function in patients with HFpEF.

The overall rationale for this proposal is that age-related inflammation promotes LV remodeling in HFpEF and can thus be used as a novel therapeutic target for HFpEF. Attenuation of inflammation can be achieved non-pharmacologically using autonomic neuromodulation (transcutaneous vagus nerve stimulation; LLTS). It is well accepted that existing animal models do not sufficiently recapitulate all the features of the HFpEF syndrome, to allow drug and device testing in animals before application to human studies [5]. Therefore, we will use a human study in this proposal, which will provide proof-of-concept clinical evidence of the efficacy of LLTS as a novel therapy for HFpEF. Our objective is to examine the effects of LLTS in patients with HFpEF on diastolic dysfunction, exercise capacity and inflammation. Our central hypothesis is that daily short-term intermittent LLTS will reverse diastolic dysfunction underlying the HFpEF phenotype in patients, through suppression of inflammation.

C. Preliminary studies

Neuromodulation suppresses AF and inflammation in humans:

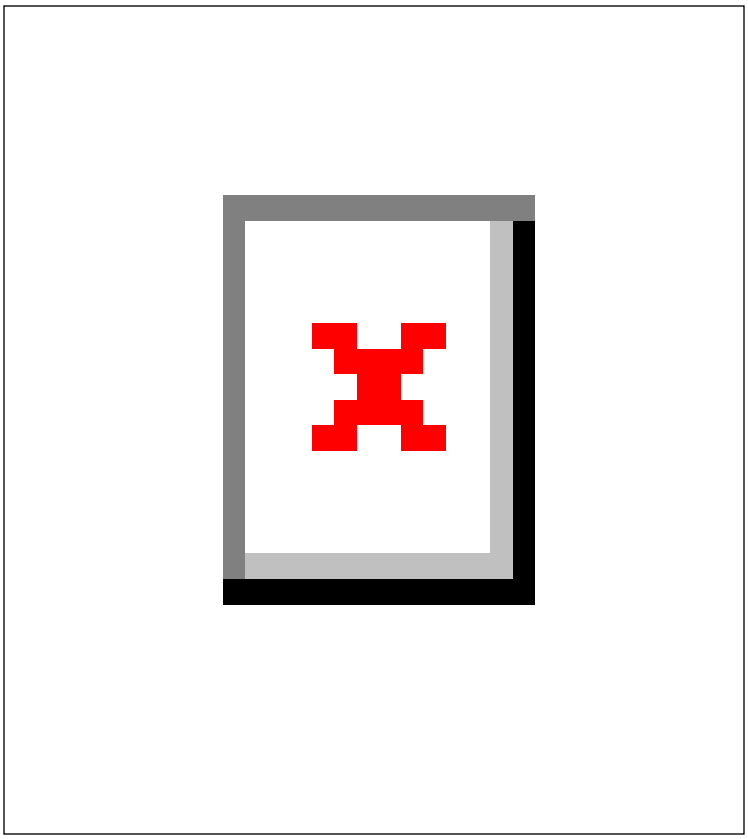
We have recently shown that transcutaneous electrical stimulation of the auricular branch of the vagus nerve at the tragus (low-level tragus stimulation; LLTS) in humans has antiarrhythmic and anti-inflammatory effects [19]. Forty patients with paroxysmal AF who presented in sinus rhythm for AF ablation, 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 TNFα 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±6.5ms in the LLTS group, but not in the control (p=0.0002 for comparison between groups). Systemic (femoral vein) but not coronary sinus TNFα 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 diseases[20]. We concluded that LLTS acutely suppresses AF and decreases inflammatory cytokines in patients with paroxysmal AF, supporting the emerging paradigm of neuromodulation to treat AF [19].

Chronic LLTS administration is feasible in humans: We are currently conducting a randomized clinical trial examining the use of LLTS to decrease AF burden and inflammatory cytokines, compared to sham stimulation in patients with paroxysmal AF (NCT02548274). The patients are required to perform LLTS or sham stimulation using the Parasym device (see below for detailed description of the device; Figure 4) for 1 hour daily for 6 months, similar to the current proposal. So far, we have enrolled 26 patients in the study and the median follow up is 4 months. According to a preliminary estimate, adherence to the protocol of daily stimulation is >90%. These results show feasibility of the proposed approach, given that patients with HFpEF share similar features with patients with AF[21].

LLTS acutely ameliorates diastolic dysfunction in humans: We aimed to evaluate the acute effects of LLTS on diastolic dysfunction in a prospective, randomized, double-blind, 2x2 cross-over pilot study. Patients with normal LV ejection fraction and evidence of echocardiographic diastolic dysfunction were included. Patients received 2 separate, 1-hour sessions, at least 1 day apart, of active and sham LLTS, with the sequence of the sessions being randomized. LLTS (20Hz, 1mA below the discomfort threshold) was performed using a transcutaneous electrical nerve stimulation (TENS) device with electrodes attached to the tragus of the ear. Sham stimulation was performed at the earlobe, which is devoid of vagal innervation. Echocardiography was performed after 1 hour of LLTS or sham stimulation to assess diastolic function. A 5-minute ECG was performed at baseline and at 1 hour of stimulation to assess HRV. HRV parameters included: 1. time domain: standard deviation of normal RR intervals (SDNN); 2. frequency domain: powers of high (0.15 to 0.4 Hz; HF) and low (0.04 to 0.15 Hz; LF) ranges, and the ratio of LF to HF power (LF/HF). Ten patients were enrolled in the study (mean age 71.4±7.6 years; 60% female). Global longitudinal strain decreased by 2.7±1.4% in the LLTS group compared to sham (-22.4±2.4% vs. -19.7±2.4%, respectively; p=0.05), representing a favorable and clinically significant response (Figure 3). LF/HF at 1 hour decreased in the LLTS group (-1.0±0.4) and increased in the sham group (2.3±0.6) compared to baseline indicating a favorable modulation of

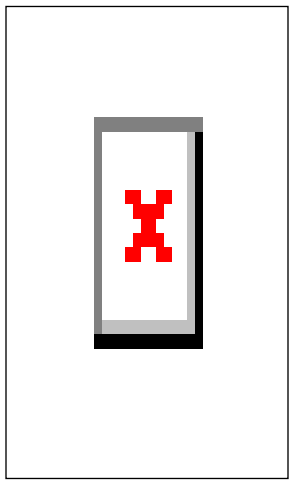


the sympathovagal balance. We concluded that LLTS acutely ameliorates diastolic dysfunction by modulating the autonomic tone, suggesting that such a treatment may be used chronically to improve diastolic function in patients with HFpEF.

D. Research design and Methods

Aim 1. To examine the effect of intermittent (1 hour daily for 3 months) LLTS on diastolic dysfunction, exercise capacity and inflammation, relative to sham stimulation, in patients with HFpEF.

This is a prospective double-blind randomized controlled pilot study. Patients with HFpEF, defined according to the latest guidelines [22] as signs and symptoms of heart failure, LV ejection fraction ≥50%, brain natriuretic peptide ≥35pg/mL and echocardiographic evidence of diastolic dysfunction (left atrial volume index ≥34mL/m^2, mitral E-wave velocity/mitral annular velocity ratio [E/e']≥13 and e'<9cm/s) and 2 of the following 4 comorbidities: 1. age≥65, 2. diabetes, 3. hypertension and 4. obesity, defined as body mass index ≥30kg/m^2, will be eligible for enrollment in the study. The rationale for inclusion of patients with these comorbidities is that by selecting a population with prominent proinflammatory state ("metabolic HFpEF phenotype"[6]), the effectiveness of LLTS will be enhanced. Patients will be excluded if they have any of the following:



1. LV ejection fraction <40%
2. significant valvular disorder (i.e., prosthetic valve or hemodynamically significant valvular diseases)
3. recent (<30 days) stroke, myocardial infarction or hospitalization for heart failure
4. severe heart failure (class IV)
5. end stage kidney disease
6. recurrent vasovagal syncope
7. history of vagotomy
8. pregnancy
9. sick sinus syndrome and 2nd or 3rd degree AV block (without a pacemaker).

Patients will be recruited from the outpatient clinics of the University of Oklahoma Health Sciences Center. Subjects will also be recruited through use of advertisement flyers distributed by campus email and posted in OUHSC clinics. Patients who view the study on ClinlicalTrials.gov and have interest in study participation can approach us. They will contact the study coordinator using the telephone number that has been provided on the website. In addition, patients from the Oklahoma Heart Hospital, who meet all study inclusion criteria, will be referred to us by Dr. Nicole Tran, Cardiologist. After informed consent, patients will be randomly assigned (1:1) to active or sham LLTS, stratified by gender. The randomization sequence will be created using randomly chosen block sizes of 4 or 6 and will be implemented through the online REDCap data system. The Parasym device (Parasym Inc) 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 4). This device was deemed as non-significant risk by the FDA in our ongoing study (NCT02548274). Moreover, in our ongoing study using this device in

a different population (paroxysmal AF), we have not seen any adverse events related to the device over 6 months. The same TENS protocol will be followed in sham LLTS arm, but the electrode will be placed on the ear lobe, which is devoid of vagal innervation [23]. The TENS unit will be set at a pulse width of 200 μs and a pulse frequency of 20 Hz. Amplitude will be titrated to the level of discomfort threshold, and will be set at 1 mA below that threshold. TENS will be applied for 1 hour daily for 3 months. After individual training, participants will apply TENS by themselves as part of their daily routine. Participants will be given a TENS unit to use at home. They will be instructed to return the unit at the end of the study. Participants will be requested to keep a daily log with the time and duration of TENS application, amplitude settings and any comments related to each daily session to monitor adherence. To ensure rigor and reduce biased self-report and outcome measurement, patients and investigators collecting study measurements 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 [23]. 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, who is not collecting study measurements, will be unblinded to treatment allocation and will instruct the patients on the proper use of the device. Based on our experience from our ongoing study using LLTS in patients with paroxysmal AF, adherence to the stimulation protocol is >90%.

Echocardiography will be performed at baseline and at 3 months to assess diastolic function (Acuson SC2000, Siemens). Two-dimensional long axis and short axis LV images will be obtained and Pulse-wave Doppler spectra of mitral inflow (E and A waves) and mitral annulus tissue Doppler spectra will be recorded. The early diastolic mitral annulus velocity (e') will be used to assess diastolic function as previously described [24,25]. The ratio of early to late mitral inflow Doppler velocity (E/A ratio) is a marker of LV diastolic relaxation and stiffness, and the E/e' ratio correlates well with LV filling pressures [24,25]. In addition, LV diastolic strain, a sensitive marker of diastolic LV function, which independently predicts outcomes in patients with HFpEF [25,26], will be obtained off-line using a speckle-tracking algorithm (Acuson SC2000 eSie VVI ™). Analysis and interpretation of the echocardiographic data will be performed in a blinded fashion. A 6-minute walk test, a well validated measure of exercise capacity in heart failure [27], will be performed at baseline and 3 months. A 5-min ECG and a 10 second ECG will be performed at baseline and at 3 months to measure heart rate variability, a marker of vagosympathetic balance. To ensure rigor and avoid bias, participants will be encouraged to return for the 3-month post-treatment assessment regardless of their adherence status.

Aim 2. To examine the effect of intermittent (1 hour daily for 3 months) LLTS on inflammatory cytokines relative to sham stimulation, in patients with HFpEF.

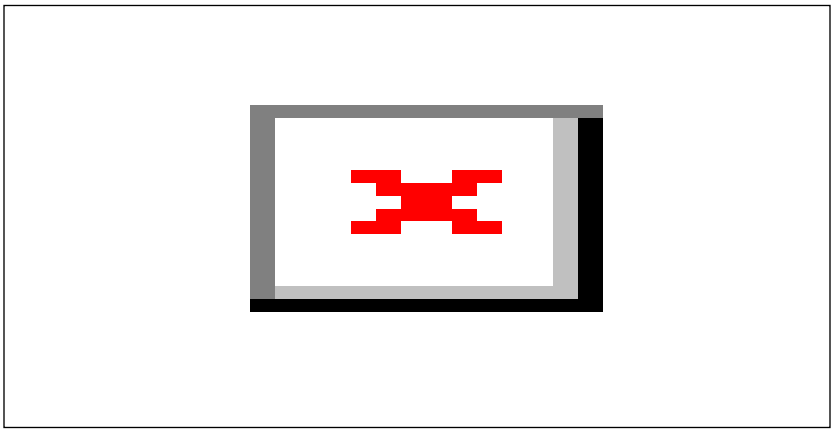
The same group assignment described in Aim 1 will be used for Aim 2. Blood samples (10ml) will be collected at baseline and at 3 months for cytokine measurement. Samples will be centrifuged (4000g for 10 min), and serum will be stored in aliquots at -20°C until assayed. Patients' serum will be saved frozen and processed in batches of 10 to 12. Inflammatory cytokines, including TNF-α, IL-1β, IL-6 and IL-18 will be measured using commercially available multiplex assays. In addition, RNA will be isolated from whole blood using the TempusTM RNA isolation tubes (ThermoFisher Scientific Inc., Waltham, MA), cDNA will be generated and gene expression of inflammatory cytokines and chemokines will be quantified using a Qiagen RT² Profiler PCR Array (PAHS-150Z), relative to the geometric mean of 5 stable reference genes. The investigators

performing the cytokine assays will be blinded to group assignment to ensure rigor. All assays will be run at the Humphrey Lab at the University of Oklahoma Health Sciences Center.

In addition, 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 pathway [12,14-17]. If this pathway is important in HFpEF as well, we expect that the decrease in cytokines will be correlated with the effect on echocardiographic markers of diastolic dysfunction and/or improvement in exercise capacity.

We intend to recruit 72 patients. Patients will be enrolled in the study over 15 months and the total duration of the study including follow-up will be 18 months, allowing for 3 months of startup and 3 months close-out. Patients will be followed with 3 outpatient visits (baseline, 1 month and 3 months). During the baseline visit, a complete history and physical examination will be done by one of the investigators. In addition, patients will be instructed on how to use the TENS device and attach the electrode clip on the respective site based on their randomization group, 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.

At each follow-up visit, patients will have a brief history and physical examination, and any cardiovascular events will be recorded. In addition, the discomfort threshold for tragus stimulation will be obtained to ensure that patients are receiving adequate stimulation. Patients will undergo echocardiography at baseline and 3 months to assess diastolic function. A 6-minute walk test will be performed at baseline and 3 months to assess exercise capacity. A 5-min ECG and 10 second ECG will be performed at baseline and 3 months. Blood samples (10cc) will be collected from patients at baseline and 3 months for inflammatory cytokine measurement. At the baseline and 3 months subjects will be asked to complete the Minnesota Living With Heart Failure Questionnaire. At the 1 month visit, the patients will be interviewed by the unblinded physician or clinical coordinator to address any issues with TENS administration at home, and to assess their adherence to the stimulation protocol. A schematic representation of the study design is shown in Figure 5.



Data collection and analysis

Data will be collected by study personnel. 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. 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. The final data will be pooled and analyzed by the investigators.

Definition of outcomes

The coprimary outcomes at 3 months are 1) the ratio of the early mitral inflow Doppler velocity to the early diastolic mitral annulus velocity (E/e') and 2) global longitudinal strain (GLS). Secondary outcomes include other echocardiographic parameters [early diastolic mitral annulus velocity (e'), left atrial volume index, tricuspid regurgitation Doppler velocity, ratio of early to late mitral inflow Doppler velocity (E/A), global circumferential strain], 6-minute walk distance, quality of life (assessed by the Minnesota Living with Heart Failure Questionnaire. To ensure rigor, analysis and interpretation of the echocardiographic data, the 6-minute walk test data and inflammatory cytokine data will be performed in a blinded fashion.

Duration of participation:

Enrolled patients will participate in the study for 3 months.

Patient enrollment:

A maximum of 72 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 18 months.

Data collection and analysis:

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

E. <u>Statistical methods</u>

Markers of inflammation, 6-minute walk distance and echocardiographic parameters will be compared between groups using repeated measures ANOVA, with 2 time points (baseline, 3 months) and 3 terms included in the model (group effect, time effect, group by time interaction). Significant interactions will be followed by time trend analyses stratified by intervention group. Transformations will be used as appropriate to satisfy modeling assumptions. Analyses will be based on the intention-to-treat principle. A separate per-protocol analysis, analyzing data from all patients who adhered to the TENS protocol at least 80% of the time, will also be performed. Sex will be investigated as a biologic variable by including sex, and interactions with sex, in the ANOVA model. Modification by sex will be explored and interpreted cautiously given that the pilot study is not powered to detect modification by sex. Gene expression array data will be analyzed using a Mann-Whitney U test, adjusting for multiple comparisons using the Benjamini-Hochberg method. Statistical significance will be set at $p<0.025$ for the 2 coprimary outcomes and at $p<0.05$ for all other data.

No formal interim efficacy analyses will be performed. Data quality and protocol implementation, as well as adverse events, will be summarized every six months for the DSMB.

Sample size and power calculations:

For purposes of sample size calculations we have simplified the analysis to focus on the difference in the E/e' ratio between the 2 intervention groups. Assuming a mean ± standard deviation E/e'

ratio 11.1±4.4 [28] and 30% reduction in the LLTS group [19], 72 patients would provide 80% power to detect the specified effect sizes at a two-sided α level of 0.025. The same sample size, under similar assumptions and a baseline GLS 16.1±2.2, would provide 90% power to detect an absolute 1.8% difference in GLS between the 2 groups.

F. Gender/Minority/Pediatric Inclusion for Research

Participants will be age 18 or older. Race, minority status and gender will not affect enrollment.

G. Human participants

72 participants of any gender/ethnic group, age 18 to 90 will be included.

Inclusion Criteria:

1. Male and female patients older than 18 year old, diagnosed with HFpEF, defined according to the latest guidelines [22] as signs and symptoms of heart failure, LV ejection fraction ≥50%, brain natriuretic peptide ≥35pg/mL and echocardiographic evidence of diastolic dysfunction (left atrial volume index ≥34mL/m^2, mitral E-wave velocity/mitral annular velocity ratio [E/e']≥13 and e'<9cm/s)
2. Two of the following 4 comorbidities: 1. age≥65, 2. diabetes, 3. hypertension and 4. obesity, defined as body mass index ≥30kg/m^2

Exclusion Criteria:

1. Left ventricular dysfunction (Left ventricular ejection fraction <40%)
2. Significant valvular disorder (i.e., prosthetic valve or hemodynamically significant valvular diseases)
3. Recent (<30 days) stroke, myocardial infarction or hospitalization for heart failure
4. Severe heart failure (class IV)
5. End stage kidney disease
6. Recurrent vasovagal syncope
7. History of vagotomy
8. Pregnancy
9. Sick sinus syndrome and 2nd or 3rd degree AV block (without a pacemaker).

2. ***Sources of research material:***

The research information will consist of echocardiographic parameters, ECG data, 6-minute walk distance, HRV parameters and inflammatory markers.

3. ***Plans for recruitment and consent procedures to be followed:***

a. ***Location where consent is most likely to take place:***

Patients will be recruited from the outpatient clinics of the University of Oklahoma Health Sciences Center. Patients who view the study on ClinlicalTrials.gov and have interest in study participation will approach us. They will contact the study coordinator using the telephone number that has been provided on the website. In addition, patients from the Oklahoma Heart Hospital, who meet all study inclusion criteria, will be referred to us by Dr. Nicole Tran, Cardiologist. The consent process will likely take place at the OU Physicians Cardiovascular Institute. 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. Only English-speaking persons will be recruited. Non-English-speaking adults constitute less than 1% of the patients referred for ablation.

c. ***Measures to decrease coercion of participants:***

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

3. **Risks and assess likelihood and seriousness:**

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 [29,30] [31]. 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 [32] and in 22 healthy volunteers [33]. 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 [29]. 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 arrhythmiogenic effects of tVNS [32] [29] [31] [30]. 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 [34].

The risks of the study are considered minimal. The potential benefits in knowledge and determining whether there may be potential usefulness of this treatment therefore justify these risks. TENS is a noninvasive, well- tolerated modality that has been extensively used for treatment of pain in various settings. TENS has not been associated with side effects more that 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 TENS for vagus nerve stimulation in healthy individuals [35] and in patients with tinnitus [32] and epilepsy [36] 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. The TENS unit used in this study has been deemed non-significant risk by the FDA.

5. ***Procedures for protecting against or minimizing potential risks:***

Risk should be no greater than minimal risk. The TENS unit used in this study has been deemed non-significant risk by the FDA.

a. ***Measures instituted to protect the privacy and/or confidentiality of participant PHI:***

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.

Certificate of Confidentialty:

To help safeguard the privacy of participants, the study is covered by a Certificate of Confidentiality from the National Institutes of Health.

6. ***Potential benefits and importance to the participants and others:***

Heart failure with preserved ejection fraction (HFpEF) has become a major public health concern. Epidemiological studies have shown that the prevalence and hospitalizations related to HFpEF are increasing, and the growing elderly population is expected to further worsen these trends. Outcomes of patients with HFpEF are poor, and so far, no treatment has been shown to decrease morbidity or mortality. If this therapy proves to be effective in reversing HFpEF, both study participants and others may benefit from this non-invasive treatment option with less risk involved. The results of this study may provide the basis for the design of human studies using this modality to target selected populations with HFpEF.

7. ***Why risks are reasonable in relation to benefits:***

Heart failure with preserved ejection fraction (HFpEF) has become a major public health concern. Epidemiological studies have shown that the prevalence and hospitalizations related to HFpEF are increasing, and the growing elderly population is expected to further worsen these trends. Outcomes of patients with HFpEF are poor, and so far, no treatment has been shown to decrease morbidity or mortality. These proof-of-concept investigations will establish the first evidence of the effects of LLTS on HFpEF and may provide the basis for the design of further human studies using this modality to target selected populations with HFpEF. In light of the increasing number of patients with HFpEF and the poor success of the currently available treatment options, an alternative approach such as the one tested in this study, may benefit a large number of patients in Oklahoma.

H. Data and Safety Monitoring Plan

1. ***Data and Safety Monitoring Plan (DSMP):***

An independent Data Safety Monitoring Board (DSMB) committee consisting of one cardiologist and one 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 3-6 months.

a. ***Reporting mechanisms for adverse events to the IRB, FDA, and NIH:***

Clinical staff will track the following 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 detailing any AEs that might occur.

e***. Interim efficacy analysis is not applicable to this study.***

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

- Institutional studies provide:
  a. Chair, members

  Dr. Beau Hawkins

  Dr. Ding Kai

  b. Frequency of safety reviews:

  After each study

I. Literature sited

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