BMJ Open

Study protocol for a randomised double-blinded, sham-controlled, prospective, cross-over clinical trial of vagal neuromodulation for pain treatment in patients with chronic pancreatitis

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-029546
Article Type:	Protocol
Date Submitted by the Author:	31-Jan-2019
Complete List of Authors:	Muthulingam, Janusiya Anajan; Aalborg University Hospital , Mech-Sense, Department of Radiology; Aalborg University, Department of Clinical Medicine Olesen, Søren; Aalborg University Hospital, Mech-Sense, Department of Gastroenterology and Hepatology; Aalborg University, Department of Clinical Medicine Hansen, Tine; Aalborg University Hospital, Denmark, Mech-Sense, Department of Radiology; Aalborg University, Department of Clinical Medicine Brock, Christina; Aalborg University, Department of Clinical Medicine; Aalborg University Hospital Drewes, Asbjørn; Mech-Sense, Department of Gastroenterology and Hepatology, Aalborg University Hospital, ; Department of Clinical Medicine, Aalborg University, Frøkjær, Jens Brøndum; Mech-Sense, Department of Radiology, Aalborg University Hospital; Aalborg University, Department of Clinical Medicine
Keywords:	Vagal nerve, Viscera, Chronic pancreatitis, Clinical trials < THERAPEUTICS, PAIN MANAGEMENT

Study protocol for a randomised double-blinded, sham-controlled, prospective, cross-over clinical trial of vagal neuromodulation for pain treatment in patients with chronic pancreatitis

Janusiya Anajan Muthulingam[1,2], Søren Schou Olesen[2,3], Tine Maria Hansen[1,2], Christina Brock[2,4], Asbjørn Mohr Drewes[2,3] and Jens Brøndum Frøkjær[1,2]

1 Mech-Sense, Department of Radiology, Aalborg University Hospital, Aalborg, Denmark

2 Department of Clinical Medicine, Aalborg University, Aalborg, Denmark

3 Centre for Pancreatic Diseases, Department of Gastroenterology & Hepatology, Aalborg University Hospital, Aalborg, Denmark

4 Mech-Sense, Department of Gastroenterology & Hepatology, Aalborg University Hospital, Aalborg, Denmark

Trial registration number: The study is registered at www.clinicaltrials.gov: NCT03357029

Protocol version: Protocol version 6, 13th of September 2017

Funding: The study is conducted as a sponsor-investigator initiated study with financial support from Independent Research Fund Denmark (DFF – 7016-00073).

Study Sponsor-investigator and corresponding author: Professor Jens Brøndum Frøkjær, MD, PhD
Mech-Sense, Department of Radiology
Aalborg University Hospital
P.O. Box 365, DK-9100 Aalborg, Denmark
Telephone: +45 97665105
E-mail: jebf@rn.dk

Contributors: JBF, JM, SSO, TMH, CB and AMD conceived and designed the study and participated in logistical planning of the study. JM drafted the initial version of the manuscript and is collecting the data. All authors made significant contributions to the development and conceptualization of the protocol. All authors reviewed the draft versions of the manuscript and have read and approved the final manuscript.

Competing interests: None

Word count: Abstract: 242 words; main text: 3874 words; Figures 3; table 1; References 44

Abstract

Introduction: The management of chronic pancreatitis (CP) is challenging and requires a personalized approach focused on the individual patient's main symptoms. Abdominal pain is the most prominent symptom in CP, where central pain mechanisms, including sensitization and impaired pain modulation, often are involved. Recent clinical studies suggest that vagal nerve stimulation (VNS) induce analgesic effects through modulation of central pain pathways. This study aims to investigate the effect of two-weeks transcutaneous VNS (t-VNS) on clinical pain in CP patients, in comparison to the effect of sham treatment.

Methods and analysis: Twenty-one CP patients will be enrolled in this randomized, double-blinded, single-centre, sham-controlled, cross-over study. The study has two treatment periods: A two-week active t-VNS using GammaCore® device and a two-week treatment with a sham device. During both treatment periods, the patients are instructed to self-administer VNS bilaterally to the cervical vagal area, three times per day. Treatment periods will be separated by two weeks. During the study period patients will record their daily pain experience in a diary (primary clinical endpoint). In addition, patients will complete questionnaires, undergo brain magnetic resonance imaging (MRI) and quantitative sensory testing before and after the two treatments to investigate mechanisms underlying VNS effects. The data will be analysed using the principle of intention-to-treat.

Ethics and dissemination: The regional Ethics committee has approved the study: N-20170023. Results of the trial will be submitted for publication in peer-reviewed journals.

Trial registration: The study is registered at www.clinicaltrials.gov: NCT03357029

Key words: Vagal nerve; Viscera; Pain; Chronic pancreatitis; Clinical trials

Strengths and limitations of the study:

- This is the first study to examine the analgesic effect of transcutaneous vagal nerve stimulation (t-VNS) in chronic pancreatitis patients with abdominal pain.
- A randomized double-blinded, sham-controlled, prospective cross-over design will be used with both clinical and experimental outcomes, which allow for exploration of the mechanisms underlying putative clinical effects.
- The study investigates the effect of two weeks of t-VNS treatment; hence, further studies are needed to explore long-term effects.
- The single-centre design may limit generalizability of the study results.

INTRODUCTION

Chronic pancreatitis (CP) is a disease characterized by progressive pancreatic inflammation and fibrosis, resulting in damage to and loss of exocrine (acinar), endocrine (islet cells), and ductal cells[1]. Chronic abdominal pain is the dominating symptom in CP and is present in up to 70% of patients[2]. Pain is associated with reduced quality of life, increased hospitalization frequencies and thus a significant socioeconomic burden[3].

The aetiology of pain in CP is increasingly better understood and often involves multiple mechanisms in the individual patient. In addition to local pathology in the pancreatic gland and its surrounding tissues, central pain pathways undergo neuroplastic changes during the course of CP. These involve sensitization of central pain pathways, functional and structural reorganization of the brain as well as impaired efficacy of endogenous pain modulatory pathways. These neural abnormalities can be targeted by different pharmacological therapies, but their effect is often limited and associated with significant side-effects in many patients. This has led to an increased interest in complementary treatment modalities for pain in patients with CP. In a model of oesophageal hyperalgesia, we have shown that physiological deep breathing enhanced vagal tone, which in response increased the pain detection threshold[4]. In addition, this effect was abolished by atropine administration thereby proving that enhanced parasympathetic tone leads to prevention of oesophageal pain hypersensitivity[4]. Also, we have previously shown an improved gastrointestinal motility and decreased pain sensitivity following non-invasive VNS of the auricular branch of the vagal nerve in conjunction with a deep-breathing approach in healthy subjects[5]. Another non-pharmacological treatment modality is transcutaneous vagal nerve stimulation (t-VNS), in which short bursts of electrical energy are directed onto the vagal nerve at the neck[6] (Figure 1). T-VNS has been shown to induce analgesic[7,8] and anti-inflammatory effects in healthy individuals[9] and different diseases. Also, the non-pharmacological treatment is FDA-approved for the preventive treatment of cluster headache and migraine[10].

This study aims to examine the analgesic effect of a two-week t-VNS in patients with CP and to explore the underlying analgesic mechanisms using advanced neuroimaging techniques and quantitative sensory testing (QST). We hypothesized that two weeks t-VNS treatment will induce clinically relevant pain relief compared to sham treatment, and that these effects are mediated via modulation of central pain pathways. To answer the overall study aims, we have two clinical and two experimental objectives:

1) The primary clinical objective is to assess the effect of t-VNS on the daily pain experience documented in a diary.
2) Secondary clinical objectives are to document changes in quality of life and daily functioning.
3) The primary experimental objective is to assess the effect of t-VNS on A) resting state brain function assessed by magnetic resonance imaging (MRI), and B) brain metabolites assessed by magnetic resonance spectroscopy.

4) Secondary experimental objectives are to assess the effect of t-VNS on A) experimental pain stimuli documented by QST, B) cardiac vagal tone and C) pro-inflammatory cytokine profiles obtained from blood samples.

METHODS AND ANALYSES:

Study design

Randomised, single-centre, double-blinded, prospective, sham-controlled, cross-over study. The study was approved by the North Denmark Region Committee on Health Research Ethics with the protocol number N-20170023 and has been registered with ClinicalTrials.gov (NCT03357029). The trial will be performed at Aalborg University Hospital and will be reported according to the Consolidated Standards of Reporting Trials (CONSORT)[11]. The study protocol follows the Standard Protocol Items: Recommendations for Interval Trials (SPIRIT) statement[12].

All patients undertake the t-VNS treatment using an active GammaCore-S, 300 treatments (10009-00603) device (ElectroCore LLC; Basking Ridge, NJ, USA) and sham treatment using a sham-device (10009-00603 P) which is identical in appearance GammaCore.

Half of the patients will be randomized to start with two-week t-VNS treatment, followed by a two-week washout period. Then, this group will be reallocated to sham treatment. The other half of patients will do the study periods in opposite order (sham treatment followed by t-VNS treatment). The two-week washout period has been used in previous studies of trans-cranial neuromodulation[13] and was shown to be sufficient to reset the effects of neuromodulation[14]. Each patient will be scheduled for four identical hospital visits (before and after each treatment period). The visits consist of 1) Fulfilment of questionnaires, 2) Collection of blood samples, 3) brain MRI scan, 4) QST, and 5) assessment of CVT (Figure 2).

Study participants

Patients will be recruited via personal correspondence and during visits at the outpatient clinic. Patients who agree to participate in the study and fill in an informed consent will be invited to participate in the study. A screening session and physical examination prior to inclusion will be conducted by a medical doctor including relevant medical and medication history and screening against the eligibility criteria.

Inclusion criteria and exclusion criteria

Patients from the age of 18 years will be included in the study. They will have a clinical diagnosis of CP based on the Mayo clinical diagnostic criteria[15]. The patients must suffer from chronic abdominal pain characteristics for CP, meet the criteria for chronic pain (pain $\geq$ 3 days per week for at least 3 months) and must consider their pain as insufficiently treated with their prescribed analgesic treatment. Additionally, the patients must be willing and able to comply with the scheduled visits, treatment plan, laboratory tests, and other study procedures. Finally, the patient must sign the informed consent and power of attorney document.

Patients will be excluded if they have any clinically significant abnormalities that may increase the risk associated with trial participation or may interfere with the interpretation of the trial results. Also, patients with alcohol and illegal drug dependence patients, cardiovascular diseases, low blood pressure (<100/60mmHg), elevated intracranial pressure will be excluded. Additionally, patients who are participating in another intervention study, patients who are pregnant or intend to become pregnant, and patients who suffer from painful conditions other than CP that make them unable to distinguish the pain associated with CP from chronic pain of other origin will be excluded. Patients will also be excluded if there are any contraindications for MRI (incl. cardiac pacemaker, implantable metallic components, etc.), have known neuropathy, or previous vagal nerve surgery.

Participants can withdraw from the study at any time they may wish. Patients will be withdrawn from the study if they do not meet for the scheduled study visits or miss a treatment period, and if they do not maintain inclusion/exclusion criteria.

Interventions

Study interventions are t-VNS treatment and sham treatment (Figure 2 & 3).

Patients will be thoroughly instructed to use the device, and when the healthcare providers are confident that the patient is capable to use the device independently, the device will be handed over to the patient. T-VNS is administered by using a handheld device (GammaCore; ElectroCore LLC; Basking Ridge, NJ, USA), which consists of a battery powered portable stimulator with a digitally controlled user-interface that controls the signal amplitude and two gel covered (Sigma gel, Parker Laboratories, NJ, USA) contact electrodes which deliver electrical stimulation to the cervical vagal nerve. One dose corresponds to 120 seconds of t-VNS to the left cervical vagal nerve followed by 120 seconds of t-VNS to the right cervical vagal nerve, with the amplitude of simulation titrated to achieve mild pulling of the ipsilateral oral commissure[16]. The patient self-administers the treatment, using the device at home three times per day (morning, afternoon, and evening) for two weeks. Previous studies with Gamma-Core, have shown that three doses per day have been effective [10,17].

The stimulation device is positioned anterior to the sternocleidomastoid muscle, over the carotid artery as this runs in close proximity with the vagal nerve. The active Gamma-Core device produces a low-voltage electrical signal comprising a 5 kHz sine wave burst lasting for 1 millisecond (five sine waves, each lasting 0.2 millisecond), with such bursts repeated once every 40 milliseconds (25 Hz), generating a 24 V peak voltage and 60 mA peak output current.

To mimic the sensation of the active treatment, the sham-device will provide vibration[18]. The appearance, weight, visual and audible feedback, and user application are identical for the sham and t-VNS devices. However, the sham device produces a low-frequency (0.1 Hz) biphasic signal that does not stimulate the vagal

nerve or generally cause muscle contractions[19]. Additionally, both devices will display a numeric value between 1 and 40, signifying the intensity of the stimulation. The maximum intensity per stimulation is 40 for both devices (Figure 1).

Compliance will be assessed by reading the remaining doses displayed at the device after each treatment period. Additionally, the patients will be asked to keep a record of the stimulation intensity of the doses applied at each stimulation. In addition, questions on compliance will be asked after each treatment period. Finally, adherence will be recorded by patients' diary.

During the study periods the patient will continue their standard care, without changes in their current pain treatment.

Randomization, sequence generation and allocation concealment

Once eligibility and consent have been approved and completed, randomization will occur using a randomization list generated by an automatic web-based randomization program. Patients will be randomly assigned to VNS/sham or sham/VNS using block randomisation, allowing seven patients at the time to be randomised in equal proportions for the order of active t-VNS or sham stimulation. The randomization order will be kept in closed envelopes; therefore, patients will get their assignment according to the order of entrance in the study. This process will be carried out by a member of the research team who is not involved in the recruitment process or conduction of the study.

An unblinded researcher will be involved in delivering the medical device according to the randomisation schedule. The sequence will follow a 1:1 sequential design, in a double-blinded fashion. Additionally, the outcome assessor (data analyst) will be blinded during the statistical analyses of primary experimental outcomes. A series of numbered, sealed, envelopes will be used to ensure concealed allocation.

Blinding

Both, active and sham devices are labelled with a serial number and not outwardly identified as active or sham. All researchers involved in the data collection and MRI analysis will be blinded to the treatment allocation group until after analyses are performed at the completion of the trial. Additionally, all patients are blinded, and they do not know that the sham treatment is an inactive treatment. Particularly, patients will be informed that they have to undergo two different treatments, and the purpose of this study is to investigate the most effective treatment.

Manufacturing and preparation of the medical devices are handled by an external good manufacturing practice-accredited facility (ElectroCore). As the patients do not know that the sham treatment is an inactive treatment, we will not be able to ask the patient "do you think you received active or inactive treatment?", thus we will not be able asses and determine if the blinding was effective.

Unblinding is only permissible if a patient experiences any serious adverse events and that the investigator/doctor judge that it is essential to know the treatment allocation in order to treat the patient appropriately.

Primary clinical outcome measures

The primary clinical efficacy parameter to be evaluated is 30% pain relief. This is assessed as changes in the daily experience of pain, which will be measured using a patient pain diary based on the numeric rating scale (NRS) (1 = no pain, 10 = worst pain imaginable). Patients will be asked to score daily pain levels in the diary for eight weeks (including one week before the first treatment period and one week after the last study period, Figure 2 and Figure 3), with one NRS value for the average pain over the previous 24 hours and one NRS value for the worst pain over the previous 24 hours.

Secondary clinical outcome measures

Quality of life questionnaire, C30, version 3.0 (QoLQ-C30)[20], the brief pain inventory – short form (BPI-SF) questionnaire[21], and Patient Global Impression of Changes[22] questionnaire (PGIC) are secondary clinical outcomes. Patient will complete QoLQ-C30 and BPI-SF questionnaire before and after each treatment period, while the PGIC questionnaire will only be fulfilled after the treatment periods. The QoLQ-C30 questionnaire is composed of both multi-item scales and single-item measures. These include five functional scales, three symptom scales, a global health status, and six single items. The BPI-SF questionnaire rapidly assess the severity of pain and its impact on daily functioning. Finally, the PGIC questionnaire evaluates all aspects of patients' health and assess if there has been an improvement or decline in the overall clinical status.

Primary experimental outcome measures

Resting state functional MRI will be employed to detect brain activity and functional connectivity changes based on BOLD signals before and after treatment of each patient. Additionally, magnetic resonance spectroscopy in anterior cingulate cortex, prefrontal cortex, parietal, and insula will also be performed in order to investigate changes in brain metabolites before and after each treatment.

MRI data will be acquired on a 3 Tesla MR scanner (Signa HDxt, General Electric, Milwaukee, WI, USA) equipped with an 8 channel standard head coil. Scan time for a structural scan will be 5½ minutes. Following parameters will be used for the structural scan: 150 slices, FOV 250 mm, echo time 3.6 millisecond, repetition time 9.0 millisecond, flip angle 14°, resolution 0.78x0.78 mm, matrix size 320x320 mm, slice thickness 1 mm, full head coverage, with no gap. Functional scans will be acquired with following parameters: gradient echo, echo planar (Gr-EPI), 192 volumes, 37-40 slices, FOV=240 mm, echo time=30 millisecond, repletion time=2000 millisecond, flip angle=90 ^{0}, matrix size=64x64, resolution=3.75x3.75 mm, slice thickness 3.8 mm, no gap, axial slices. The scan time for functional MRI will be 6 minutes and 32 seconds. Additionally, MRI spectroscopy will be used to estimate brain metabolites in the anterior cingulate cortex, prefrontal cortex, parietal, and insula. For MRI spectroscopy, single voxel PRESS (Point RESolved Spectroscopy) will be acquired. Following parameters will be used: Echo time=30 millisecond, repetition time=2000 millisecond, scan time will be 5 minutes, and the total number of scans will be 128. Bandwidth will be 5,000 Hz. A 20x20x20 mm voxel of interest will be positioned on a sagittal T2-weighted fast spin echo sequence. Repletion time= 4600 millisecond and echo time=102 millisecond, matrix 384x256, slice thickness 3 mm, gap 0.3 mm), in the midline in the ACC with the inferior border along the anterior-posterior commissure line.

Secondary experimental outcome measures

Secondary outcomes are changes in QST, CVT and pro-inflammatory cytokine profiles.

QST includes temporal summation[23], pressure pain thresholds[23,24] and conditioned pain modulation (CPM)[25]. Temporal summation demonstrates an increase perception of pain to repetitive pain stimuli[23]. Temporal summation will be recorded in the dermatome T10 (pancreatic area) and control area (dominant forearm) using the PinPrick stimulator, 256 mN (MRC Systems GmbH Medizintechnische Systeme, Germany).

The pressure pain threshold and pressure pain tolerance will be determined by pressing an electronic pressure algometer (Somedic AB, Stockholm, Sweden) on specified muscle groups: C5 - clavicula, T10 – dorsum, T10 – abdomen, L1 – anterior superior iliac spine, and rectus femoris. Also, pressure pain threshold and pressure pain tolerance will be measured on bone. For the muscle pressure stimulation, the probe has a surface area of 1 cm^2. Pressure will be increased at a rate of 30 kPa/sec until the pressure pain threshold is reached. For the bone pressure stimulation, a probe with 3.1 mm^2 (Aalborg University, Denmark) will be applied.

CPM is a clinically measurable form of descending pain modulation[25] that can be induced experimentally by a conditioning stimulus (the cold pressor test) and quantified by applying a “test-pain” (pressure stimulation of the right quadriceps musculature) before and after its induction[26]. The patient will lower their dominant hand in cold water (2°C for maximum two minutes). The difference in pressure stimulus intensity (pain threshold) before and after induction of cold pressor pain provides a quantitative index of CPM capacity for

the individual patient. The techniques used for pressure stimulation and cold pressor test described above will be combined to measure CPM.

CVT is a beat-to-beat measure of brainstem efferent vagal activity, which is assessed by heart rate variability measurement and reflects the contribution of the vagal nerve to cardiac functioning. In this particular test, changes in R-R interval would be measured non-invasively using eMotion Faros 180 device[27].

Blood samples are collected to explore changes in pro-inflammatory cytokines profiles. 26 ml blood is collected, and the following inflammatory state and macrophage markers will be assessed: interferon-G, interleukin-8 (IL-8), IL-10, IL-12p70, IL-13, IL-1b, IL-2, IL-4, IL-6, tumour necrosis factor- α (TNF-α), monocyte chemoattractant protein-1 (MCP-1), and high sensitivity C-reactive protein (hs-CRP).

Statistical power

The study is powered to detect a minimal difference between the sham treatment and the active treatment of 30% on the average clinical pain score at the end of the two study periods. Based on a standard deviation of 40% we determine that a study with 16 patients in a cross-over design is needed, with a power of 80%, and the use of a two-sided significance level of 0.05 (alpha). This calculation (standard deviation) is based on data from a study with CP patients, who received pregabalin treatment, which related to an improvement in clinical measures of the pain score[28]. To allow for a dropout rate of 25%, we will aim to recruit 21 CP patients. The sample size was calculated using statistical software package STATA 15.0 (StataCorp LP, College Station)[29].

Harms and adverse events

We do not anticipate this project causing any harm or discomfort to the patients, and we will ensure that our patients participate in the study voluntarily.

Information about adverse events and serious adverse events will be collected from the date of inclusion and in all following contacts with the study subject throughout the project. Adverse events will be documented on the patient file and on the electronic case report form. All types of adverse events will be notified to the device manufacturer ElectroCore and to the Danish Health Authorities by use of Manufactures Incident Report Form.

Data collection and data management

All instruments in the questionnaires are validated[20,21]. Additionally, all data collectors are highly experienced registered research nurses, radiographers, and researchers who have been trained in good clinical practice

(GCP). There will be regular meetings between the data collectors, monitor, principal investigator and other co-researchers involved in the project. All paper protocols will be kept safe and transferred to a computerised database. The questionnaires will be checked for errors and missing data by research staff. Data entries are double-checked against the paper questionnaires.

During trial conduct, the Good Clinical Practice unit (GCP, Aalborg, Denmark) will conduct periodic monitoring of all signed consents at monitoring visits to ensure that the protocol and GCP standards are followed. The monitors may review source documents and medical records to confirm that data recorded on Case Report Form is accurate. Thus, GCP monitoring includes all signed consents, signed power of attorney, and AE.

Criteria for the termination of the trial is, when patients according to the sample size with valid data are recorded. If the study fails to recruit adequate patients according to the sample size by end of 2019, the study will be terminated.

Data analysis

Both descriptive and analytical statistics will be used in order to compare groups and for analyses of outcomes over time including changes therein. All data will be presented as mean ± standard deviation and summarized in frequency tables, unless otherwise indicated. We will use Research Electronic Data Capture (REDCap)[30] to store the data and the statistical software package STATA to perform statistical analysis. We will use mixed ANOVA for the inferential statistic of the parametric data, with Tukey's and/or Bonferroni post hoc tests for the primary clinical endpoints. Significance level will be set as $\alpha \leq 0.05$.

The principal analysis of clinical endpoints will be by intention-to-treat, meaning that all randomized patients are included in their initially assigned study arm, regardless of adherence to study protocol. Experimental endpoints will be analysed by per-protocol, meaning that only patients completing the experimental setup are included. The primary endpoints will be compared between the treatment groups.

Analysis of MRI data: We will use standard pre-processing procedures in Statistical Parametric Mapping (SPM) (http://www.fil.ion.ucl.ac.uk/spm/) before conducting the statistical analysis. Moreover, we will use a mixed effects design in which within-subject effects between the two treatments (before and after both treatments) responses brain activity and group effects will be modelled. For MR spectroscopy, specific metabolites changes will be assessed in pain related brain regions[31].

The rest of the data, like demographic data, changes in circulating cytokines, and others, will be used descriptively and as input to regression and mixed model analyses. The final statistical analysis plan, providing

details of the analysis and presentation of the results will be finalized before initiating any statistical analysis.

Patient and public involvement

The study was designed based on the need for new therapeutic options for CP patients and the literature relating to pain management in chronic pancreatitis, as described in the introduction. The primary outcomes, such as pain scores and MRI brain scans were deliberately chosen in order to assess the potential effect of t-VNS treatment both subjectively (patient-oriented) and objectively. Furthermore, no patients were directly involved in the design, recruitment to or conduct of the study. However, an expert/chief doctor specialized in chronic pancreatitis disease is an associated investigator of the study (SSO). The results and findings gathered from this study will be provided to the patients on request in the form of a written report.

There was no public involvement in the study design.

DISCUSSION

To the best of our knowledge, there are no randomized, sham controlled, studies investigating the effect of t-VNS on clinical pain in patients with CP. We expect the study to provide clinical evidence of the analgesic effect of VNS and to elucidate its underlying mechanisms of action. This may pave the road for non-pharmacological treatment of pain associated with CP and the findings of the study may be generalizable to chronic pain conditions *per se*.

Previous studies have shown structural and functional alterations of the CNS in CP patients with abdominal pain[32–36]. The CNS mechanisms may have the ability to recover by targeting treatment at plasticity mechanisms and reorganization of neuronal pathways leading to improvement of clinical symptoms[37]. VNS treatment has emerged promising technique in stimulating neural reorganization and synaptic plasticity in cortical and subcortical networks, leading to modulation of serotoninergic and noradrenergic pain inhibitory pathways[38]. Those mechanisms might alter and regenerate the neural connectivity in regions responsible for pain[39–41]. In addition, the vagal nerve serves as an essential transmitter of inflammatory signals in immune-to-neuronal communication[42–44]. Afferent fibers of the vagal nerve relay information from viscera to the nucleus tractus solitaries in the brainstem, where it 'senses' pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α. Information is then projected to the parvocellular zone of the paraventricular nucleus of the hypothalamus, and therefore comparison of functional alterations in the CNS and circulating levels of pro-inflammatory cytokines may provide evidence of an existing association. Some limitations about the study should be discussed. Firstly, the patients are very heterogeneous, they may suffer from co-morbidities and may receive other pharmacological therapies, which may bias the results. Secondly, the researchers may involuntarily become unblinded since the active treatment will deliver facial contractions while this is not present during sham treatment. Thirdly, the relatively low number of patients may hamper the results; however, we eliminate the inter-individual variability because of the cross-over design.

ETHICS APPROVAL, CONSENT TO PARTICIPATE AND DISSEMINATION

The procedures set out in this study protocol, pertaining to conduct the study in compliance with Good Clinical Practice (CPMP/ICH/135/95), designated Standard Operating Procedures, the Danish Health and Medicines Authority, the Research Ethics Committee in Denmark, and within the principles of the World Medical Association, Declaration of Helsinki amended by the 52nd General Assembly, Edinburgh, Scotland, October 2000, clarified by the General Assembly in Washington 2002, Tokyo 2004, and Seoul 2008 and Fortaleza 2013 as outlined herein.

Investigators (i.e. medical doctors) will obtain informed consent from each patient. We will conduct this study under the rules of Resolution 466/12 and Declaration of Helsinki. Data will be stored electronically in REDCap database, with secure and restricted access. Data transfer will be encrypted and any information capable of identifying individuals removed. Results gathered from this protocol will be presented at national and international conferences and will be published in peer-reviewed journals. All confidential patient data will be protected, and patient identity will not be disclosed. Further dissemination of the data set can be decided by the principal investigator.

Only researchers involved in the data collection and/or data analysis will have access to the final dataset. However, the principal investigator allows direct access to all source data and documents at monitoring, and inspection from the North Denmark Region Committee on Health Research Ethics, the Danish Health and Medicines Authority or from other countries' health authorities.

Trial status

The recruitment of the study started in January 2018. As of January 2019, a total of 13 patients have completed the study.

References

1. Muniraj T, Aslanian HR, Farrell J, Jamidar PA. Chronic pancreatitis, a comprehensive review and update. Part I: Epidemiology, etiology, risk factors, genetics, pathophysiology, and clinical features. *Disease-a-Month*. 2014;60(12):530-550. doi:10.1016/j.disamonth.2014.11.002.

2. Pham A, Forsmark C. Chronic pancreatitis: review and update of etiology, risk factors, and management. *F1000Research*. 2018;7(May):607. doi:10.12688/f1000research.12852.1.

3. Mullady DK, Yadav D, Amann ST, et al. Type of pain, pain-associated complications, quality of life, disability and resource utilisation in chronic pancreatitis: a prospective cohort study. *Gut*. 2011;60(1):77-84. doi:10.1136/gut.2010.213835.

4. Botha C, Farmer AD, Nilsson M, et al. Preliminary report: modulation of parasympathetic nervous system tone influences oesophageal pain hypersensitivity. *Gut*. 2015;64(4):611-617. doi:10.1136/gutjnl-2013-306698.

5. Frøkjær JB, Bergmann S, Brock C, et al. Modulation of vagal tone enhances gastroduodenal motility and reduces somatic pain sensitivity. *Neurogastroenterol Motil*. 2016;28(4):592-598. doi:10.1111/nmo.12760.

6. Johnson RL, Wilson CG. A review of vagus nerve stimulation as a therapeutic intervention. *J Inflamm Res*. 2018;11:203-213. doi:10.2147/JIR.S163248.

7. Frokjaer JB, Bergmann S, Brock C, et al. Modulation of vagal tone enhances gastroduodenal motility and reduces somatic pain sensitivity. *Neurogastroenterol Motil*. 2016;28(4):592-598. doi:10.1111/nmo.12760.

8. Napadow V, Edwards RR, Cahalan CM, et al. Evoked Pain Analgesia in Chronic Pelvic Pain Patients Using Respiratory-Gated Auricular Vagal Afferent Nerve Stimulation. *Pain Med (United States)*. 2012;13(6):777-789. doi:10.1111/j.1526-4637.2012.01385.x.

9. Brock C, Brock B, Aziz Q, et al. Transcutaneous cervical vagal nerve stimulation modulates cardiac vagal tone and tumor necrosis factor-alpha. *Neurogastroenterol Motil*. 2017;29(5):4-7. doi:10.1111/nmo.12999.

10. Gaul C, Diener H-C, Silver N, et al. Non-invasive vagus nerve stimulation for PREVention and Acute treatment of chronic cluster headache (PREVA): A randomised controlled study. *Cephalalgia*. 2016;36(6):534-546. doi:10.1177/0333102415607070.

11. Moher D, Hopewell S, Schulz KF, et al. CONSORT 2010 explanation and elaboration: Updated guidelines for reporting parallel group randomised trials. *Int J Surg*. 2012 10(1): 28-55.

doi:10.1016/j.ijsu.2011.10.001.

12. Chan AW, Tetzlaff JM, Gøtzsche PC, et al. SPIRIT 2013 explanation and elaboration: guidance for protocols of clinical trials. *BMJ*. 2013 Jan8;346:e7586. doi:10.1136/bmj.e7586.

13. Vitória A, Araújo L De, Ribeiro V, et al. Effects of high-frequency transcranial magnetic stimulation on functional performance in individuals with incomplete spinal cord injury : study protocol for a randomized controlled trial. 2017: Nov 6; 18 (1):522. doi:10.1186/s13063-017-2280-1.

14. Kuppuswamy A, Balasubramaniam A V, Maksimovic R, et al. Clinical Neurophysiology Action of 5 Hz repetitive transcranial magnetic stimulation on sensory , motor and autonomic function in human spinal cord injury. *Clin Neurophysiol*. 2011;122(12):2452-2461. doi:10.1016/j.clinph.2011.04.022.

15. Layer P, Yamamoto H, Kalthoff L, Clain JE, Bakken LJ, DiMagno EP. The different courses of early- and late-onset idiopathic and alcoholic chronic pancreatitis. *Gastroenterology*. 1994;107(5):1481-1487. doi:10.1016/0016-5085(94)90553-3.

16. Strickland I, Mwamburi M, Davis S, et al. Noninvasive vagus nerve stimulation in a primary care setting: effects on quality of life and utilization measures in multimorbidity patients with or without primary headache. *Am J Manag Care*. 2018;24(24 Suppl):S517-S526.

17. A.D. N, J.C.A. M, E. T, M.H. R, P.J. G. Initial use of a novel noninvasive vagus nerve stimulator for cluster headache treatment. *Neurology*. 2015;84(12):1249-1253.

18. De Icco R, Martinelli D, Bitetto V, et al. Peripheral vagal nerve stimulation modulates the nociceptive withdrawal reflex in healthy subjects: A randomized, cross-over, sham-controlled study. *Cephalalgia*. 2018;38(10):1658-1664. doi:10.1177/0333102417742347.

19. Silberstein SD, Mechtler LL, Kudrow DB, et al. Non???Invasive Vagus Nerve Stimulation for the ACute Treatment of Cluster Headache: Findings From the Randomized, Double-Blind, Sham-Controlled ACT1 Study. *Headache*. 2016;56(8):1317-1332. doi:10.1111/head.12896.

20. Fayers P. M. et al. EORTC QLQ-C30 Scoring Manual The EORTC QLQ-C30 Introduction. *EORTC QLQ-C30 Scoring Man EORTC QLQ-C30 Introd*. 2001;30:1-67. doi:D/2001/6136/001.

21. Cleeland CS. Brief Pain Inventory (BPI). *Cleel CS MD Anderson Cancer Cent*. 1982;1100:6. doi:10.1007/978-1-4419-9893-4_13.

22. Farrar JT, Young JP, LaMoreaux L, Werth JL, Poole RM. Clinical importance of changes in chronic pain intensity measured on an 11-point numerical pain rating scale. *Pain*. 2001;94(2):149-158. doi:10.1016/S0304-3959(01)00349-9.

23. Arendt-Nielsen L, Morlion B, Perrot S, et al. Assessment and manifestation of central sensitisation across different chronic pain conditions. *Eur J Pain (United Kingdom)*. 2018;22(2):216-241. doi:10.1002/ejp.1140.

24. Olesen SS, Bouwense SAW, Wildersmith OHG, Van Goor H, Drewes AM. Pregabalin reduces pain in patients with chronic pancreatitis in a randomized, controlled trial. *Gastroenterology*. 2011 Aug; 141 (2): 536-543. doi:10.1053/j.gastro.2011.04.003.

25. Kennedy DL, Kemp HI, Ridout D, Yarnitsky D, Rice ASC. Reliability of conditioned pain modulation: A systematic review. *Pain*. 2016;157(11):2410-2419. doi:10.1097/j.pain.0000000000000689.

26. Yarnitsky D, Bouhassira D, Drewes AM, et al. Recommendations on practice of conditioned pain modulation (CPM) testing. *Eur J Pain (United Kingdom)*. 2015;19(6):805-806. doi:10.1002/ejp.605.

27. Farmer AD, Coen SJ, Kano M, et al. Normal values and reproducibility of the real-time index of vagal tone in healthy humans: a multi-center study. *Ann Gastroenterol Q Publ Hell Soc Gastroenterol*. 2014;27(4):362-368.

28. Trial C, Olesen SS, Bouwense SAW, Smith OHGW, Goor HVAN. CLINICAL — PANCREAS Pregabalin Reduces Pain in Patients With Chronic Pancreatitis in a. *YGAST*. 2011;141(2):536-543. doi:10.1053/j.gastro.2011.04.003.

29. College Station TSL. StataCorp. 2015. *Stata Stat Softw Release 13*. 2013.

30. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)-A metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform*. 2009 Apr;42(2):377-81. doi:10.1016/j.jbi.2008.08.010.

31. Hansen TM, Olesen AE, Simonsen CW, Drewes AM, Frokjaer JB. Cingulate metabolites during pain and morphine treatment as assessed by magnetic resonance spectroscopy. *J Pain Res*. 2014;7:269-276. doi:10.2147/JPR.S61193.

32. Dimcevski G, Sami SAK, Funch-Jensen P, et al. {A figure is presented}Pain in Chronic Pancreatitis: The Role of Reorganization in the Central Nervous System. *Gastroenterology*. 2007;132(4):1546-1556. doi:10.1053/j.gastro.2007.01.037.

33. Frøkjær JB, Olesen SS, Gram M, et al. Altered brain microstructure assessed by diffusion tensor imaging in patients with chronic pancreatitis. *Gut*. 2011;60(11):1554-1562. doi:10.1136/gut.2010.236620.

34. Frøkjær JB, Bouwense SAW, Olesen SS, et al. Reduced Cortical Thickness of Brain Areas Involved in Pain Processing in Patients With Chronic Pancreatitis. *Clin Gastroenterol Hepatol*. 2012;10(4):434-438. doi:10.1016/j.cgh.2011.11.024.

35. Lelic D, Olesen SS, Graversen C, Brock C, Valeriani M, Drewes AM. Electrophysiology as a tool to unravel the origin of pancreatic pain. *World J Gastrointest Pathophysiol*. 2014;5(1):33-39. doi:10.4291/wjgp.v5.i1.33.

36. Muthulingam J, Olesen SS, Hansen TM, et al. Progression of Structural Brain Changes in Patients With Chronic Pancreatitis and Its Association to Chronic Pain: A 7-Year Longitudinal Follow-up Study. *Pancreas*. 2018;47(10):1267-1276. doi:10.1097/MPA.0000000000001151.

37. Apkarian A V, Hashmi JA, Baliki MN. Pain and the brain: Specificity and plasticity of the brain in clinical chronic pain. *Pain*. 2011;152(3 Suppl):S49-64. doi:10.1016/j.pain.2010.11.010.

38. Garcia RG, Lin RL, Lee J, et al. Modulation of brainstem activity and connectivity by respiratory-gated auricular vagal afferent nerve stimulation in migraine patients. *Pain*. 2017;158(8):1461-1472. doi:10.1097/j.pain.0000000000000930.

39. O'Connell NE, Marston L, Spencer S, et al. Non-invasive brain stimulation techniques for chronic pain. *Cochrane database Syst Rev*. 2018;3(9):CD008208. doi:10.1002/14651858.CD008208.pub2.

40. Seminowicz DA, Wideman TH, Naso L, et al. Effective Treatment of Chronic Low Back Pain in Humans Reverses Abnormal Brain Anatomy and Function. *J Neurosci*. 2011;31(20):7540-7550. doi:10.1523/JNEUROSCI.5280-10.2011.

41. Čeko M, Shir Y, Ouellet JA, Ware MA, Stone LS, Seminowicz DA. Partial recovery of abnormal insula and dorsolateral prefrontal connectivity to cognitive networks in chronic low back pain after treatment. *Hum Brain Mapp*. 2015;36(6):2075-2092. doi:10.1002/hbm.22757.

42. Bonaz B, Sinniger V, Pellissier S. Vagal tone: Effects on sensitivity, motility, and inflammation. *Neurogastroenterol Motil*. 2016;28(4):455-462. doi:10.1111/nmo.12817.

43. Watkins LR, Maier SF, Goehler LE. Cytokine-to-brain communication: A review & analysis of alternative mechanisms. *Life Sci*. 1995;57(11):1011-1026. doi:10.1016/0024-3205(95)02047-M.

44. Hosoi T, Okuma Y, Matsuda T, Nomura Y. Novel pathway for LPS-induced afferent vagus nerve activation: Possible role of nodose ganglion. *Auton Neurosci Basic Clin*. 2005;120(1-2):104-107. doi:10.1016/j.autneu.2004.11.012.

Legends for illustrations

- Figure 1: Mode of action of transcutaneous vagal nerve stimulation (t-VNS). (1) Pain arises in the periphery e.g. pancreas and a signal is sent to the spinal cord. This leads to ascending activation of the spinal neurons (2). In the brain, pain is processed in higher cortical centres (3). T-VNS, it is expected to block the perception of pain in the cerebral cortex, by stimulating nucleus tractus solitarius and thereby decrease glutamate level. Simultaneously, the net-descending inhibition will be activated as a result of top-down input from cortex and the limbic system (4).
- Figure 2: Schematic flowchart of the interventional study design enabling comparison of the modulatory effect to self-administered of transcutaneous vagal nerve stimulation (t-VNS) in patients with chronic pancreatitis. Chronic pancreatitis patients will be randomly assigned to one of two double blinded treatments: (1) two weeks of t-VNS, two weeks of wash-out, and two weeks of sham treatment; or (2) two weeks of sham treatment, two weeks of washout, and two weeks of t-VNS. Evaluation of the two treatments will be assessed by collecting pain diary, pain questionnaires, MRI scan, blood sample, cardiac vagal tone, and pain assessments. QST=Quantitative sensory testing. CVT=Cardiac vagal tone. MRI=Magnetic resonance imaging.
- Figure 3: SPIRIT Figure.

Table 1: Trial characteristics based on WHO Trial Registration Data Set

Data category	Trial Information
Primary Registry and Trial Identifying Number	ClinicalTrials.gov (NCT03357029)
Date of Registration in Primary Registry	November 29, 2017
Secondary Identifying Numbers	North Denmark Region Committee on Health Research Ethics: protocol number N-20170023
Source(s) of Monetary or Material Support	The study is conducted as a sponsor-investigator initiated study with financial support from Independent Research Fund Denmark (DFF – 7016-00073).
Primary Sponsor	JBF
Secondary Sponsor	NA
Contact for Public Queries	JBF
Contact for Scientific Queries	JBF
Public title	Neuromodulation in Patients with Painful Chronic Pancreatitis
Scientific title	Study protocol for a randomized double-blinded, sham-controlled, prospective, cross-over clinical trial of vagal neuromodulation for pain treatment in patients with chronic pancreatitis
Country of recruitment	Denmark
Healthy conditions(s) or problems studied	Chronic pancreatitis
Interventions	Two-week transcutaneous vagal nerve stimulation (t-VNS) on the cervical vagal area (Self-administering vagal nerve stimulation bilaterally to the cervical vagal area, the times per day).
Key inclusion and exclusion criteria	Inclusion criteria: Age ≥18 years; Patients with a diagnosis of CP diagnosed using the Mayo Clinic diagnostic criteria.; The participants must be able to read and understand Danish.; The patients must suffer from chronic abdominal pain characteristic for CP, meet the criteria for chronic pain (pain ≥ 3 days per week in at least 3 months) and must consider their pain as insufficiently treated with their usual analgesic treatment. ; Personally, signed and dated informed consent document and the Power of attorney document; Patients willing and able to comply with the scheduled visits, treatment plan, laboratory tests and other trial procedures. Exclusion criteria: Patients with any clinically significant abnormalities that in the opinion of the investigator may increase the risk associated with trial participation or may interfere with the interpretation of the trial results. ; Alcohol dependence; Illegal drug dependencies; Participating in another study where investigational drug is used, ; Patients must not suffer from painful conditions other than CP that make them unable to distinguish the pain associated with CP from chronic pain of other origin.; Cardiovascular diseases ; Low blood pressure < 100/60, Not able to understand or follow the instructions, ; Any condition with elevated intracranial pressure.; Female patients who are pregnant; Contraindications for MRI; Previous surgery on vagal nerve.; Known neuropathy.
Study type	Interventional allocation: randomized Masking: double-blind Assignment: cross-over Primary purpose: treatment

Date of first enrolment	January 2018
Target sample size	21
Recruitment status	Recruiting
Primary outcome(s)	Change in NRS scores in pain diary
Key Secondary outcomes (s)	Aassessment of the effect of t-VNS on A) resting state brain function assessed by MRI, and B) brain metabolites assessed by MR spectroscopy.

546

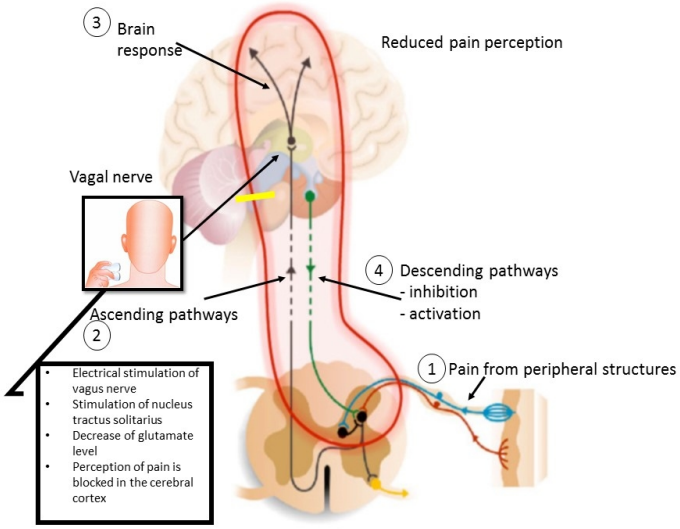

Figure 1: Mode of action of transcutaneous vagal nerve stimulation (t-VNS). (1) Pain arises in the periphery e.g. pancreas and a signal is sent to the spinal cord. This leads to ascending activation of the spinal neurons (2). In the brain, pain is processed in higher cortical centres (3). T-VNS, it is expected to block the perception of pain in the cerebral cortex, by stimulating nucleus tractus solitarius and thereby decrease glutamate level. Simultaneously, the net-descending inhibition will be activated as a result of top-down input from cortex and the limbic system (4).

338x190mm (96 x 96 DPI)

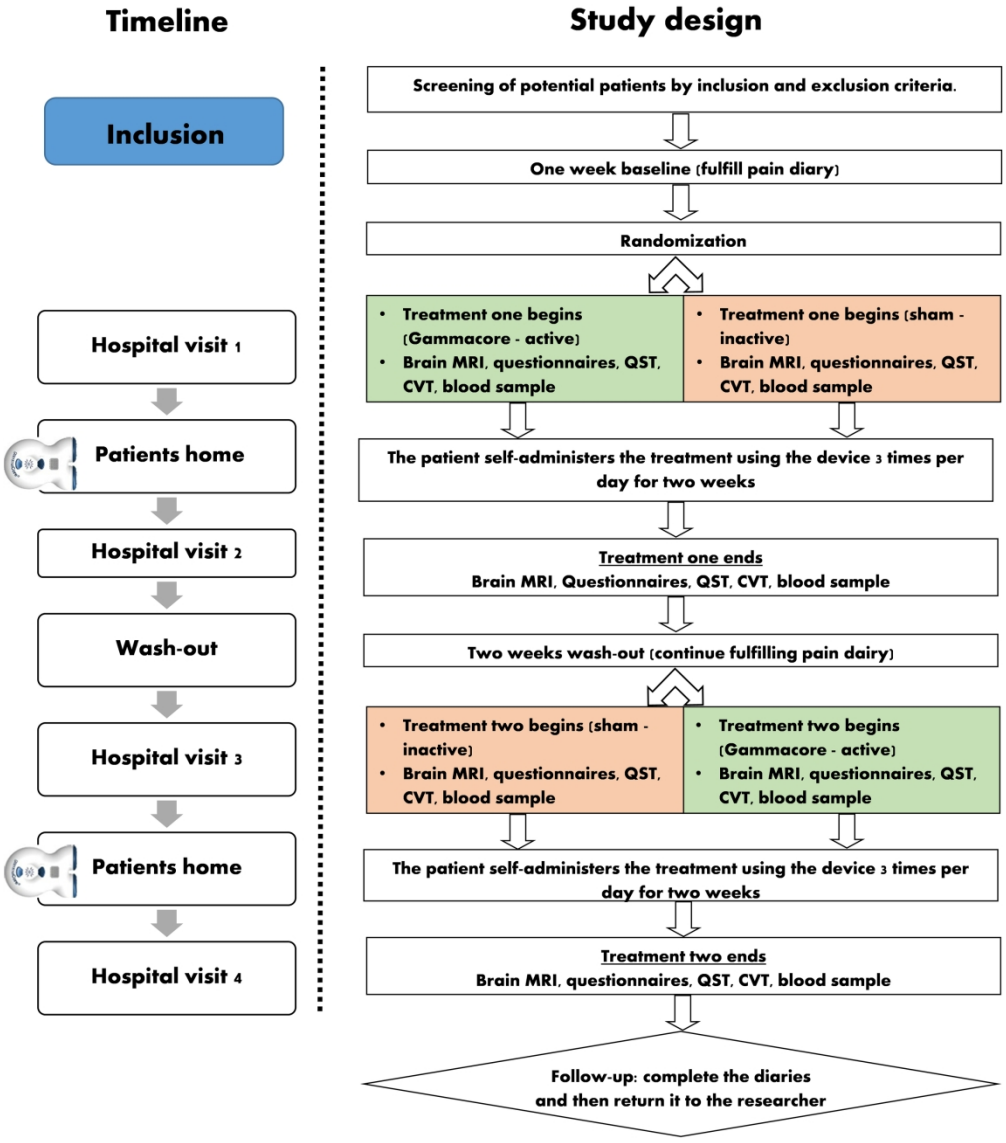

Figure 2: Schematic flowchart of the interventional study design enabling comparison of the modulatory effect to self-administered of transcutaneous vagal nerve stimulation (t-VNS) in patients with chronic pancreatitis. Chronic pancreatitis patients will be randomly assigned to one of two double blinded treatments: (1) two weeks of t-VNS, two weeks of wash-out, and two weeks of sham treatment; or (2) two weeks of sham treatment, two weeks of washout, and two weeks of t-VNS. Evaluation of the two treatments will be assessed by collecting pain diary, pain questionnaires, MRI scan, blood sample, cardiac vagal tone, and pain assessments. QST=Quantitative sensory testing. CVT=Cardiac vagal tone. MRI=Magnetic resonance imaging.

220x253mm (300 x 300 DPI)

	STUDY PERIOD								
	Enrolment & screening	Baseline & allocation	Post-allocation						Follow-up
TIMEPOINT**	T_{-1}	0	T_1	T_2	T_3	T_4	T_5	T_6	T_7
Week	0	1	2	3	4	5	6	7	8
ENROLMENT:									
Eligibility screen	X								
Informed consent	X								
Allocation		X							
INTERVENTIONS:									
[transcutaneous VNS (active)]			X	X	Washout		X	X	
[Sham treatment]			X	X			X	X	
ASSESSMENTS:									
Pain diary		X	X	X	X	X	X	X	X
QORTC-QLQ-C30 questionnaire			X	X			X	X	
BPI-SF Questionnaire			X	X			X	X	
PGIC questionnaire				X				X	
MRI			X	X			X	X	
CVT			X	X			X	X	
Blood samples			X	X			X	X	
QST			X	X			X	X	
[Adverse events]			X	X	X		X	X	

Figure 3: SPIRIT Figure.

87x104mm (300 x 300 DPI)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	____1_____
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	_____2_____
	2b	All items from the World Health Organization Trial Registration Data Set	__Table 1__
Protocol version	3	Date and version identifier	_____1_____
Funding	4	Sources and types of financial, material, and other support	_____1___
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	_____1____
	5b	Name and contact information for the trial sponsor	_____1_____
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	____1_____
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	Not applicable

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	__4-5, 7-8, 11_
	6b	Explanation for choice of comparators	___7-9_____
Objectives	7	Specific objectives or hypotheses	___4-5_____
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	__6_____
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	_____ 6_____
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	____6-7_____
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	___7-8_____
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	_____7_____
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	__ 8_____
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	___7_____
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	___9-11_____
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	___Figure 2 & 3_

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	__11_____
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	__6_____
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	____8_____
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	__8_____
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	__8_____
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	___8,9_____
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	___9_____
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	__7-12_____
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	Not applicable

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	___11,12_____
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	____12_____
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	Not applicable
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	__12_____
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	____12_____
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	__12_____
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	___12_____
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	Not applicable
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	____6_____
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	Not applicable

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	___14_____
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	___14_____
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	___14_____
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	_____1_____
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	___14_____
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	____14_____
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	___14_____
	31b	Authorship eligibility guidelines and any intended use of professional writers	Not applicable
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	Not applicable
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	See rules of the Ethical Committee
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	Not applicable

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Study protocol for a randomised double-blinded, sham-controlled, prospective, cross-over clinical trial of vagal neuromodulation for pain treatment in patients with chronic pancreatitis

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-029546.R1
Article Type:	Protocol
Date Submitted by the Author:	04-Jun-2019
Complete List of Authors:	Muthulingam, Janusiya Anajan; Aalborg University Hospital , Mech-Sense, Department of Radiology; Aalborg University, Department of Clinical Medicine Olesen, Søren; Aalborg University Hospital, Mech-Sense, Department of Gastroenterology and Hepatology; Aalborg University, Department of Clinical Medicine Hansen, Tine; Aalborg University Hospital, Denmark, Mech-Sense, Department of Radiology; Aalborg University, Department of Clinical Medicine Brock, Christina; Aalborg University, Department of Clinical Medicine; Aalborg University Hospital Drewes, Asbjørn; Mech-Sense, Department of Gastroenterology and Hepatology, Aalborg University Hospital, ; Department of Clinical Medicine, Aalborg University, Frøkjær, Jens Brøndum; Mech-Sense, Department of Radiology, Aalborg University Hospital; Aalborg University, Department of Clinical Medicine
Primary Subject Heading:	Gastroenterology and hepatology
Secondary Subject Heading:	Neurology
Keywords:	Viscera, Clinical trials < THERAPEUTICS, Gastrointestinal disease, Chronic pain, Transcutaneous electrical nerve stimulation

Study protocol for a randomised double-blinded, sham-controlled, prospective, cross-over clinical trial of vagal neuromodulation for pain treatment in patients with chronic pancreatitis

Janusiya Anajan Muthulingam[1,2], Søren Schou Olesen[2,3], Tine Maria Hansen[1,2], Christina Brock[2,4], Asbjørn Mohr Drewes[2,3] and Jens Brøndum Frøkjær[1,2]

1 Mech-Sense, Department of Radiology, Aalborg University Hospital, Aalborg, Denmark

2 Department of Clinical Medicine, Aalborg University, Aalborg, Denmark

3 Centre for Pancreatic Diseases, Department of Gastroenterology & Hepatology, Aalborg University Hospital, Aalborg, Denmark

4 Mech-Sense, Department of Gastroenterology & Hepatology, Aalborg University Hospital, Aalborg, Denmark

Trial registration number: The study is registered at www.clinicaltrials.gov: NCT03357029

Protocol version: Protocol version 6, 13th of September 2017

Funding: The study is conducted as a sponsor-investigator initiated study with financial support from Independent Research Fund Denmark (DFF – 7016-00073).

Study Sponsor-investigator and corresponding author: Professor Jens Brøndum Frøkjær, MD, PhD
Mech-Sense, Department of Radiology
Aalborg University Hospital
P.O. Box 365, DK-9100 Aalborg, Denmark
Telephone: +45 97665105
E-mail: jebf@rn.dk

Contributors: JBF, JM, SSO, TMH, CB and AMD conceived and designed the study and participated in logistical planning of the study. JM drafted the initial version of the manuscript and is collecting the data. All authors made significant contributions to the development and conceptualization of the protocol. All authors reviewed the draft versions of the manuscript and have read and approved the final manuscript.

Competing interests: None

Word count: Abstract: 243 words; main text: 4226 words; Figures 3; table 1; References 50

Abstract

Introduction: The management of chronic pancreatitis (CP) is challenging and requires a personalized approach focused on the individual patient's main symptoms. Abdominal pain is the most prominent symptom in CP, where central pain mechanisms, including sensitization and impaired pain modulation, often are involved. Recent clinical studies suggest that vagal nerve stimulation (VNS) induce analgesic effects through modulation of central pain pathways. This study aims to investigate the effect of two-weeks transcutaneous VNS (t-VNS) on clinical pain in CP patients, in comparison to the effect of sham treatment.

Methods and analysis: Twenty-one CP patients will be enrolled in this randomized, double-blinded, single-centre, sham-controlled, cross-over study. The study has two treatment periods: A two-week active t-VNS using GammaCore® device and a two-week treatment with a sham device. During both treatment periods, the patients are instructed to self-administer VNS bilaterally to the cervical vagal area, three times per day. Treatment periods will be separated by two weeks. During the study period patients will record their daily pain experience in a diary (primary clinical endpoint). In addition, all subjects will undergo testing which will include magnetic resonance imaging (MRI), quantitative sensory testing (QST), cardiac vagal tone (CVT) assessment and collecting blood samples, before and after the two treatments to investigate mechanisms underlying VNS effects. The data will be analysed using the principle of intention-to-treat.

Ethics and dissemination: The regional Ethics committee has approved the study: N-20170023. Results of the trial will be submitted for publication in peer-reviewed journals.

Trial registration: The study is registered at www.clinicaltrials.gov: NCT03357029

Key words: Transcutaneous electrical nerve stimulation; Viscera; Chronic Pain; Gastrointestinal disease; Clinical trials

Strengths and limitations of the study:

- This is the first study to examine the analgesic effect of transcutaneous vagal nerve stimulation (t-VNS) in chronic pancreatitis patients with abdominal pain.
- A randomized double-blinded, sham-controlled, prospective cross-over design will be used with both clinical and experimental outcomes, which allow for exploration of the mechanisms underlying putative clinical effects.
- The study investigates the effect of two weeks of t-VNS treatment; hence, further studies are needed to explore long-term effects.
- The single-centre design may limit generalizability of the study results.

INTRODUCTION

Chronic pancreatitis (CP) is a disease characterized by progressive pancreatic inflammation and fibrosis, resulting in damage to and loss of exocrine (acinar), endocrine (islet cells), and ductal cells[1]. Chronic abdominal pain is the dominating symptom in CP and is present in up to 70% of patients[2]. Pain is associated with reduced quality of life, increased hospitalization frequencies and thus a significant socioeconomic burden[3].

The aetiology of pain in CP is increasingly better understood and often involves multiple mechanisms in the individual patient. In addition to local pathology in the pancreatic gland and its surrounding tissues, central pain pathways undergo neuroplastic changes during the course of CP. These involve sensitization of central pain pathways, functional and structural reorganization of the brain as well as impaired efficacy of endogenous pain modulatory pathways. These neural abnormalities can be targeted by different pharmacological therapies, but their effect is often limited and associated with significant side-effects in many patients. This has led to an increased interest in complementary treatment modalities for pain in patients with CP. In a model of oesophageal hyperalgesia, we have shown that physiological deep breathing enhanced vagal tone, which in response increased the pain detection threshold[4]. In addition, this effect was abolished by atropine administration thereby proving that enhanced parasympathetic tone leads to prevention of oesophageal pain hypersensitivity[4]. Also, we have previously shown an improved gastrointestinal motility and decreased pain sensitivity following non-invasive VNS of the auricular branch of the vagal nerve in conjunction with a deep-breathing approach in healthy subjects[5]. Another non-pharmacological treatment modality is transcutaneous vagal nerve stimulation (t-VNS), in which short bursts of electrical energy are directed onto the vagal nerve at the neck[6] (Figure 1). T-VNS has been shown to induce analgesic[7,8] and anti-inflammatory effects in healthy individuals[9] and different diseases. The exact mechanisms by which VNS modulates chronic pain is unclear, however it has been proposed that the analgesic effect is potentially mediated by vagal afferents that inhibit spinal nociceptive reflexes and transmission[10]. Specifically, the analgesic effects is mediated through vagal afferent modulation in the nucleus tractus solitaries, raphe magnus, locus ceruleus, amygdala and periaqueductal grey, which are involved in the descending inhibition of pain[10,11]. It has also been demonstrated that VNS inhibits spinal cord neurons below C3 but excites neurons between C1 and C3, suggesting that propriospinal neurons from high segments play an essential role in vagally mediated antinociception. Thus, VNS appears to induce neuromodulatory antinociception through peripheral and central, ascending and descending pathways[12]. Also, the non-pharmacological treatment is FDA-approved for the acute treatment in migraine patients[13].

This study aims to examine the analgesic effect of a two-week t-VNS in patients with CP and to explore the underlying analgesic mechanisms using advanced neuroimaging techniques and quantitative sensory testing (QST). We hypothesized that two weeks t-VNS treatment will induce clinically relevant pain relief compared

to sham treatment, and that these effects are mediated via modulation of central pain pathways. To answer the overall study aims, we have two clinical and two experimental objectives:

1) The primary clinical objective is to assess the effect of t-VNS on the daily pain experience documented in a pain diary in chronic pancreatitis patients.
2) Secondary clinical objectives are to document changes in quality of life and daily functioning.
3) The experimental objective is to assess the effect of t-VNS on A) resting state brain function assessed by magnetic resonance imaging (MRI), and B) brain metabolites assessed by magnetic resonance spectroscopy.
4) Secondary experimental objectives are to assess the effect of t-VNS on A) experimental pain stimuli documented by QST, B) cardiac vagal tone and C) pro-inflammatory cytokine profiles obtained from blood samples.

METHODS AND ANALYSES:

Study design

Randomised, single-centre, double-blinded, prospective, sham-controlled, cross-over study. The study was approved by the North Denmark Region Committee on Health Research Ethics with the protocol number N-20170023 and has been registered with ClinicalTrials.gov (NCT03357029). The trial will be performed at Aalborg University Hospital and will be reported according to the Consolidated Standards of Reporting Trials (CONSORT)[14]. The study protocol follows the Standard Protocol Items: Recommendations for Interval Trials (SPIRIT) statement[15].

All patients undertake the t-VNS treatment using an active GammaCore-S, 300 treatments (10009-00603) device (ElectroCore LLC; Basking Ridge, NJ, USA) and sham treatment using a sham-device (10009-00603 P) which is identical in appearance GammaCore.

Half of the patients will be randomized to start with two-week t-VNS treatment, followed by a two-week washout period. Then, this group will be reallocated to sham treatment. The other half of patients will do the study periods in opposite order (sham treatment followed by t-VNS treatment). The two-week washout period has been used in previous studies of trans-cranial neuromodulation[16] and was shown to be sufficient to reset the effects of neuromodulation[17]. Each patient will be scheduled for four identical hospital visits (before and after each treatment period). The visits consist of 1) Fulfilment of questionnaires, 2) Collection of blood samples, 3) brain MRI scan, 4) QST, and 5) assessment of CVT (Figure 2, Table 1).

Study participants

Patients will be recruited via personal correspondence and during visits at the outpatient clinic. Patients who agree to participate in the study and fill in an informed consent will be invited to participate in the study. A screening session and physical examination prior to inclusion will be conducted by a medical doctor including relevant medical and medication history and screening against the eligibility criteria. All patients will be asked to continue their medication during the entire study, and any changes needed in pain medication will be noted in the diary.

Inclusion criteria and exclusion criteria

Patients from the age of 18 years will be included in the study. They will have a clinical diagnosis of CP based on the Mayo clinical diagnostic criteria[18]. All aetiological types of CP patients would be included (incl. alcohol, nicotine, hereditary, efferent duct factors, and immunological aetiologies). The patients must suffer from chronic abdominal pain characteristics for CP, meet the criteria for chronic pain (pain ≥ 3 days per week for at least 3 months) and must consider their pain as insufficiently treated with their prescribed analgesic

treatment. Additionally, the patients must be willing and able to comply with the scheduled visits, treatment plan, laboratory tests, and other study procedures. Finally, the patient must sign the informed consent and power of attorney document.

Patients will be excluded if they have any clinically significant abnormalities that may increase the risk associated with trial participation or may interfere with the interpretation of the trial results. Also, patients with alcohol and illegal drug dependence patients, cardiovascular diseases, low blood pressure (<100/60mmHg), elevated intracranial pressure will be excluded. Additionally, patients who are participating in another intervention study, patients who are pregnant or intend to become pregnant, and patients who suffer from painful conditions other than CP that make them unable to distinguish the pain associated with CP from chronic pain of other origin will be excluded. Patients will also be excluded if there are any contraindications for MRI (incl. cardiac pacemaker, implantable metallic components, etc.), have known neuropathy, or previous vagal nerve surgery (Table 1).

Participants can withdraw from the study at any time they may wish. Patients will be withdrawn from the study if they do not meet for the scheduled study visits or miss a treatment period, and if they do not maintain inclusion/exclusion criteria.

Interventions

Study interventions are t-VNS treatment and sham treatment (Figure 2 & 3). Prior to receiving the t-VNS treatment/sham treatment, the standard care must be stable.

Patients will be thoroughly instructed to use the device, and when the healthcare providers are confident that the patient is capable to use the device independently, the device will be handed over to the patient. T-VNS is administered by using a handheld device (GammaCore; ElectroCore LLC; Basking Ridge, NJ, USA), which consists of a battery powered portable stimulator with a digitally controlled user-interface that controls the signal amplitude and two gel covered (Sigma gel, Parker Laboratories, NJ, USA) contact electrodes which deliver electrical stimulation to the cervical vagal nerve. One dose corresponds to 120 seconds of t-VNS to the left cervical vagal nerve followed by 120 seconds of t-VNS to the right cervical vagal nerve, with the amplitude of simulation titrated to achieve mild pulling of the ipsilateral oral commissure[19]. Bilateral stimulation has shown to be effective in previous studies with GammaCore[20,21]. The patient self-administers the treatment, using the device at home three times per day (morning, afternoon, and evening) for two weeks. Previous studies with Gamma-Core, have shown that three doses per day have been effective [13,22].

The stimulation device is positioned anterior to the sternocleidomastoid muscle, over the carotid artery as this runs in close proximity with the vagal nerve. The active Gamma-Core device produces a low-voltage electrical signal comprising a 5 kHz sine wave burst lasting for 1 millisecond (five sine waves, each lasting 0.2

millisecond), with such bursts repeated once every 40 milliseconds (25 Hz), generating a 24 V peak voltage and 60 mA peak output current. Those parameters have been used to activate the vagal nerve in electrophysiological studies[20,21].

To mimic the sensation of the active treatment, the sham-device will provide vibration[23]. The appearance, weight, visual and audible feedback, and user application are identical for the sham and t-VNS devices. However, the sham device produces a low-frequency (0.1 Hz) biphasic signal that does not stimulate the vagal nerve or generally cause muscle contractions[24]. Additionally, both devices will display a numeric value between 1 and 40, signifying the intensity of the stimulation. The maximum intensity per stimulation is 40 for both devices (Figure 1). The intensity of the stimulation can vary from patient to patient. The intensity for stimulation is reached by increasing the stimulation to the maximum the patient can tolerate without excessive pain. Some patients can tolerate less than other patients depending on the pain level. Therefore the dosage of every stimulation is patient-dependent [25].

Compliance will be assessed by reading the remaining doses displayed at the device after each treatment period. Additionally, the patients will be asked to keep a record of the stimulation intensity of the doses applied at each stimulation. In addition, questions on compliance will be asked after each treatment period. Finally, adherence will be recorded by patients' diary.

During the study periods the patient will continue their standard care, without changes in their current pain treatment.

Randomization, sequence generation and allocation concealment

Once eligibility and consent have been approved and completed, randomization will occur using a randomization list generated by an automatic web-based randomization program. Patients will be randomly assigned to VNS/sham or sham/VNS using block randomisation, allowing seven patients at the time to be randomised in equal proportions for the order of active t-VNS or sham stimulation. The randomization order will be kept in closed envelopes; therefore, patients will get their assignment according to the order of entrance in the study. This process will be carried out by a member of the research team who is not involved in the recruitment process or conduction of the study.

An unblinded researcher will be involved in delivering the medical device according to the randomisation schedule. The sequence will follow a 1:1 sequential design, in a double-blinded fashion. Additionally, the outcome assessor (data analyst) will be blinded during the statistical analyses of experimental outcomes. A series of numbered, sealed, envelopes will be used to ensure concealed allocation.

Blinding

Both, active and sham devices are labelled with a serial number and not outwardly identified as active or sham. All researchers involved in the data collection and MRI analysis will be blinded to the treatment allocation group until after analyses are performed at the completion of the trial. Additionally, all patients are blinded, and they do not know that the sham treatment is an inactive treatment. Particularly, patients will be informed that they have to undergo two different interventions with two different devices, and the purpose of this study is to investigate the most effective treatment.

Manufacturing and preparation of the medical devices are handled by an external good manufacturing practice-accredited facility (ElectroCore). As the patients do not know that the sham treatment is an inactive treatment, we will not be able to ask the patient "do you think you received active or inactive treatment?", thus we will not be able asses and determine if the blinding was effective.

Unblinding is only permissible if a patient experiences any serious adverse events and that the investigator/doctor judge that it is essential to know the treatment allocation in order to treat the patient appropriately.

Primary clinical outcome measures

The primary clinical efficacy parameter to be evaluated is 30% pain relief. This is assessed as changes in the daily experience of pain, which will be measured using a patient pain diary based on the numeric rating scale (NRS) (1 = no pain, 10 = worst pain imaginable). Patients will be asked to score daily pain levels in the diary for eight weeks (including one week before the first treatment period and one week after the last study period, Figure 2 and Figure 3), with one NRS value for the average pain over the previous 24 hours and one NRS value for the worst pain over the previous 24 hours.

Secondary clinical outcome measures

Quality of life questionnaire, C30, version 3.0 (QoLQ-C30)[26], the brief pain inventory – short form (BPI-SF) questionnaire[27], and Patient Global Impression of Changes[28] questionnaire (PGIC) are secondary clinical outcomes. Patient will complete QoLQ-C30 and BPI-SF questionnaire before and after each treatment period, while the PGIC questionnaire will only be fulfilled after the treatment periods. The QoLQ-C30 questionnaire is composed of both multi-item scales and single-item measures. These include five functional scales, three symptom scales, a global health status, and six single items. The BPI-SF questionnaire rapidly assess the severity of pain and its impact on daily functioning. Finally, the PGIC questionnaire evaluates all aspects of patients' health and assess if there has been an improvement or decline in the overall clinical status.

Experimental outcome measures

Resting state functional MRI will be employed to detect brain activity and functional connectivity changes based on BOLD signals before and after treatment of each patient. Additionally, magnetic resonance spectroscopy in anterior cingulate cortex, prefrontal cortex, parietal, and insula will also be performed in order to investigate changes in brain metabolites before and after each treatment.

MRI data will be acquired on a 3 Tesla MR scanner (Signa HDxt, General Electric, Milwaukee, WI, USA) equipped with an 8 channel standard head coil. Scan time for a structural scan will be 5½ minutes. Following parameters will be used for the structural scan: 150 slices, FOV 250 mm, echo time 3.6 millisecond, repetition time 9.0 millisecond, flip angle 14°, resolution 0.78x0.78 mm, matrix size 320x320 mm, slice thickness 1 mm, full head coverage, with no gap. Functional scans will be acquired with following parameters: gradient echo, echo planar (Gr-EPI), 192 volumes, 37-40 slices, FOV=240 mm, echo time=30 millisecond, repletion time=2000 millisecond, flip angle=90 ^{0}, matrix size=64x64, resolution=3.75x3.75 mm, slice thickness 3.8 mm, no gap, axial slices. The scan time for functional MRI will be 6 minutes and 32 seconds. Additionally, MRI spectroscopy will be used to estimate brain metabolites in the anterior cingulate cortex, prefrontal cortex, parietal, and insula. For MRI spectroscopy, single voxel PRESS (Point RESolved Spectroscopy) will be acquired. Following parameters will be used: Echo time=30 millisecond, repetition time=2000 millisecond, scan time will be 5 minutes, and the total number of scans will be 128. Bandwidth will be 5,000 Hz. A 20x20x20 mm voxel of interest will be positioned on a sagittal T2-weighted fast spin echo sequence. Repletion time= 4600 millisecond and echo time=102 millisecond, matrix 384x256, slice thickness 3 mm, gap 0.3 mm), in the midline in the ACC with the inferior border along the anterior-posterior commissure line.

Secondary experimental outcome measures

Secondary outcomes are changes in QST, CVT and pro-inflammatory cytokine profiles.

QST includes temporal summation[29], pressure pain thresholds[29,30] and conditioned pain modulation (CPM)[31]. Temporal summation demonstrates an increase perception of pain to repetitive pain stimuli[29]. Temporal summation will be recorded in the dermatome T10 (pancreatic area) and control area (dominant forearm) using the PinPrick stimulator, 256 mN (MRC Systems GmbH Medizintechnische Systeme, Germany).

The pressure pain threshold and pressure pain tolerance will be determined by pressing an electronic pressure algometer (Somedic AB, Stockholm, Sweden) on specified muscle groups: C5 - clavicula, T10 – dorsum, T10 – abdomen, L1 – anterior superior iliac spine, and rectus femoris. Also, pressure pain threshold and pressure pain tolerance will be measured on bone. For the muscle pressure stimulation, the probe has a surface area of

1 cm^2. Pressure will be increased at a rate of 30 kPa/sec until the pressure pain threshold is reached. For the bone pressure stimulation, a probe with 3.1 mm^2 (Aalborg University, Denmark) will be applied.

CPM is a clinically measurable form of descending pain modulation[31] that can be induced experimentally by a conditioning stimulus (the cold pressor test) and quantified by applying a "test-pain" (pressure stimulation of the right quadriceps musculature) before and after its induction[32]. The patient will lower their dominant hand in cold water (2°C for maximum two minutes). The difference in pressure stimulus intensity (pain threshold) before and after induction of cold pressor pain provides a quantitative index of CPM capacity for the individual patient. The techniques used for pressure stimulation and cold pressor test described above will be combined to measure CPM.

CVT is a beat-to-beat measure of brainstem efferent vagal activity, which is assessed by heart rate variability measurement and reflects the contribution of the vagal nerve to cardiac functioning. In this particular test, changes in R-R interval would be measured non-invasively using eMotion Faros 180 device[33].

Blood samples are collected to explore changes in pro-inflammatory cytokines profiles. 26 ml blood is collected, and the following inflammatory state and macrophage markers will be assessed: interferon-G, interleukin-8 (IL-8), IL-10, IL-12p70, IL-13, IL-1b, IL-2, IL-4, IL-6, tumour necrosis factor- α (TNF-α), monocyte chemoattractant protein-1 (MCP-1), and high sensitivity C-reactive protein (hs-CRP).

Statistical power

The study is powered to detect a minimal difference between the sham treatment and the active treatment of 30% on the average clinical pain score at the end of the two study periods. Based on a standard deviation of 40% we determine that a study with 16 patients in a cross-over design is needed, with a power of 80%, and the use of a two-sided significance level of 0.05 (alpha). This calculation (standard deviation) is based on data from a study with CP patients, who received pregabalin treatment, which related to an improvement in clinical measures of the pain score[34]. To allow for a dropout rate of 25%, we will aim to recruit 21 CP patients. The sample size was calculated using statistical software package STATA 15.0 (StataCorp LP, College Station)[35].

Harms and adverse events

We do not anticipate this project causing any harm or discomfort to the patients, and we will ensure that our patients participate in the study voluntarily.

Information about adverse events and serious adverse events will be collected from the date of inclusion and in all following contacts with the study subject throughout the project. Adverse events will be documented on the patient file and on the electronic case report form. All types of adverse events will be notified to the device manufacturer ElectroCore and to the Danish Health Authorities by use of Manufactures Incident Report Form.

Data collection and data management

All instruments in the questionnaires are validated[26,27]. Additionally, all data collectors are highly experienced registered research nurses, radiographers, and researchers who have been trained in good clinical practice (GCP). There will be regular meetings between the data collectors, monitor, principal investigator and other co-researchers involved in the project. All paper protocols will be kept safe and transferred to a computerised database. The questionnaires will be checked for errors and missing data by research staff. Data entries are double-checked against the paper questionnaires.

During trial conduct, the Good Clinical Practice unit (GCP, Aalborg, Denmark) will conduct periodic monitoring of all signed consents at monitoring visits to ensure that the protocol and GCP standards are followed. The monitors may review source documents and medical records to confirm that data recorded on Case Report Form is accurate. Thus, GCP monitoring includes all signed consents, signed power of attorney, and AE.

Criteria for the termination of the trial is, when patients according to the sample size with valid data are recorded. If the study fails to recruit adequate patients according to the sample size by end of 2019, the study will be terminated.

Data analysis

Both descriptive and analytical statistics will be used in order to compare groups and for analyses of outcomes over time including changes therein. All data will be presented as mean ± standard deviation and summarized in frequency tables, unless otherwise indicated. We will use Research Electronic Data Capture (REDCap)[36] to store the data and the statistical software package STATA to perform statistical analysis. We will use mixed ANOVA for the inferential statistic of the parametric data, with Tukey's and/or Bonferroni post hoc tests for the primary clinical endpoints. Significance level will be set as $\alpha \leq 0.05$.

The principal analysis of clinical endpoints will be by intention-to-treat, meaning that all randomized patients are included in their initially assigned study arm, regardless of adherence to study protocol. Experimental endpoints will be analysed by per-protocol, meaning that only patients completing the experimental setup are included. The primary endpoints will be compared between the treatment groups.

Analysis of MRI data: We will use standard pre-processing procedures in Statistical Parametric Mapping (SPM) (http://www.fil.ion.ucl.ac.uk/spm/) before conducting the statistical analysis. Moreover, we will use a mixed effects design in which within-subject effects between the two treatments (before and after both treatments) responses brain activity and group effects will be modelled. For MR spectroscopy, specific metabolites changes will be assessed in pain related brain regions[37].

The rest of the data, like demographic data, changes in circulating cytokines, and others, will be used descriptively and as input to regression and mixed model analyses. The final statistical analysis plan, providing details of the analysis and presentation of the results will be finalized before initiating any statistical analysis.

Patient and public involvement

The study was designed based on the need for new therapeutic options for CP patients and the literature relating to pain management in chronic pancreatitis, as described in the introduction. The outcomes, such as pain scores and MRI brain scans were deliberately chosen in order to assess the potential effect of t-VNS treatment both subjectively (patient-oriented) and objectively. Furthermore, no patients were directly involved in the design, recruitment to or conduct of the study. However, an expert/chief doctor specialized in chronic pancreatitis disease is an associated investigator of the study (SSO). The results and findings gathered from this study will be provided to the patients on request in the form of a written report.

There was no public involvement in the study design.

DISCUSSION

To the best of our knowledge, there are no randomized, sham controlled, studies investigating the effect of t-VNS on clinical pain in patients with CP. We expect the study to provide clinical evidence of the analgesic effect of VNS and to elucidate its underlying mechanisms of action. This may pave the road for non-pharmacological treatment of pain associated with CP and the findings of the study may be generalizable to chronic pain conditions *per se*.

Previous studies have shown structural and functional alterations of the CNS in CP patients with abdominal pain[38–42]. The CNS mechanisms may have the ability to recover by targeting treatment at plasticity mechanisms and reorganization of neuronal pathways leading to improvement of clinical symptoms[43]. VNS treatment has emerged promising technique in stimulating neural reorganization and synaptic plasticity in cortical and subcortical networks, leading to modulation of serotoninergic and noradrenergic pain inhibitory pathways[44]. Those mechanisms might alter and regenerate the neural connectivity in regions responsible for pain[45–47]. In addition, the vagal nerve serves as an essential transmitter of inflammatory signals in immune-to-neuronal communication[48–50]. Afferent fibers of the vagal nerve relay information from viscera to the nucleus tractus solitaries in the brainstem, where it 'senses' pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α. Information is then projected to the parvocellular zone of the paraventricular nucleus of the hypothalamus, and therefore comparison of functional alterations in the CNS and circulating levels of pro-inflammatory cytokines may provide evidence of an existing association. Some limitations about the study should be discussed. Firstly, the patient group is very heterogeneous, they may suffer from co-morbidities and may receive other pharmacological therapies, which may bias the results and consequently makes it difficult to assess the isolated effect of the VNS treatment. Secondly, the researchers may involuntarily become unblinded since the active treatment will deliver facial contractions while this is not present during sham treatment. Thirdly, the relatively low number of patients may hamper the results including the explorative secondary outcomes; however, we eliminate the inter-individual variability because of the cross-over design. Finally, although, all the patients will be trained to use the device correctly according to manufactures' protocol, it is uncertain whether the patients will applicate the device correctly.

Regarding expected outcome, we hypothesize that VNS will reduce the pain in CP patients and induce changes in pain associated brain networks as well in the autonomic, inflammatory parameters and in the sensory system. Also, we expect that the neuromodulation will improve the overall quality of life in CP patients.

ETHICS APPROVAL, CONSENT TO PARTICIPATE AND DISSEMINATION

The procedures set out in this study protocol, pertaining to conduct the study in compliance with Good Clinical Practice (CPMP/ICH/135/95), designated Standard Operating Procedures, the Danish Health and Medicines Authority, the Research Ethics Committee in Denmark, and within the principles of the World Medical Association, Declaration of Helsinki amended by the 52nd General Assembly, Edinburgh, Scotland, October 2000, clarified by the General Assembly in Washington 2002, Tokyo 2004, and Seoul 2008 and Fortaleza 2013 as outlined herein.

Investigators (i.e. medical doctors) will obtain informed consent from each patient. We will conduct this study under the rules of Resolution 466/12 and Declaration of Helsinki. Data will be stored electronically in REDCap database, with secure and restricted access. Data transfer will be encrypted and any information capable of identifying individuals removed. Results gathered from this protocol will be presented at national and international conferences and will be published in peer-reviewed journals. All confidential patient data will be protected, and patient identity will not be disclosed. Further dissemination of the data set can be decided by the principal investigator.

Only researchers involved in the data collection and/or data analysis will have access to the final dataset. However, the principal investigator allows direct access to all source data and documents at monitoring, and inspection from the North Denmark Region Committee on Health Research Ethics, the Danish Health and Medicines Authority or from other countries' health authorities.

Trial status

The recruitment of the study started in January 2018. As of January 2019, a total of 13 patients have completed the study.

References

1. Muniraj T, Aslanian HR, Farrell J, Jamidar PA. Chronic pancreatitis, a comprehensive review and update. Part I: Epidemiology, etiology, risk factors, genetics, pathophysiology, and clinical features. *Disease-a-Month*. 2014;60(12):530-550. doi:10.1016/j.disamonth.2014.11.002.

2. Pham A, Forsmark C. Chronic pancreatitis: review and update of etiology, risk factors, and management. *F1000Research*. 2018;7(May):607. doi:10.12688/f1000research.12852.1.

3. Mullady DK, Yadav D, Amann ST, et al. Type of pain, pain-associated complications, quality of life, disability and resource utilisation in chronic pancreatitis: a prospective cohort study. *Gut*. 2011;60(1):77-84. doi:10.1136/gut.2010.213835.

4. Botha C, Farmer AD, Nilsson M, et al. Preliminary report: modulation of parasympathetic nervous system tone influences oesophageal pain hypersensitivity. *Gut*. 2015;64(4):611-617. doi:10.1136/gutjnl-2013-306698.

5. Frøkjær JB, Bergmann S, Brock C, et al. Modulation of vagal tone enhances gastroduodenal motility and reduces somatic pain sensitivity. *Neurogastroenterol Motil*. 2016;28(4):592-598. doi:10.1111/nmo.12760.

6. Johnson RL, Wilson CG. A review of vagus nerve stimulation as a therapeutic intervention. *J Inflamm Res*. 2018;11:203-213. doi:10.2147/JIR.S163248.

7. Frokjaer JB, Bergmann S, Brock C, et al. Modulation of vagal tone enhances gastroduodenal motility and reduces somatic pain sensitivity. *Neurogastroenterol Motil*. 2016;28(4):592-598. doi:10.1111/nmo.12760.

8. Napadow V, Edwards RR, Cahalan CM, et al. Evoked Pain Analgesia in Chronic Pelvic Pain Patients Using Respiratory-Gated Auricular Vagal Afferent Nerve Stimulation. *Pain Med (United States)*. 2012;13(6):777-789. doi:10.1111/j.1526-4637.2012.01385.x.

9. Brock C, Brock B, Aziz Q, et al. Transcutaneous cervical vagal nerve stimulation modulates cardiac vagal tone and tumor necrosis factor-alpha. *Neurogastroenterol Motil*. 2017;29(5):4-7. doi:10.1111/nmo.12999.

10. Chakravarthy K, Chaudhry H, Williams K, Christo PJ. Review of the Uses of Vagal Nerve Stimulation in Chronic Pain Management. *Curr Pain Headache Rep*. 2015;19(12). doi:10.1007/s11916-015-0528-6.

11. Frangos E, Ellrich J, Komisaruk BR. Non-invasive access to the vagus nerve central projections via electrical stimulation of the external ear: FMRI evidence in humans. *Brain Stimul*. 2015;8(3):624-636. doi:10.1016/j.brs.2014.11.018.

12. Yuan H, Silberstein SD. Vagus Nerve and Vagus Nerve Stimulation, a Comprehensive Review: Part II. *Headache*. 2016;56(2):259-266. doi:10.1111/head.12650.

13. Gaul C, Diener H-C, Silver N, et al. Non-invasive vagus nerve stimulation for PREVention and Acute treatment of chronic cluster headache (PREVA): A randomised controlled study. *Cephalalgia*. 2016;36(6):534-546. doi:10.1177/0333102415607070.

14. Moher D, Hopewell S, Schulz KF, et al. CONSORT 2010 explanation and elaboration: Updated guidelines for reporting parallel group randomised trials. *Int J Surg*. 2012. doi:10.1016/j.ijsu.2011.10.001.

15. Chan AW, Tetzlaff JM, Gøtzsche PC, et al. SPIRIT 2013 explanation and elaboration: guidance for protocols of clinical trials. *BMJ*. 2013. doi:10.1136/bmj.e7586.

16. Vitória A, Araújo L De, Ribeiro V, et al. Effects of high-frequency transcranial magnetic stimulation on functional performance in individuals with incomplete spinal cord injury : study protocol for a randomized controlled trial. 2017:1-11. doi:10.1186/s13063-017-2280-1.

17. Kuppuswamy A, Balasubramaniam A V, Maksimovic R, et al. Clinical Neurophysiology Action of 5 Hz repetitive transcranial magnetic stimulation on sensory , motor and autonomic function in human spinal cord injury. *Clin Neurophysiol*. 2011;122(12):2452-2461. doi:10.1016/j.clinph.2011.04.022.

18. Layer P, Yamamoto H, Kalthoff L, Clain JE, Bakken LJ, DiMagno EP. The different courses of early- and late-onset idiopathic and alcoholic chronic pancreatitis. *Gastroenterology*. 1994;107(5):1481-1487. doi:10.1016/0016-5085(94)90553-3.

19. Strickland I, Mwamburi M, Davis S, et al. Noninvasive vagus nerve stimulation in a primary care setting: effects on quality of life and utilization measures in multimorbidity patients with or without primary headache. *Am J Manag Care*. 2018;24(24 Suppl):S517-S526.

20. Grazzi L, Tassorelli C, Tommaso M De, Pierangeli G, Martelletti P. Practical and clinical utility of non-invasive vagus nerve stimulation (nVNS) for the acute treatment of migraine : a post hoc analysis of the randomized , sham-controlled , double-blind PRESTO trial. 2018;1:1-9.

21. Tarn J, Legg S, Mitchell S, Simon B, Ng WF. The Effects of Noninvasive Vagus Nerve Stimulation on Fatigue and Immune Responses in Patients With Primary Sjögren's Syndrome. *Neuromodulation*. 2018;2018. doi:10.1111/ner.12879.

22. A.D. N, J.C.A. M, E. T, M.H. R, P.J. G. Initial use of a novel noninvasive vagus nerve stimulator for cluster headache treatment. *Neurology*. 2015;84(12):1249-1253.

23. De Icco R, Martinelli D, Bitetto V, et al. Peripheral vagal nerve stimulation modulates the nociceptive withdrawal reflex in healthy subjects: A randomized, cross-over, sham-controlled study. *Cephalalgia*. 2018;38(10):1658-1664. doi:10.1177/0333102417742347.

24. Silberstein SD, Mechtler LL, Kudrow DB, et al. Non???Invasive Vagus Nerve Stimulation for the ACute Treatment of Cluster Headache: Findings From the Randomized, Double-Blind, Sham-Controlled ACT1 Study. *Headache*. 2016;56(8):1317-1332. doi:10.1111/head.12896.

25. Use I, Risks P. Instructions for Use for gammaCore ® -S 1. :1-28.

26. Fayers P. M. et al. EORTC QLQ-C30 Scoring Manual The EORTC QLQ-C30 Introduction. *EORTC QLQ-C30 Scoring Man EORTC QLQ-C30 Introd*. 2001;30:1-67. doi:D/2001/6136/001.

27. Cleeland CS. Brief Pain Inventory (BPI). *Cleel CS MD Anderson Cancer Cent*. 1982;1100:6. doi:10.1007/978-1-4419-9893-4_13.

28. Farrar JT, Young JP, LaMoreaux L, Werth JL, Poole RM. Clinical importance of changes in chronic pain intensity measured on an 11-point numerical pain rating scale. *Pain*. 2001;94(2):149-158. doi:10.1016/S0304-3959(01)00349-9.

29. Arendt-Nielsen L, Morlion B, Perrot S, et al. Assessment and manifestation of central sensitisation across different chronic pain conditions. *Eur J Pain (United Kingdom)*. 2018;22(2):216-241. doi:10.1002/ejp.1140.

30. Olesen SS, Bouwense SAW, Wildersmith OHG, Van Goor H, Drewes AM. Pregabalin reduces pain in patients with chronic pancreatitis in a randomized, controlled trial. *Gastroenterology*. 2011. doi:10.1053/j.gastro.2011.04.003.

31. Kennedy DL, Kemp HI, Ridout D, Yarnitsky D, Rice ASC. Reliability of conditioned pain modulation: A systematic review. *Pain*. 2016;157(11):2410-2419. doi:10.1097/j.pain.0000000000000689.

32. Yarnitsky D, Bouhassira D, Drewes AM, et al. Recommendations on practice of conditioned pain modulation (CPM) testing. *Eur J Pain (United Kingdom)*. 2015;19(6):805-806. doi:10.1002/ejp.605.

33. Farmer AD, Coen SJ, Kano M, et al. Normal values and reproducibility of the real-time index of vagal tone in healthy humans: a multi-center study. *Ann Gastroenterol Q Publ Hell Soc Gastroenterol*. 2014;27(4):362-368.

34. Trial C, Olesen SS, Bouwense SAW, Smith OHGW, Goor HVAN. CLINICAL — PANCREAS Pregabalin Reduces Pain in Patients With Chronic Pancreatitis in a. *YGAST*. 2011;141(2):536-543. doi:10.1053/j.gastro.2011.04.003.

35. College Station TSL. StataCorp. 2015. *Stata Stat Softw Release 13*. 2013.

36. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)-A metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform*. 2009. doi:10.1016/j.jbi.2008.08.010.

37. Hansen TM, Olesen AE, Simonsen CW, Drewes AM, Frokjaer JB. Cingulate metabolites during pain and morphine treatment as assessed by magnetic resonance spectroscopy. *J Pain Res*. 2014;7:269-276. doi:10.2147/JPR.S61193.

38. Dimcevski G, Sami SAK, Funch-Jensen P, et al. {A figure is presented}Pain in Chronic Pancreatitis: The Role of Reorganization in the Central Nervous System. *Gastroenterology*. 2007;132(4):1546-1556. doi:10.1053/j.gastro.2007.01.037.

39. Frøkjær JB, Olesen SS, Gram M, et al. Altered brain microstructure assessed by diffusion tensor imaging in patients with chronic pancreatitis. *Gut*. 2011;60(11):1554-1562. doi:10.1136/gut.2010.236620.

40. Frøkjær JB, Bouwense SAW, Olesen SS, et al. Reduced Cortical Thickness of Brain Areas Involved in Pain Processing in Patients With Chronic Pancreatitis. *Clin Gastroenterol Hepatol*. 2012;10(4):434-438. doi:10.1016/j.cgh.2011.11.024.

41. Lelic D, Olesen SS, Graversen C, Brock C, Valeriani M, Drewes AM. Electrophysiology as a tool to unravel the origin of pancreatic pain. *World J Gastrointest Pathophysiol*. 2014;5(1):33-39. doi:10.4291/wjgp.v5.i1.33.

42. Muthulingam J, Olesen SS, Hansen TM, et al. Progression of Structural Brain Changes in Patients With Chronic Pancreatitis and Its Association to Chronic Pain: A 7-Year Longitudinal Follow-up Study. *Pancreas*. 2018;47(10):1267-1276. doi:10.1097/MPA.0000000000001151.

43. Apkarian A V, Hashmi JA, Baliki MN. Pain and the brain: Specificity and plasticity of the brain in clinical chronic pain. *Pain*. 2011;152(3 Suppl):S49-64. doi:10.1016/j.pain.2010.11.010.

44. Garcia RG, Lin RL, Lee J, et al. Modulation of brainstem activity and connectivity by respiratory-gated auricular vagal afferent nerve stimulation in migraine patients. *Pain*. 2017;158(8):1461-1472. doi:10.1097/j.pain.0000000000000930.

45. O'Connell NE, Marston L, Spencer S, et al. Non-invasive brain stimulation techniques for chronic pain. *Cochrane database Syst Rev*. 2018;3(9):CD008208. doi:10.1002/14651858.CD008208.pub2.

46. Seminowicz DA, Wideman TH, Naso L, et al. Effective Treatment of Chronic Low Back Pain in Humans Reverses Abnormal Brain Anatomy and Function. *J Neurosci*. 2011;31(20):7540-7550. doi:10.1523/JNEUROSCI.5280-10.2011.

47. Čeko M, Shir Y, Ouellet JA, Ware MA, Stone LS, Seminowicz DA. Partial recovery of abnormal insula and dorsolateral

prefrontal connectivity to cognitive networks in chronic low back pain after treatment. *Hum Brain Mapp*. 2015;36(6):2075-2092. doi:10.1002/hbm.22757.

48. Bonaz B, Sinniger V, Pellissier S. Vagal tone: Effects on sensitivity, motility, and inflammation. *Neurogastroenterol Motil*. 2016;28(4):455-462. doi:10.1111/nmo.12817.

49. Watkins LR, Maier SF, Goehler LE. Cytokine-to-brain communication: A review & analysis of alternative mechanisms. *Life Sci*. 1995;57(11):1011-1026. doi:10.1016/0024-3205(95)02047-M.

50. Hosoi T, Okuma Y, Matsuda T, Nomura Y. Novel pathway for LPS-induced afferent vagus nerve activation: Possible role of nodose ganglion. *Auton Neurosci Basic Clin*. 2005;120(1-2):104-107. doi:10.1016/j.autneu.2004.11.012.

Legends for illustrations

- Figure 1: Mode of action of transcutaneous vagal nerve stimulation (t-VNS). (1) Pain arises in the periphery e.g. pancreas and a signal is sent to the spinal cord. This leads to ascending activation of the spinal neurons (2). In the brain, pain is processed in higher cortical centres (3). T-VNS, it is expected to block the perception of pain in the cerebral cortex, by stimulating nucleus tractus solitarius and thereby decrease glutamate level. Simultaneously, the net-descending inhibition will be activated as a result of top-down input from cortex and the limbic system (4).
- Figure 2: Schematic flowchart of the interventional study design enabling comparison of the modulatory effect to self-administered of transcutaneous vagal nerve stimulation (t-VNS) in patients with chronic pancreatitis. Chronic pancreatitis patients will be randomly assigned to one of two double blinded treatments: (1) two weeks of t-VNS, two weeks of wash-out, and two weeks of sham treatment; or (2) two weeks of sham treatment, two weeks of washout, and two weeks of t-VNS. Evaluation of the two treatments will be assessed by collecting pain diary, pain questionnaires, MRI scan, blood sample, cardiac vagal tone, and pain assessments. QST=Quantitative sensory testing. CVT=Cardiac vagal tone. MRI=Magnetic resonance imaging.
- Figure 3: SPIRIT Figure.

555 **Table 1: Trial characteristics based on WHO Trial Registration Data Set**

Data category	Trial Information
Primary Registry and Trial Identifying Number	ClinicalTrials.gov (NCT03357029)
Date of Registration in Primary Registry	November 29, 2017
Secondary Identifying Numbers	North Denmark Region Committee on Health Research Ethics: protocol number N-20170023
Source(s) of Monetary or Material Support	The study is conducted as a sponsor-investigator initiated study with financial support from Independent Research Fund Denmark (DFF – 7016-00073).
Primary Sponsor	JBF
Secondary Sponsor	NA
Contact for Public Queries	JBF
Contact for Scientific Queries	JBF
Public title	Neuromodulation in Patients with Painful Chronic Pancreatitis
Scientific title	Study protocol for a randomized double-blinded, sham-controlled, prospective, cross-over clinical trial of vagal neuromodulation for pain treatment in patients with chronic pancreatitis
Country of recruitment	Denmark
Healthy conditions(s) or problems studied	Chronic pancreatitis
Interventions	Two-week transcutaneous vagal nerve stimulation (t-VNS) on the cervical vagal area (Self-administering vagal nerve stimulation bilaterally to the cervical vagal area, the times per day).
Key inclusion and exclusion criteria	Inclusion criteria: Age ≥18 years; Patients with a diagnosis of CP diagnosed using the Mayo Clinic diagnostic criteria.; The participants must be able to read and understand Danish.; The patients must suffer from chronic abdominal pain characteristic for CP, meet the criteria for chronic pain (pain ≥ 3 days per week in at least 3 months) and must consider their pain as insufficiently treated with their usual analgesic treatment. ; Personally, signed and dated informed consent document and the Power of attorney document; Patients willing and able to comply with the scheduled visits, treatment plan, laboratory tests and other trial procedures. Exclusion criteria: Patients with any clinically significant abnormalities that in the opinion of the investigator may increase the risk associated with trial participation or may interfere with the interpretation of the trial results. ; Alcohol dependence; Illegal drug dependencies; Participating in another study where investigational drug is used, ; Patients must not suffer from painful conditions other than CP that make them unable to distinguish the pain associated with CP from chronic pain of other origin.; Cardiovascular diseases ; Low blood pressure < 100/60, Not able to understand or follow the instructions, ; Any condition with elevated intracranial pressure.; Female patients who are pregnant; Contraindications for MRI; Previous surgery on vagal nerve.; Known neuropathy.
Study type	Interventional allocation: randomized Masking: double-blind Assignment: cross-over Primary purpose: treatment

Date of first enrolment	January 2018
Target sample size	21
Recruitment status	Recruiting
Primary outcome(s)	Change in NRS scores in pain diary
Key Secondary outcomes (s)	Aassessment of the effect of t-VNS on A) resting state brain function assessed by MRI, and B) brain metabolites assessed by MR spectroscopy.

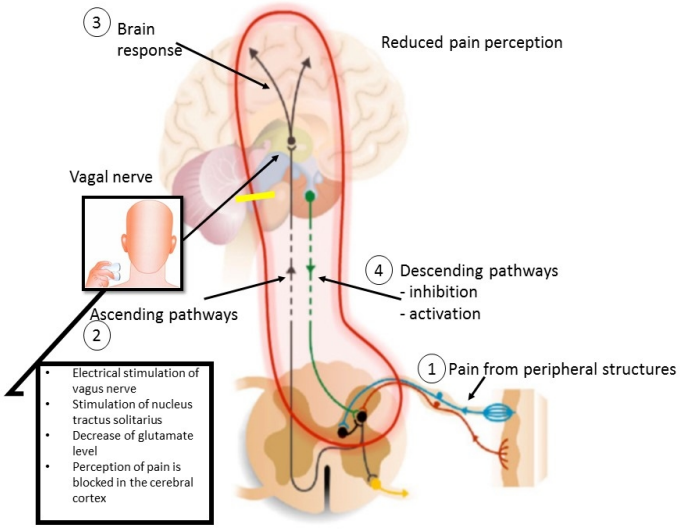

Figure 1: Mode of action of transcutaneous vagal nerve stimulation (t-VNS). (1) Pain arises in the periphery e.g. pancreas and a signal is sent to the spinal cord. This leads to ascending activation of the spinal neurons (2). In the brain, pain is processed in higher cortical centres (3). T-VNS, it is expected to block the perception of pain in the cerebral cortex, by stimulating nucleus tractus solitarius and thereby decrease glutamate level. Simultaneously, the net-descending inhibition will be activated as a result of top-down input from cortex and the limbic system (4).

338x190mm (96 x 96 DPI)

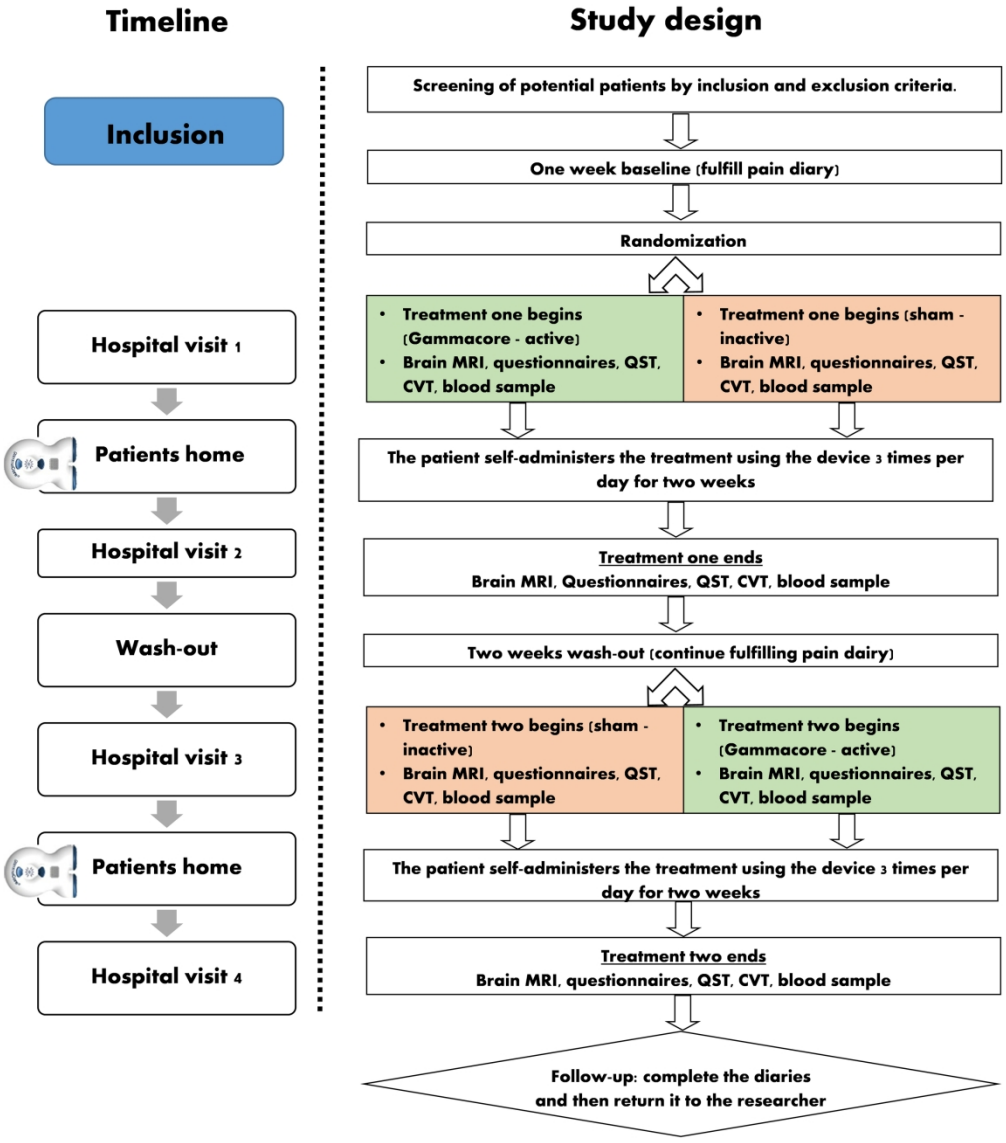

Figure 2: Schematic flowchart of the interventional study design enabling comparison of the modulatory effect to self-administered of transcutaneous vagal nerve stimulation (t-VNS) in patients with chronic pancreatitis. Chronic pancreatitis patients will be randomly assigned to one of two double blinded treatments: (1) two weeks of t-VNS, two weeks of wash-out, and two weeks of sham treatment; or (2) two weeks of sham treatment, two weeks of washout, and two weeks of t-VNS. Evaluation of the two treatments will be assessed by collecting pain diary, pain questionnaires, MRI scan, blood sample, cardiac vagal tone, and pain assessments. QST=Quantitative sensory testing. CVT=Cardiac vagal tone. MRI=Magnetic resonance imaging.

220x253mm (300 x 300 DPI)

	STUDY PERIOD								
	Enrolment & screening	Baseline & allocation	Post-allocation						Follow-up
TIMEPOINT**	T_{-1}	0	T_1	T_2	T_3	T_4	T_5	T_6	T_7
Week	0	1	2	3	4	5	6	7	8
ENROLMENT:									
Eligibility screen	X								
Informed consent	X								
Allocation		X							
INTERVENTIONS:									
[transcutaneous VNS (active)]			X	X	Washout		X	X	
[Sham treatment]			X	X			X	X	
ASSESSMENTS:									
Pain diary		X	X	X	X	X	X	X	X
QORTC-QLQ-C30 questionnaire			X	X			X	X	
BPI-SF Questionnaire			X	X			X	X	
PGIC questionnaire				X				X	
MRI			X	X			X	X	
CVT			X	X			X	X	
Blood samples			X	X			X	X	
QST			X	X			X	X	
[Adverse events]			X	X	X		X	X	

Figure 3: SPIRIT Figure.

87x104mm (300 x 300 DPI)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	____1_____
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	_____2_____
	2b	All items from the World Health Organization Trial Registration Data Set	__Table 1__
Protocol version	3	Date and version identifier	_____1_____
Funding	4	Sources and types of financial, material, and other support	_____1___
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	_____1____
	5b	Name and contact information for the trial sponsor	_____1_____
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	____1_____
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	Not applicable

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	__4-5, 7-8, 11_
	6b	Explanation for choice of comparators	___7-9_____
Objectives	7	Specific objectives or hypotheses	___4-5_____
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	__6_____
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	_____ 6_____
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	____6-7_____
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	___7-8_____
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	_____7_____
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	__ 8_____
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	___7_____
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	___9-11_____
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	___Figure 2 & 3_

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	__11_____
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	__6_____
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	____8_____
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	__8_____
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	__8_____
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	___8,9_____
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	___9_____
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	__7-12_____
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	Not applicable

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	___11,12_____
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	____12_____
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	Not applicable
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	__12_____
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	____12_____
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	__12_____
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	___12_____
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	Not applicable
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	____6_____
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	Not applicable

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	___14_____
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	___14_____
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	___14_____
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	_____1_____
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	___14_____
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	____14_____
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	___14_____
	31b	Authorship eligibility guidelines and any intended use of professional writers	Not applicable
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	Not applicable
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	See rules of the Ethical Committee
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	Not applicable

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.