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

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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, Aalborg University, Frøkjær, Jens Brøndum; Mech-Sense, Department of Clinical Medicine
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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

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3 <u>Abstract</u>

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.

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

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

53 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 ets. explore long-term effects.
- The single-centre design may limit generalizability of the study results.

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INTRODUCTION

64 Chronic pancreatitis (CP) is a disease characterized by progressive pancreatic inflammation and fibrosis, 65 resulting in damage to and loss of exocrine (acinar), endocrine (islet cells), and ductal cells¹. Chronic 66 abdominal pain is the dominating symptom in CP and is present in up to 70% of patients². Pain is associated 67 with reduced quality of life, increased hospitalization frequencies and thus a significant socioeconomic 68 burden³.

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 threshold⁴. In addition, this effect was abolished by atropine administration thereby proving that enhanced parasympathetic tone leads to prevention of oesophageal pain hypersensitivity⁴. 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 subjects⁵. 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 neck⁶ (Figure 1). T-VNS has been shown to induce analgesic^{7,8} and anti-inflammatory effects in healthy individuals⁹ and different diseases. Also, the non-pharmacological treatment is FDA-approved for the preventive treatment of cluster headache and migraine¹⁰.

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:

- 91 1) The primary clinical objective is to assess the effect of t-VNS on the daily pain experience documented in
 92 a diary.
- ⁵⁴ 93 2) Secondary clinical objectives are to document changes in quality of life and daily functioning.
- 56 94 3) The primary experimental objective is to assess the effect of t-VNS on A) resting state brain function
 57 95 assessed by magnetic resonance imaging (MRI), and B) brain metabolites assessed by magnetic resonance
 59 96 spectroscopy.

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

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100 **METHODS AND ANALYSES:**

Study design 101

Randomised, single-centre, double-blinded, prospective, sham-controlled, cross-over study. The study was 102 103 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 12 104 105 Aalborg University Hospital and will be reported according to the Consolidated Standards of Reporting Trials 15 106 (CONSORT)¹¹. The study protocol follows the Standard Protocol Items: Recommendations for Interval Trials ₁₇ 107 (SPIRIT) statement¹².

19 108 All patients undertake the t-VNS treatment using an active GammaCore-S, 300 treatments (10009-00603) 109 device (ElectroCore LLC; Basking Ridge, NJ, USA) and sham treatment using a sham-device (10009-00603 22 110 P) which is identical in appearance GammaCore.

₂₄ 111 Half of the patients will be randomized to start with two-week t-VNS treatment, followed by a two-week ²⁵ 112 washout period. Then, this group will be reallocated to sham treatment. The other half of patients will do the 27 113 study periods in opposite order (sham treatment followed by t-VNS treatment). The two-week washout 114 period has been used in previous studies of trans-cranial neuromodulation¹³ and was shown to be sufficient 30 115 to reset the effects of neuromodulation¹⁴. Each patient will be scheduled for four identical hospital visits 32¹116 (before and after each treatment period). The visits consist of 1) Fulfilment of questionnaires, 2) Collection ³³ 117 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 43 122 screening session and physical examination prior to inclusion will be conducted by a medical doctor including 45 123 relevant medical and medication history and screening against the eligibility criteria.

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Inclusion criteria and exclusion criteria

50 51 126 Patients from the age of 18 years will be included in the study. They will have a clinical diagnosis of CP based ⁵² 127 on the Mayo clinical diagnostic criteria¹⁵. The patients must suffer from chronic abdominal pain characteristics 53 54 128 for CP, meet the criteria for chronic pain (pain \geq 3 days per week for at least 3 months) and must consider their 55 56 129 pain as insufficiently treated with their prescribed analgesic treatment. Additionally, the patients must be 57 130 willing and able to comply with the scheduled visits, treatment plan, laboratory tests, and other study 58 ₅₉ 131 procedures. Finally, the patient must sign the informed consent and power of attorney document.

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Patients will be excluded if they have any clinically significant abnormalities that may increase the risk 132 associated with trial participation or may interfere with the interpretation of the trial results. Also, patients with 133 alcohol and illegal drug dependence patients, cardiovascular diseases, low blood pressure (<100/60mmHg), 134 elevated intracranial pressure will be excluded. Additionally, patients who are participating in another 135 ₁₁ 136 intervention study, patients who are pregnant or intend to become pregnant, and patients who suffer from 137 painful conditions other than CP that make them unable to distinguish the pain associated with CP from chronic 14 138 pain of other origin will be excluded. Patients will also be excluded if there are any contraindications for MRI 139 (incl. cardiac pacemaker, implantable metallic components, etc.), have known neuropathy, or previous vagal 17 140 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.

146 Interventions

₂₉ 147 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 31 148 149 the patient is capable to use the device independently, the device will be handed over to the patient. T-VNS 33 34 150 is administered by using a handheld device (GammaCore; ElectroCore LLC; Basking Ridge, NJ, USA), which 36 151 consists of a battery powered portable stimulator with a digitally controlled user-interface that controls the ³⁷ 152 signal amplitude and two gel covered (Sigma gel, Parker Laboratories, NJ, USA) contact electrodes which 38 39 153 deliver electrical stimulation to the cervical vagal nerve. One dose corresponds to 120 seconds of t-VNS to 40 154 the left cervical vagal nerve followed by 120 seconds of t-VNS to the right cervical vagal nerve, with the 42 155 amplitude of simulation titrated to achieve mild pulling of the ipsilateral oral commissure¹⁶. The patient self-44 156 administers the treatment, using the device at home three times per day (morning, afternoon, and evening) for ⁴⁵ 157 two weeks. Previous studies with Gamma-Core, have shown that three doses per day have been effective ^{10,17}.

47 The stimulation device is positioned anterior to the sternocleidomastoid muscle, over the carotid artery as this 158 48 49 159 runs in close proximity with the vagal nerve. The active Gamma-Core device produces a low-voltage electrical 50 160 signal comprising a 5 kHz sine wave burst lasting for 1 millisecond (five sine waves, each lasting 0.2 51 52 161 millisecond), with such bursts repeated once every 40 milliseconds (25 Hz), generating a 24 V peak voltage 53 54 162 and 60 mA peak output current.

To mimic the sensation of the active treatment, the sham-device will provide vibration¹⁸. The appearance, 56 163 57 57 58 164 weight, visual and audible feedback, and user application are identical for the sham and t-VNS devices. ⁵⁹ 165 However, the sham device produces a low-frequency (0.1 Hz) biphasic signal that does not stimulate the vagal 60

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nerve or generally cause muscle contractions¹⁹. Additionally, both devices will display a numeric value 166 between 1 and 40, signifying the intensity of the stimulation. The maximum intensity per stimulation is 40 for 167 168 both devices (Figure 1).

₁₀ 169 Compliance will be assessed by reading the remaining doses displayed at the device after each treatment 170 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, 13 171 172 adherence will be recorded by patients' diary.

₁₇ 173 During the study periods the patient will continue their standard care, without changes in their current pain ¹⁸ 174 treatment.

22 176 Randomization, sequence generation and allocation concealment

177 Once eligibility and consent have been approved and completed, randomization will occur using a 26 178 randomization list generated by an automatic web-based randomization program. Patients will be randomly 27 28 179 assigned to VNS/sham or sham/VNS using block randomisation, allowing seven patients at the time to be 29 180 randomised in equal proportions for the order of active t-VNS or sham stimulation. The randomization order ₃₁ 181 will be kept in closed envelopes; therefore, patients will get their assignment according to the order of entrance 182 in the study. This process will be carried out by a member of the research team who is not involved in the 34 183 recruitment process or conduction of the study.

36 184 An unblinded researcher will be involved in delivering the medical device according to the randomisation ₃₈ 185 schedule. The sequence will follow a 1:1 sequential design, in a double-blinded fashion. Additionally, the ³⁹ 186 outcome assessor (data analyst) will be blinded during the statistical analyses of primary experimental 41 187 outcomes. A series of numbered, sealed, envelopes will be used to ensure concealed allocation.

189 Blinding

47 48¹⁹⁰ Both, active and sham devices are labelled with a serial number and not outwardly identified as active or sham. ⁴⁹ 191 All researchers involved in the data collection and MRI analysis will be blinded to the treatment allocation 50 51 192 group until after analyses are performed at the completion of the trial. Additionally, all patients are blinded, 52 193 and they do not know that the sham treatment is an inactive treatment. Particularly, patients will be informed 53 54 194 that they have to undergo two different treatments, and the purpose of this study is to investigate the most 56 195 55 effective treatment.

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Manufacturing and preparation of the medical devices are handled by an external good manufacturing practice-196 accredited facility (ElectroCore). As the patients do not know that the sham treatment is an inactive treatment, 197 198 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. 199

200 Unblinding is only permissible if a patient experiences any serious adverse events and that the 13 201 investigator/doctor judge that it is essential to know the treatment allocation in order to treat the patient 202 appropriately.

16 ₁₇ 203

19 204 Primary clinical outcome measures

²¹ 205 The primary clinical efficacy parameter to be evaluated is 30% pain relief. This is assessed as changes in the 23 206 daily experience of pain, which will be measured using a patient pain diary based on the numeric rating scale 207 (NRS) (1 = no pain, 10 = worst pain imaginable). Patients will be asked to score daily pain levels in the diary 26 208 for eight weeks (including one week before the first treatment period and one week after the last study period, 27 28 209 Figure 2 and Figure 3), with one NRS value for the average pain over the previous 24 hours and one NRS 29 210 value for the worst pain over the previous 24 hours.

₃₄ 212 Secondary clinical outcome measures

Quality of life questionnaire, C30, version 3.0 (QoLQ-C30)²⁰, the brief pain inventory – short form (BPI-SF) 36 213 214 questionnaire²¹, and Patient Global Impression of Changes²² questionnaire (PGIC) are secondary clinical 39 215 outcomes. Patient will complete QoLQ-C30 and BPI-SF questionnaire before and after each treatment period, 40 41 216 while the PGIC questionnaire will only be fulfilled after the treatment periods. The QoLQ-C30 questionnaire ⁴² 217 is composed of both multi-item scales and single-item measures. These include five functional scales, three 44 218 symptom scales, a global health status, and six single items. The BPI-SF questionnaire rapidly assess the 219 severity of pain and its impact on daily functioning. Finally, the PGIC questionnaire evaluates all aspects of 47 220 patients' health and assess if there has been an improvement or decline in the overall clinical status.

51 222 Primary experimental outcome measures 52

₅₄ 223 Resting state functional MRI will be employed to detect brain activity and functional connectivity changes ⁵⁵ 224 based on BOLD signals before and after treatment of each patient. Additionally, magnetic resonance 57 225 spectroscopy in anterior cingulate cortex, prefrontal cortex, parietal, and insula will also be performed in order 58 226 to investigate changes in brain metabolites before and after each treatment. 59

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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°, 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, 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.

243 Secondary experimental outcome measures

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

QST includes temporal summation²³, pressure pain thresholds^{23,24} and conditioned pain modulation (CPM)²⁵.
Temporal summation demonstrates an increase perception of pain to repetitive pain stimuli²³. 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 cm². 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 mm² (Aalborg University, Denmark) will be applied.

CPM is a clinically measurable form of descending pain modulation²⁵ 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 induction²⁶. 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 context of the cold pressor pain provides a quantitative index of CPM capacity for

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

262 CVT is a beat-to-beat measure of brainstem efferent vagal activity, which is assessed by heart rate variability
 263 measurement and reflects the contribution of the vagal nerve to cardiac functioning. In this particular test,
 264 changes in R-R interval would be measured non-invasively using eMotion Faros 180 device²⁷.

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).

270 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 score²⁸. 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.

9 Harms and adverse events

We do not anticipate this project causing any harm or discomfort to the patients, and we will ensure that ourpatients 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.

8 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

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(GCP). There will be regular meetings between the data collectors, monitor, principal investigator and other 291 co-researchers involved in the project. All paper protocols will be kept safe and transferred to a computerised 292 293 database. The questionnaires will be checked for errors and missing data by research staff. Data entries are double-checked against the paper questionnaires. 294

295 During trial conduct, the Good Clinical Practice unit (GCP, Aalborg, Denmark) will conduct periodic 13 296 monitoring of all signed consents at monitoring visits to ensure that the protocol and GCP standards are 297 followed. The monitors may review source documents and medical records to confirm that data recorded on 16 298 Case Report Form is accurate. Thus, GCP monitoring includes all signed consents, signed power of attorney, 18²⁹⁹ and AE.

20 300 Criteria for the termination of the trial is, when patients according to the sample size with valid data are 301 recorded. If the study fails to recruit adequate patients according to the sample size by end of 2019, the study 23 302 will be terminated.

-' 28 304 **Data analysis**

29 305 Both descriptive and analytical statistics will be used in order to compare groups and for analyses of outcomes ₃₁ 306 over time including changes therein. All data will be presented as mean ± standard deviation and summarized 307 in frequency tables, unless otherwise indicated. We will use Research Electronic Data Capture (REDCap)³⁰ to 34 308 store the data and the statistical software package STATA to perform statistical analysis. We will use mixed 309 ANOVA for the inferential statistic of the parametric data, with Tukey's and/or Bonferroni post hoc tests for 37 310 the primary clinical endpoints. Significance level will be set as $\alpha \leq 0.05$.

40 312 The principal analysis of clinical endpoints will be by intention-to-treat, meaning that all randomized patients 42 313 are included in their initially assigned study arm, regardless of adherence to study protocol. Experimental ⁴³ 314 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. 45 315

⁴⁷ 316 Analysis of MRI data: We will use standard pre-processing procedures in Statistical Parametric Mapping 49 317 (SPM) (http://www.fil.ion.ucl.ac.uk/spm/) before conducting the statistical analysis. Moreover, we will use a ⁵⁰ 318 mixed effects design in which within-subject effects between the two treatments (before and after both 52 319 treatments) responses brain activity and group effects will be modelled. For MR spectroscopy, specific 53 54 320 metabolites changes will be assessed in pain related brain regions³¹.

56 57 322 The rest of the data, like demographic data, changes in circulating cytokines, and others, will be used ⁵⁸ 323 descriptively and as input to regression and mixed model analyses. The final statistical analysis plan, providing 59

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2 3 4 5 7 324 7 325	details of the analysis and presentation of the results will be finalized before initiating any statistical analysis.
8 9 326	Patient and public involvement
10 327	The study was designed based on the need for new therapeutic options for CP patients and the literature relating
11 12 328	to pain management in chronic pancreatitis, as described in the introduction. The primary outcomes, such as
¹³ 329 14	pain scores and MRI brain scans were deliberately chosen in order to assess the potential effect of t-VNS
15 330	treatment both subjectively (patient-oriented) and objectively. Furthermore, no patients were directly involved
16 17 331	in the design, recruitment to or conduct of the study. However, an expert/chief doctor specialized in chronic
18 332	pancreatitis disease is an associated investigator of the study (SSO). The results and findings gathered from
19 20 333	this study will be provided to the patients on request in the form of a written report.
21 22 334	There was no public involvement in the study design.
22 554 23	There was no public involvement in the study design.
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336 **DISCUSSION**

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To the best of our knowledge, there are no randomized, sham controlled, studies investigating the effect of t-337 VNS on clinical pain in patients with CP. We expect the study to provide clinical evidence of the analgesic 338 10 339 effect of VNS and to elucidate its underlying mechanisms of action. This may pave the road for non-340 pharmacological treatment of pain associated with CP and the findings of the study may be generalizable to 13 341 chronic pain conditions per se.

15 342 Previous studies have shown structural and functional alterations of the CNS in CP patients with abdominal 16 17 343 pain^{32–36}. The CNS mechanisms may have the ability to recover by targeting treatment at plasticity mechanisms 18 344 and reorganization of neuronal pathways leading to improvement of clinical symptoms³⁷. VNS treatment has 19 20 345 emerged promising technique in stimulating neural reorganization and synaptic plasticity in cortical and 21 346 subcortical networks, leading to modulation of serotoninergic and noradrenergic pain inhibitory pathways³⁸. 22 23 347 Those mechanisms might alter and regenerate the neural connectivity in regions responsible for pain^{39–41}. In 24 25 348 addition, the vagal nerve serves as an essential transmitter of inflammatory signals in immune-to-neuronal 26 ₃₄₉ communication⁴²⁻⁴⁴. Afferent fibers of the vagal nerve relay information from viscera to the nucleus tractus 27 28 350 solitaries in the brainstem, where it 'senses' pro-inflammatory cytokines such as IL-1, IL-6, and TNF- α . 29 351 Information is then projected to the parvocellular zone of the paraventricular nucleus of the hypothalamus, and 30 therefore comparison of functional alterations in the CNS and circulating levels of pro-inflammatory cytokines 353 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 358 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 ₁₀ 370 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 13 372 2000, clarified by the General Assembly in Washington 2002, Tokyo 2004, and Seoul 2008 and Fortaleza 2013 as outlined herein.

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₁₇ 374 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 20 376 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 23 378 international conferences and will be published in peer-reviewed journals. All confidential patient data will be 25 379 protected, and patient identity will not be disclosed. Further dissemination of the data set can be decided by 26 380 the principal investigator.

Only researchers involved in the data collection and/or data analysis will have access to the final dataset. 30 382 However, the principal investigator allows direct access to all source data and documents at monitoring, and 32 383 inspection from the North Denmark Region Committee on Health Research Ethics, the Danish Health and ³³ 384 Medicines Authority or from other countries' health authorities.

₃₈ 386 **Trial status**

The recruitment of the study started in January 2018. As of January 2019, a total of 13 patients have completed 40 387 the study.

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Legends for illustrations 511

Figure 1: Mode of action of transcutaneous vagal nerve stimulation (t-VNS). (1) Pain arises in the 512 periphery e.g. pancreas and a signal is sent to the spinal cord. This leads to ascending activation of the 513 10 514 spinal neurons (2). In the brain, pain is processed in higher cortical centres (3). T-VNS, it is expected to 11 515 block the perception of pain in the cerebral cortex, by stimulating nucleus tractus solitarius and thereby 12 13 516 decrease glutamate level. Simultaneously, the net-descending inhibition will be activated as a result of top-14 517 down input from cortex and the limbic system (4). 15

16 518 Figure 2: Schematic flowchart of the interventional study design enabling comparison of the modulatory 17 effect to self-administered of transcutaneous vagal nerve stimulation (t-VNS) in patients with chronic 18 519 19 520 pancreatitis. Chronic pancreatitis patients will be randomly assigned to one of two double blinded 20 treatments: (1) two weeks of t-VNS, two weeks of wash-out, and two weeks of sham treatment; or (2) two 21 521 22 522 weeks of sham treatment, two weeks of washout, and two weeks of t-VNS. Evaluation of the two 23 24 523 treatments will be assessed by collecting pain diary, pain questionnaires, MRI scan, blood sample, cardiac 25 26 524 vagal tone, and pain assessments. QST=Quantitative sensory testing. CVT=Cardiac vagal tone. ²⁷ 525 MRI=Magnetic resonance imaging. 28

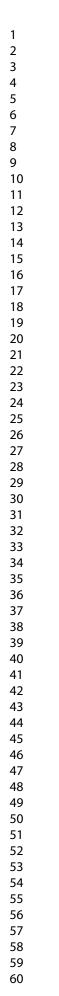
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Figure 3: SPIRIT Figure. 29 526

5 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 Denma (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-controlle prospective, cross-over clinical trial of vagal neuromodulation f 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 of diagnosed using the Mayo Clinic diagnostic criteria.; T participants must be able to read and understand Danish.; T patients must suffer from chronic abdominal pain characteristic CP, meet the criteria for chronic pain (pain ≥ 3 days per week in least 3 months) and must consider their pain as insufficient treated with their usual analgesic treatment. ; Personally, sign and dated informed consent document and the Power of attorn document; Patients willing and able to comply with the schedul visits, treatment plan, laboratory tests and other trial procedure Exclusion criteria: Patients with any clinically significated abnormalities that in the opinion of the investigator may increated risk associated with trial participation or may interfere with interpretation of the trial results. ; Alcohol dependence; Illegal drift dependencies; Participating in another study where investigation drug is used, ; Patients must not suffer from painful conditions of the instructions, ; Any condition with elevated intracram pressure.; Female patients who are pregnant; Contraindications MRI; Previous surgery on vagal nerve.; Known neuropathy.
Study type	Interventional allocation: randomized Masking: double-blind Assignment: cross-over

Date of first enrolment
Target sample size
Recruitment status
Primary outcome(s)
Key Secondary outcomes (s)



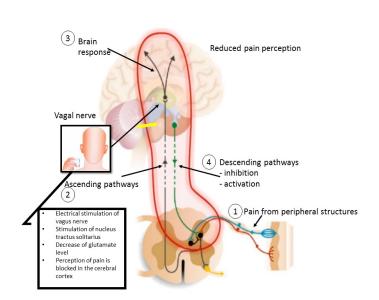


Figure 1: Mode of action of transcutaneous vagal nerve stimulation (t-VNS). (1) Pain arises in the periphery e.g. pancreas and a signal is sent to the spinal cord. This leads to ascending activation of the spinal neurons (2). In the brain, pain is processed in higher cortical centres (3). T-VNS, it is 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).

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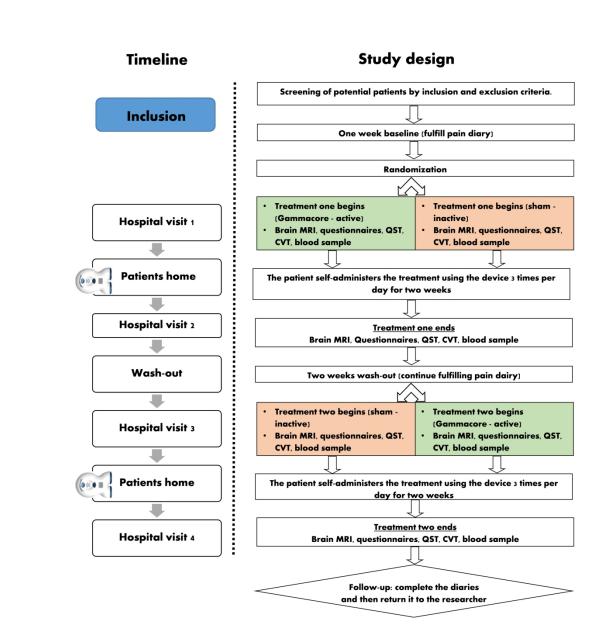


Figure 2: Schematic flowchart of the interventional study design enabling comparison of the modulatory effect to self-administered of transcutaneous vagal nerve stimulation (t-VNS) in patients with chronic pancreatitis. Chronic pancreatitis patients will be randomly assigned to one of two double blinded 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.

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	STUDY PERIOD								
	Enrolment & Baseline & Post-allocation							Follow- up	
TIMEPOINT**	T- 1	0	T 1	T ₂	T ₃	T4	T5	T 6	T 7
Week	0	1	2	3	4	5	6	7	8
ENROLMENT:									
Eligibility screen	х								
Informed consent	х								
Allocation		х							
INTERVENTIONS:									
[transcutaneous VNS (active)]			x	x	7	···•	х	x	
[Sham treatment]			x	х	Was	→ hout	х	х	
ASSESSMENTS:									
Pain diary		Х	X	X	Х	Х	Х	Х	Х
QORTC-QLQ-C30 questionnaire			x	x			х	x	
BPI-SF			x	x			x	x	
Questionnaire			^	^					
PGIC questionnaire				x				x	
MRI			x	x			х	x	
сvт			x	x			х	x	
Blood samples			x	x			x	x	
QST			х	x			х	х	
[Adverse events]			X	X	X		Х	Х	

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	ltem No	Description	Addressed or page number
Administrative info	ormation		
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	5a	Names, affiliations, and roles of protocol contributors	1
responsibilities	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
		For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml	

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1 2	Introduction			
3 4 5	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
6 7		6b	Explanation for choice of comparators	7-9
8 9	Objectives	7	Specific objectives or hypotheses	4-5
10 11 12 13	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
14 15	Methods: Participa	nts, inte	erventions, and outcomes	
16 17 18	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
19 20 21	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
22 23 24	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7-8
25 26 27 28		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
29 30 31		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	8
32 33		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	7
34 35 36 37 38 39	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
40 41 42	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
43 44 45			For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml	:

1 2	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	
3 4 5	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	_6	
6 7	Methods: Assignm	ent of i	nterventions (for controlled trials)		
8 9	Allocation:				
10 11 12 13 14 15	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		_
16 17 18 19	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	
20 21 22	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to _ interventions	8	
23 24 25 26	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	8,9	-
27 28 29		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's _ allocated intervention during the trial	9	
30 31	Methods: Data coll	ection,	management, and analysis		
32 33 34 35 36 37	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	
38 39 40 41 42 43 44 45		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	
			For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml		3

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1 2 3 4	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
5 6 7	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
8 9		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	Not applicable
10 11 12 13		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
14 15	Methods: Monitorin	ıg		
16 17 18 19 20 21	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
21 22 23 24		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
25 26 27	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
28 29 30	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
31 32	Ethics and dissemi	nation		
33 34 35 36	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	6
37 38 39 40 41	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
42 43 44 45 46			For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml	

1 2	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	14	
3 4 5 6		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	14	
7 8 9	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	
10 11 12	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	1	
13 14 15	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	
16 17 18	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	
19 20 21 22 23	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	
24 25		31b	Authorship eligibility guidelines and any intended use of professional writers	Not applicable	
26 27 28		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	Not applicable	
29 30	Appendices				
31 32 33	Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	See rules of the Ethical Committee	
34 35 36	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	
37 38 39 40	*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 " <u>Attribution-NonCommercial-NoDerivs 3.0 Unported</u> " license.				
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Study protocol for a randomised double-blinded, shamcontrolled, prospective, cross-over clinical trial of vagal neuromodulation for pain treatment in patients with chronic pancreatitis

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Aalborg, Denmark

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Denmark

Study protocol for a randomised double-blinded, sham-controlled,

prospective, cross-over clinical trial of vagal neuromodulation for pain

Janusiya Anajan Muthulingam^{1,2}, Søren Schou Olesen^{2,3}, Tine Maria Hansen^{1,2}, Christina Brock^{2,4}, Asbjørn

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

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

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reviewed the draft versions of the manuscript and have read and approved the final manuscript.

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Mech-Sense, Department of Radiology

P.O. Box 365, DK-9100 Aalborg, Denmark

Aalborg University Hospital

Telephone: +45 97665105

E-mail: jebf@rn.dk

Study Sponsor-investigator and corresponding author: Professor Jens Brøndum Frøkjær, MD, PhD

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

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

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treatment in patients with chronic pancreatitis

Mohr Drewes^{2,3} and Jens Brøndum Frøkjær^{1,2}

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33 <u>Abstract</u>

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.

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

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

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

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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 sets.
 ign may limit genera. explore long-term effects.
- The single-centre design may limit generalizability of the study results.

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INTRODUCTION

 65 Chronic pancreatitis (CP) is a disease characterized by progressive pancreatic inflammation and fibrosis, 66 resulting in damage to and loss of exocrine (acinar), endocrine (islet cells), and ductal cells¹. Chronic 67 abdominal pain is the dominating symptom in CP and is present in up to 70% of patients². Pain is associated 68 with reduced quality of life, increased hospitalization frequencies and thus a significant socioeconomic 69 burden³.

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 threshold⁴. In addition, this effect was abolished by atropine administration thereby proving that enhanced parasympathetic tone leads to prevention of oesophageal pain hypersensitivity⁴. 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 subjects⁵. 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 neck⁶ (Figure 1). T-VNS has been shown to induce analgesic^{7,8} and anti-inflammatory effects in healthy individuals⁹ 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 transmission¹⁰. 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 pathways¹². Also, the non-pharmacological treatment is FDA-approved for the acute treatment in migraine patients¹³.

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

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3 4 g	98	to s	ham treatment, and that these effects are mediated via modulation of central pain pathways. To answer the
5			erall study aims, we have two clinical and two experimental objectives:
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8 10 9		1)	The primary clinical objective is to assess the effect of t-VNS on the daily pain experience documented in
10 10			a pain diary in chronic pancreatitis patients.
11 10 12)2	2)	Secondary clinical objectives are to document changes in quality of life and daily functioning.
13 10		3)	The experimental objective is to assess the effect of t-VNS on A) resting state brain function assessed by
14 15 10)4		magnetic resonance imaging (MRI), and B) brain metabolites assessed by magnetic resonance
16 10			spectroscopy.
17 18 10)6	4)	Secondary experimental objectives are to assess the effect of t-VNS on A) experimental pain stimuli
¹⁹ 10)7		documented by QST, B) cardiac vagal tone and C) pro-inflammatory cytokine profiles obtained from blood samples.
20 21 10)8		blood samples.
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9 <u>METHODS AND ANALYSES:</u>

110 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) statement¹⁵.

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 neuromodulation¹⁶ and was shown to be sufficient
to reset the effects of neuromodulation¹⁷. 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).

28 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.

50 135 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 criteria¹⁸. 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 \ge 3 days per week for at least 3 months) and must consider their pain as insufficiently treated with their prescribed analgesic

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treatment. Additionally, the patients must be willing and able to comply with the scheduled visits, treatment 141 plan, laboratory tests, and other study procedures. Finally, the patient must sign the informed consent and 142 power of attorney document. 143

Patients will be excluded if they have any clinically significant abnormalities that may increase the risk 144 associated with trial participation or may interfere with the interpretation of the trial results. Also, patients with 146 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 148 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 150 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 159 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 163 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 168 amplitude of simulation titrated to achieve mild pulling of the ipsilateral oral commissure¹⁹. Bilateral stimulation has shown to be effective in previous studies with GammaCore^{20,21}. The patient self-administers 50 169 170 the treatment, using the device at home three times per day (morning, afternoon, and evening) for two weeks. 52 Previous studies with Gamma-Core, have shown that three doses per day have been effective ^{13,22}. 53 171

⁵⁵ 172 The stimulation device is positioned anterior to the sternocleidomastoid muscle, over the carotid artery as this 56 57 173 runs in close proximity with the vagal nerve. The active Gamma-Core device produces a low-voltage electrical 58 174 signal comprising a 5 kHz sine wave burst lasting for 1 millisecond (five sine waves, each lasting 0.2 59

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millisecond), with such bursts repeated once every 40 milliseconds (25 Hz), generating a 24 V peak voltage 175 and 60 mA peak output current. Those parameters have been used to activate the vagal nerve in 176 177 electrophysiological studies^{20,21}.

₁₀ 178 To mimic the sensation of the active treatment, the sham-device will provide vibration²³. The appearance, 179 weight, visual and audible feedback, and user application are identical for the sham and t-VNS devices. 13 180 However, the sham device produces a low-frequency (0.1 Hz) biphasic signal that does not stimulate the vagal 181 nerve or generally cause muscle contractions²⁴. Additionally, both devices will display a numeric value 16 182 between 1 and 40, signifying the intensity of the stimulation. The maximum intensity per stimulation is 40 for 18 ¹⁸³ both devices (Figure 1). The intensity of the stimulation can vary from patient to patient. The intensity for 19 184 stimulation is reached by increasing the stimulation to the maximum the patient can tolerate without excessive 21 185 pain. Some patients can tolerate less than other patients depending on the pain level. Therefore the dosage of 22 186 every stimulation is patient-dependent²⁵.

₂₅ 187 Compliance will be assessed by reading the remaining doses displayed at the device after each treatment ²⁶ 188 period. Additionally, the patients will be asked to keep a record of the stimulation intensity of the doses applied 28 189 at each stimulation. In addition, questions on compliance will be asked after each treatment period. Finally, 190 adherence will be recorded by patients' diary.

₃₂ 191 During the study periods the patient will continue their standard care, without changes in their current pain ³³ 192 treatment.

36 Randomization, sequence generation and allocation concealment 37 194

³⁹ 195 Once eligibility and consent have been approved and completed, randomization will occur using a 41 196 randomization list generated by an automatic web-based randomization program. Patients will be randomly 197 assigned to VNS/sham or sham/VNS using block randomisation, allowing seven patients at the time to be 44 198 randomised in equal proportions for the order of active t-VNS or sham stimulation. The randomization order .5 46 199 will be kept in closed envelopes; therefore, patients will get their assignment according to the order of entrance 47 200 in the study. This process will be carried out by a member of the research team who is not involved in the 49 201 recruitment process or conduction of the study.

An unblinded researcher will be involved in delivering the medical device according to the randomisation 51 202 52 53 203 schedule. The sequence will follow a 1:1 sequential design, in a double-blinded fashion. Additionally, the 54 204 outcome assessor (data analyst) will be blinded during the statistical analyses of experimental outcomes. A 55 ₅₆ 205 series of numbered, sealed, envelopes will be used to ensure concealed allocation.

58 206 Blinding

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Both, active and sham devices are labelled with a serial number and not outwardly identified as active or sham. 207 All researchers involved in the data collection and MRI analysis will be blinded to the treatment allocation 208 209 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 210 ₁₁ 211 that they have to undergo two different interventions with two different devices, and the purpose of this study 12 212 is to investigate the most effective treatment.

213 Manufacturing and preparation of the medical devices are handled by an external good manufacturing practice-16 214 accredited facility (ElectroCore). As the patients do not know that the sham treatment is an inactive treatment, 18 215 we will not be able to ask the patient "do you think you received active or inactive treatment?", thus we will 216 not be able asses and determine if the blinding was effective.

217 Unblinding is only permissible if a patient experiences any serious adverse events and that the 23 218 investigator/doctor judge that it is essential to know the treatment allocation in order to treat the patient 25 219 appropriately.

29 221 Primary clinical outcome measures

222 The primary clinical efficacy parameter to be evaluated is 30% pain relief. This is assessed as changes in the 33 223 daily experience of pain, which will be measured using a patient pain diary based on the numeric rating scale 224 (NRS) (1 = no pain, 10 = worst pain imaginable). Patients will be asked to score daily pain levels in the diary 36 225 for eight weeks (including one week before the first treatment period and one week after the last study period, ₃₈ 226 Figure 2 and Figure 3), with one NRS value for the average pain over the previous 24 hours and one NRS ³⁹ 227 value for the worst pain over the previous 24 hours.

44 229 Secondary clinical outcome measures

Quality of life questionnaire, C30, version 3.0 (QoLQ-C30)²⁶, the brief pain inventory – short form (BPI-SF) 46 230 47 48^{''} 231 questionnaire²⁷, and Patient Global Impression of Changes²⁸ questionnaire (PGIC) are secondary clinical ⁴⁹ 232 outcomes. Patient will complete QoLQ-C30 and BPI-SF questionnaire before and after each treatment period, 50 ₅₁ 233 while the PGIC questionnaire will only be fulfilled after the treatment periods. The QoLQ-C30 questionnaire ⁵² 234 is composed of both multi-item scales and single-item measures. These include five functional scales, three 53 54 235 symptom scales, a global health status, and six single items. The BPI-SF questionnaire rapidly assess the 56 236 55 severity of pain and its impact on daily functioning. Finally, the PGIC questionnaire evaluates all aspects of 57 237 patients' health and assess if there has been an improvement or decline in the overall clinical status.

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238 **Experimental outcome measures**

Resting state functional MRI will be employed to detect brain activity and functional connectivity changes 239 based on BOLD signals before and after treatment of each patient. Additionally, magnetic resonance 240 10 241 spectroscopy in anterior cingulate cortex, prefrontal cortex, parietal, and insula will also be performed in order 242 to investigate changes in brain metabolites before and after each treatment.

243 MRI data will be acquired on a 3 Tesla MR scanner (Signa HDxt, General Electric, Milwaukee, WI, USA) 15 244 equipped with an 8 channel standard head coil. Scan time for a structural scan will be 5¹/₂ minutes. Following ₁₇ 245 parameters will be used for the structural scan: 150 slices, FOV 250 mm, echo time 3.6 millisecond, repetition 246 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, 20 247 248 echo planar (Gr-EPI), 192 volumes, 37-40 slices, FOV=240 mm, echo time=30 millisecond, repletion 23 249 time=2000 millisecond, flip angle=90^o, matrix size=64x64, resolution=3.75x3.75 mm, slice thickness 3.8 mm, 25²250 no gap, axial slices. The scan time for functional MRI will be 6 minutes and 32 seconds. Additionally, MRI ²⁶ 251 spectroscopy will be used to estimate brain metabolites in the anterior cingulate cortex, prefrontal cortex, 28 252 parietal, and insula. For MRI spectroscopy, single voxel PRESS (Point RESolved Spectroscopy) will be 253 acquired. Following parameters will be used: Echo time=30 millisecond, repetition time=2000 millisecond, 31 254 scan time will be 5 minutes, and the total number of scans will be 128. Bandwidth will be 5,000 Hz. A 255 20x20x20 mm voxel of interest will be positioned on a sagittal T2-weighted fast spin echo sequence. Repletion 34 256 time= 4600 millisecond and echo time=102 millisecond, matrix 384x256, slice thickness 3 mm, gap 0.3 mm), ₃₆ 257 in the midline in the ACC with the inferior border along the anterior-posterior commissure line.

⁴⁰ 259 Secondary experimental outcome measures 41

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

₄₅ 261 QST includes temporal summation²⁹, pressure pain thresholds^{29,30} and conditioned pain modulation (CPM)³¹. 262 Temporal summation demonstrates an increase perception of pain to repetitive pain stimuli²⁹. Temporal 48 263 summation will be recorded in the dermatome T10 (pancreatic area) and control area (dominant forearm) using 264 the PinPrick stimulator, 256 mN (MRC Systems GmbH Medizintechnische Systeme, Germany).

₅₂ 265 The pressure pain threshold and pressure pain tolerance will be determined by pressing an electronic pressure ⁵³ 266 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 268 pain tolerance will be measured on bone. For the muscle pressure stimulation, the probe has a surface area of

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1 cm². 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 mm² (Aalborg University, Denmark) will be applied.

CPM is a clinically measurable form of descending pain modulation³¹ 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 induction³². 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 device³³.

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).

286 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 score³⁴. 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.

4.

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.

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299 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 300 the patient file and on the electronic case report form. All types of adverse events will be notified to the device 301 302 manufacturer ElectroCore and to the Danish Health Authorities by use of Manufactures Incident Report Form.

304 Data collection and data management

305 All instruments in the questionnaires are validated^{26,27}. Additionally, all data collectors are highly experienced 16 306 registered research nurses, radiographers, and researchers who have been trained in good clinical practice ., 18 307 (GCP). There will be regular meetings between the data collectors, monitor, principal investigator and other 308 co-researchers involved in the project. All paper protocols will be kept safe and transferred to a computerised 21 309 database. The questionnaires will be checked for errors and missing data by research staff. Data entries are 310 double-checked against the paper questionnaires.

25 311 During trial conduct, the Good Clinical Practice unit (GCP, Aalborg, Denmark) will conduct periodic ²⁶ 312 monitoring of all signed consents at monitoring visits to ensure that the protocol and GCP standards are 28 313 followed. The monitors may review source documents and medical records to confirm that data recorded on 314 Case Report Form is accurate. Thus, GCP monitoring includes all signed consents, signed power of attorney, 31 315 and AE.

³³ 316 Criteria for the termination of the trial is, when patients according to the sample size with valid data are 35 317 recorded. If the study fails to recruit adequate patients according to the sample size by end of 2019, the study ³⁶ 318 will be terminated.

41 320 **Data analysis**

321 Both descriptive and analytical statistics will be used in order to compare groups and for analyses of outcomes 44 322 over time including changes therein. All data will be presented as mean ± standard deviation and summarized 46 323 in frequency tables, unless otherwise indicated. We will use Research Electronic Data Capture (REDCap)³⁶ to ⁴⁷ 324 store the data and the statistical software package STATA to perform statistical analysis. We will use mixed 49 325 ANOVA for the inferential statistic of the parametric data, with Tukey's and/or Bonferroni post hoc tests for ⁵⁰ 326 the primary clinical endpoints. Significance level will be set as $\alpha \leq 0.05$.

⁵³ 54 328 The principal analysis of clinical endpoints will be by intention-to-treat, meaning that all randomized patients 55 329 are included in their initially assigned study arm, regardless of adherence to study protocol. Experimental 57 330 endpoints will be analysed by per-protocol, meaning that only patients completing the experimental setup are ⁵⁸ 331 included. The primary endpoints will be compared between the treatment groups.

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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 11³³⁶ metabolites changes will be assessed in pain related brain regions³⁷.

14 338 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 17 340 details of the analysis and presentation of the results will be finalized before initiating any statistical analysis.

Patient and public involvement 21 342

23 343 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 26 345 and MRI brain scans were deliberately chosen in order to assess the potential effect of t-VNS treatment both 28 346 subjectively (patient-oriented) and objectively. Furthermore, no patients were directly involved in the design, 29 347 recruitment to or conduct of the study. However, an expert/chief doctor specialized in chronic pancreatitis 31 348 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.

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352 **DISCUSSION**

To the best of our knowledge, there are no randomized, sham controlled, studies investigating the effect of t-353 VNS on clinical pain in patients with CP. We expect the study to provide clinical evidence of the analgesic 354 effect of VNS and to elucidate its underlying mechanisms of action. This may pave the road for non-356 pharmacological treatment of pain associated with CP and the findings of the study may be generalizable to chronic pain conditions per se.

15 358 Previous studies have shown structural and functional alterations of the CNS in CP patients with abdominal ₁₇ 359 pain^{38–42}. The CNS mechanisms may have the ability to recover by targeting treatment at plasticity mechanisms 360 and reorganization of neuronal pathways leading to improvement of clinical symptoms⁴³. VNS treatment has 20 361 emerged promising technique in stimulating neural reorganization and synaptic plasticity in cortical and 362 subcortical networks, leading to modulation of serotoninergic and noradrenergic pain inhibitory pathways⁴⁴. 23 363 Those mechanisms might alter and regenerate the neural connectivity in regions responsible for pain^{45–47}. In 25 364 addition, the vagal nerve serves as an essential transmitter of inflammatory signals in immune-to-neuronal ²⁶ 365 communication⁴⁸⁻⁵⁰. Afferent fibers of the vagal nerve relay information from viscera to the nucleus tractus 28 366 solitaries in the brainstem, where it 'senses' pro-inflammatory cytokines such as IL-1, IL-6, and TNF- α . 367 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 31 368 369 may provide evidence of an existing association. Some limitations about the study should be discussed. Firstly, 34 370 the patient group is very heterogeneous, they may suffer from co-morbidities and may receive other ₃₆ 371 pharmacological therapies, which may bias the results and consequently makes it difficult to assess the isolated ³⁷ 372 effect of the VNS treatment. Secondly, the researchers may involuntarily become unblinded since the active 39 373 treatment will deliver facial contractions while this is not present during sham treatment. Thirdly, the relatively 374 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 42 375 44 376 will be trained to use the device correctly according to manufactures' protocol, it is uncertain whether the 45 377 patients will applicate the device correctly. 46

378 Regarding expected outcome, we hypothesize that VNS will reduce the pain in CP patients and induce changes 49 379 in pain associated brain networks as well in the autonomic, inflammatory parameters and in the sensory system. 380 Also, we expect that the neuromodulation will improve the overall quality of life in CP patients.

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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 ₁₀ 387 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 13 389 2000, clarified by the General Assembly in Washington 2002, Tokyo 2004, and Seoul 2008 and Fortaleza 2013 as outlined herein.

₁₇ 391 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 20 393 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 23 395 international conferences and will be published in peer-reviewed journals. All confidential patient data will be 25 396 protected, and patient identity will not be disclosed. Further dissemination of the data set can be decided by 26 397 the principal investigator.

Only researchers involved in the data collection and/or data analysis will have access to the final dataset. 30 399 However, the principal investigator allows direct access to all source data and documents at monitoring, and 32 400 inspection from the North Denmark Region Committee on Health Research Ethics, the Danish Health and ³³ 401 Medicines Authority or from other countries' health authorities.

₃₈ 403 **Trial status**

The recruitment of the study started in January 2018. As of January 2019, a total of 13 patients have completed 40 404 the study.

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521 Legends for illustrations

Figure 1: Mode of action of transcutaneous vagal nerve stimulation (t-VNS). (1) Pain arises in the 522 periphery e.g. pancreas and a signal is sent to the spinal cord. This leads to ascending activation of the 523 10 524 spinal neurons (2). In the brain, pain is processed in higher cortical centres (3). T-VNS, it is expected to 11 525 block the perception of pain in the cerebral cortex, by stimulating nucleus tractus solitarius and thereby 12 13 526 decrease glutamate level. Simultaneously, the net-descending inhibition will be activated as a result of top-14 527 down input from cortex and the limbic system (4). 15

16 528 Figure 2: Schematic flowchart of the interventional study design enabling comparison of the modulatory 17 effect to self-administered of transcutaneous vagal nerve stimulation (t-VNS) in patients with chronic 18 529 19 530 pancreatitis. Chronic pancreatitis patients will be randomly assigned to one of two double blinded 20 treatments: (1) two weeks of t-VNS, two weeks of wash-out, and two weeks of sham treatment; or (2) two 21 531 22 532 weeks of sham treatment, two weeks of washout, and two weeks of t-VNS. Evaluation of the two 23 24 533 treatments will be assessed by collecting pain diary, pain questionnaires, MRI scan, blood sample, cardiac 25 26 534 vagal tone, and pain assessments. QST=Quantitative sensory testing. CVT=Cardiac vagal tone. ²⁷ 535 MRI=Magnetic resonance imaging. 28

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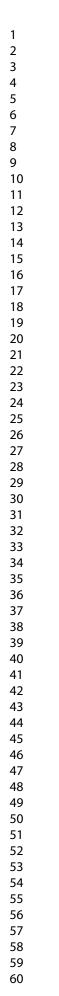
Figure 3: SPIRIT Figure. 29 536

5 <u>Table 1. That characteristics based on WHO That Registration Data Set</u>	5	Table 1:	Trial characteristics bas	sed on WHO Trial	Registration Data Set
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Data category	Trial Information
Primary Registry and Trial	ClinicalTrials.gov (NCT03357029)
Identifying Number	
Date of Registration in Primary	November 29, 2017
Registry	
Secondary Identifying Numbers	North Denmark Region Committee on Health Research Ethics:
	protocol number N-20170023
Source(s) of Monetary or Material	The study is conducted as a sponsor-investigator initiated study
Support	with financial support from Independent Research Fund Denmar
	(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	Chronic pancreatitis
studied	
Interventions	Two-week transcutaneous vagal nerve stimulation (t-VNS) on th
interventions	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 C
	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 \geq 3 days per week in least 3 months) and must consider their pain as insufficient treated with their usual analgesic treatment. ; Personally, signed and dated informed consent document and the Power of attorned document; Patients willing and able to comply with the schedule visits, treatment plan, laboratory tests and other trial procedure Exclusion criteria: Patients with any clinically significa abnormalities that in the opinion of the investigator may increase the risk associated with trial participation or may interfere with the risk associated with trial results. ; Alcohol dependence; Illegal drud dependencies; Participating in another study where investigation of the trial conditions oth than CP that make them unable to distinguish the pain associated with the sum of the pain associated with the pain associated with the sum of the pain associated with the sum of the pain associated with the sum of the pain associated with the pain associated with the sum of the pain associated with the sum of the pain associated with the pain associated w
Study type	with CP from chronic pain of other origin.; Cardiovascular disease ; Low blood pressure < 100/60, Not able to understand or follo the instructions, ; Any condition with elevated intracrani pressure.; Female patients who are pregnant; Contraindications for MRI; Previous surgery on vagal nerve.; Known neuropathy. Interventional allocation: randomized Masking: double-blind

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.

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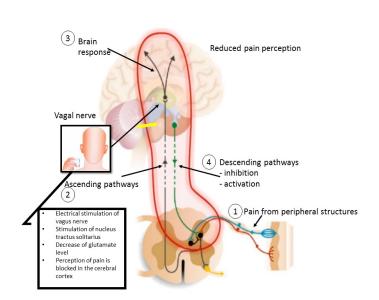


Figure 1: Mode of action of transcutaneous vagal nerve stimulation (t-VNS). (1) Pain arises in the periphery e.g. pancreas and a signal is sent to the spinal cord. This leads to ascending activation of the spinal neurons (2). In the brain, pain is processed in higher cortical centres (3). T-VNS, it is 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).

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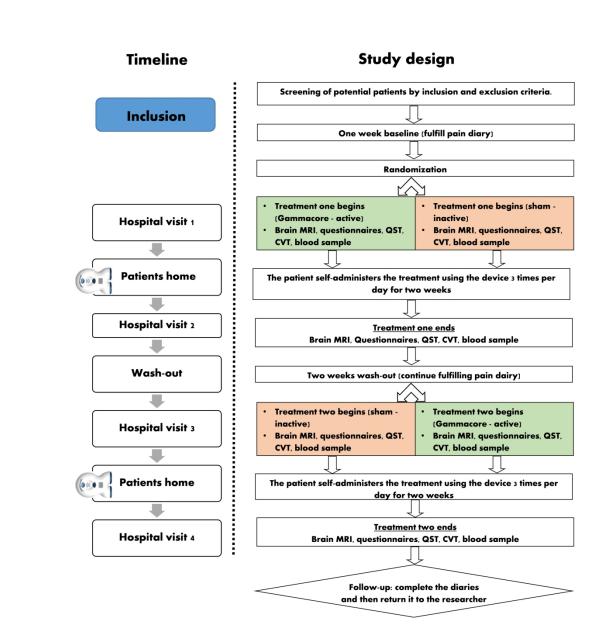


Figure 2: Schematic flowchart of the interventional study design enabling comparison of the modulatory effect to self-administered of transcutaneous vagal nerve stimulation (t-VNS) in patients with chronic pancreatitis. Chronic pancreatitis patients will be randomly assigned to one of two double blinded 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.

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		S	TUD	TUDY PERIOD					
	Enrolment & screening	Baseline & allocation		Post-allocation					Follow- up
TIMEPOINT**	T- 1	0	T 1	T ₂	T ₃	T4	T5	T 6	T 7
Week	0	1	2	3	4	5	6	7	8
ENROLMENT:									
Eligibility screen	х								
Informed consent	х								
Allocation		х							
INTERVENTIONS:									
[transcutaneous VNS (active)]			x	x	7	···•	х	x	
[Sham treatment]			x	х	Was	→ hout	х	х	
ASSESSMENTS:									
Pain diary		Х	X	X	Х	Х	Х	Х	Х
QORTC-QLQ-C30 questionnaire			x	x			х	x	
BPI-SF			x	x			x	x	
Questionnaire			^	^					
PGIC questionnaire				x				x	
MRI			x	x			х	x	
сvт			x	x			x	x	
Blood samples			x	x			x	x	
QST			х	x			х	x	
[Adverse events]			X	X	X		Х	Х	

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	ltem No	Description	Addressed or page number
Administrative info	ormation		
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	5a	Names, affiliations, and roles of protocol contributors	1
responsibilities	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
		For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml	

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1 2	Introduction						
- 3 4 5 6 7 8 9 10 11 12 13	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				
		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)				
14 15	Methods: Participa	nts, inte	erventions, and outcomes				
13 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45	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			
			For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml	:			

1 2 3	Sample size	2e 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					
4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size						
	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		_				
	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					
20 21 22 23	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to _ interventions	8					
23 24 25 26 27 28 29	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					
30 31	Methods: Data coll	ection,	management, and analysis						
32 33 34 35 36 37 38 39 40 41 42 43 44 45	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					
			For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml		3				

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1 2 3 4	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				
5 6 7	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				
8 9		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	Not applicable				
10 11 12 13 14 15		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							
16 17 18 19 20	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				
21 22 23 24 25 26 27 28 29 30		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				
31 32	Ethics and dissemination							
 33 34 35 36 37 38 39 40 41 42 43 44 45 46 	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				
			For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml					

1 2 3 4 5 6	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	
7 8 9	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	
10 11 12	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	1	
13 14 15	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	
16 17 18	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	
19 20 21 22 23	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	
24 25		31b	Authorship eligibility guidelines and any intended use of professional writers	Not applicable	
26 27 28		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	Not applicable	
29 30 31 32 33	Appendices				
	Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	See rules of the Ethical Committee	
34 35 36	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	
37 38 39 40	*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 " <u>Attribution-NonCommercial-NoDerivs 3.0 Unported</u> " license.				
41 42 43 44 45			For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml	5	