PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	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
AUTHORS	Muthulingam, Janusiya Anajan; Olesen, Søren Schou; Hansen, Tine; Brock, Christina; Drewes, Asbjørn Mohr; Frøkjær, Jens Brøndum

VERSION 1 – REVIEW

REVIEWER	Harald Stauss Burrell College of Osteopathic Medicine, Las Cruces, NM, USA
REVIEW RETURNED	25-Mar-2019

GENERAL COMMENTS	This manuscript describes a study protocol to be used to test if transcutaneous vagal nerve stimulation (t-VNS) is effective in reducing pain in patients with chronic pancreatitis. General Comments The inclusion of neurophysiological and CNS imaging techniques is certainly a strength of the study. In addition, assessment of heart rate variability (HRV) is essential to test if t-VNS really causes some vagal nerve stimulation. Ideally, HRV should also be assessed during acute application of the stimulation. Major Concerns Blinding: Since the intensity of the stimulation is individually adjusted based on muscle contractions (at least that's the way it is presented on the company webpage), the "sham" stimulation will not be perceived as a true VNS. Thus, the patients will not really be blinded for VNS or sham. Knowing that they are not really receiving VNS, may affect the pain rating in the daily diaries. Maybe a better sham procedure would be to stimulate at a different site within the neck that still causes muscle contractions but no vagus nerve stimulation. Stimulation parameters: Bursts of 5kHz sine waves are applied at 25 Hz. However, it has been demonstrated that kilo-Hertz stimulation blocks rather than stimulates nerve fibers (Patel, Y.A., Butera, R.J., 2015. Differential fiber-specific block of nerve conduction in mammalian peripheral nerves using kilohertz electrical stimulation. Journal of neurophysiology 113, 3923-3929). Is there evidence, that this device at these stimulation parameters really activates nerve fibers within the vagus nerve? Pain Medication: Are the subjects allowed to increase or decrease their pain medication during the study period? It seems unethical to not allow the subjects to adjust their pain medication to the level of experienced pain. If the patients are allowed to change the pain

	medication (or the dose of the analgesics) during the study, these changes need to be monitored and analyzed. HRV Assessment: Is the device used to assess HRV validated against the TASK force monitor or any other established clinical device? Minor Comments: Avoid keywords that are already in the title Introduction, page 4, lines 89-90: Aims cannot be answered.

REVIEWER	Bruno BONAZ Division of Gastroenterology & Liver Disease Grenoble Faculty of Medicine & Hospital France
REVIEW RETURNED	05-Apr-2019

GENERAL COMMENTS	Based on the antinociceptive effect of the vagus nerve (VN) and VN stimulation (VNS), the authors aim to investigate the effect of non-invasive transcutaneous VNS (t-VNS) on clinical pain in patients with chronic pancreatitis (CP) by comparison to sham t-VNS. This is an interesting and original study, as stated by the authors, since there are no clinical trials on VNS in CP. However, there are some points which need to be addressed: - Abstract: there is no data in the “Methods” section regarding the recording of vagal tone and plasmatic cytokines while it is detailed in the manuscript. - The number of patients is rather small for me, as specified by the authors. - Why the authors stimulate both VN while most of the studies on VNS stimulate the left VN? - The duration of the study (2 weeks) seems too short for me. Indeed, VNS is a slow acting therapy as demonstrated in the treatment of epilepsy. VNS is known to induce modifications of neuronal plasticity, which are supposed to take time. In addition, if the effect of t-VNS is delayed over time, when switching to sham treatment it could induce a bias of the results of sham stimulation. - The authors did not specify which type of CP patients they will include in the study: alcoholic CP?? - The duration of CP and thus chronic pain is supposed to vary between patients and thus the central integration of pain and central sensitization is supposed to be patient-dependent and different between patients. - The analgesic treatments may interfere with VNS. To state that “patients must consider their pain as insufficiently treated with their prescribed analgesic treatment” is rather unclear. The authors need to comment on these points. - Pain is multidimensional and rather subjective. In particular, pain is a stress and stress increases pain and may induce anxiety, depression. Questionnaires regarding stress, depression, anxiety, coping strategies should be included in the trial. - Specify if patients use to perform complementary & alternative medicines. - What about the reproducibility of t-VNS in patients? I mean, do the patients will applicate correctly, along the VN, the stimulator. One can wonder that depending on the level of application of the device along the VN and laterally to the VN, results should be different.

	- Compliance is difficult to appreciate in non-invasive VNS by comparison to invasive VNS. Reading the remaining doses does not mean that patients have applicated the device on the VN. - The background regarding the antinociceptive effect of VNS should be improved. - A chapter regarding “expected results” is missing.

REVIEWER	Roberto De Icco 1 - Headache Science Centre, IRCCS Mondino Foundation, Pavia, Italy 2 - Department of Brain and Behavioral Sciences, University of Pavia, Pavia, Italy
REVIEW RETURNED	08-Apr-2019

GENERAL COMMENTS	The protocol presented in the paper appears to be well written and sufficiently detailed. I kindly ask the authors to reply to the subsequent comments: 1) In the introduction the Authors state that t-VNS is FDA-approved for the preventive treatment of cluster headache and migraine. At state of art, in migraine t-VNS is approved only for the acute treatment. I suggest to update the introduction and the references to address this point. 2) Both in the "introduction" and in the "methods" the Authors define a "primary clinical objective" and a "primary experimental objective", anyway the power calculations is only defined for the clinical one. I suggest to remove the adjective "primary" from the experimental objective. 3) In the "Inclusion criteria and exclusion criteria", Authors state that patiens must consider their pain as unsufficiently treated. Is this evalutaion only subjetive? How do you define it? 4) In the "Interventions section" I suggest to define the period prior to enrollment during which the pharmacological treatment / standard of care must be stable. 5) The most important point to address concerns patients blinding. In line 193 of Blinding section, Authors state that patients do not know that sham treatment is inactive. I think it could be unethical to not inform patients regards the real treatment modalities and the expected effectiveness.

VERSION 1 – AUTHOR RESPONSE

Response to reviewer #1

We appreciate the helpful comments, which we find relevant and constructive. The manuscript has now been carefully evaluated (changes highlighted in the manuscript) and all comments are addressed in the point-to-point response below.

General Comments:
Reviewer wrote: The inclusion of neurophysiological and CNS imaging techniques is certainly a strength of the study. In addition, assessment of heart rate variability (HRV) is essential to test if t-

VNS really causes some vagal nerve stimulation. Ideally, HRV should also be assessed during acute application of the stimulation.

Response: We agree with the reviewer that heart rate variability (HRV) ideally should be assessed during acute application of the stimulation. However, in the current study, we are recording the cardiac vagal tone (CVT), which is derived from heat rate variability and measures beat-to-beat changes in the R-R intervals. In this study the CVT is recorded before the stimulation and right after the stimulation (1).

The patients are self-administering the stimulation on the neck, by standing in front of a mirror in order to practice to use the device, resulting in some movement artifacts and some background noise in the CVT data. Self-administering the stimulation and recording CVT data may leads to poor CVT data quality, therefore we recorded CVT before and after to test if t-VNS really causes vagal nerve stimulation.

Assessment of CVT before and after modulation have been applied in our previous study (2) showing that CP patients have increased CVT after t-VNS in comparison with sham treatment. This study measured CVT after 25 minutes and were able to detect VNS modulation. Since we are measuring before and right after the stimulation, we strongly believe that we are capable to detect that t-VNS modulates the vagal tone.

Major Concerns

Reviewer wrote: Blinding: Since the intensity of the stimulation is individually adjusted based on muscle contractions (at least that's the way it is presented on the company webpage), the "sham" stimulation will not be perceived as a true VNS. Thus, the patients will not really be blinded for VNS or sham. Knowing that they are not really receiving VNS, may affect the pain rating in the daily diaries. Maybe a better sham procedure would be to stimulate at a different site within the neck that still causes muscle contractions but no Vagus nerve stimulation.

Response: We agree that one of our study limitation is blinding given that the active device should be individually adjusted to the maximum tolerance, while it is challenging to identify the correct intensity with the sham device, as it is not providing any stimuli (only vibration). The patients will be informed that they have to undergo two different interventions with two different devices, and the purpose of this study is to investigate the most effective treatment. The patients will be informed that both devices are visually identical, however the mechanism of the two devices differs.

Several previous studies have also used the sham device as a control arm. Particularly, Silberstein et al. 2016 (3) conducted a randomized, double-blind, sham-controlled study with cluster headache patients evaluating non-invasive VNS as an acute cluster headache treatment. Silberstein and colleagues assessed the effectiveness of blinding by calculating Bang index (4) and demonstrated that blinding for both the sham group and active group became successful during the study. Therefore, we consider the sham device to be successful leading to effective blinding of the two interventions.

Reviewer wrote: Stimulation parameters: Bursts of 5 kHz sine waves are applied at 25 Hz. However, it has been demonstrated that kilo-Hertz stimulation blocks rather than stimulates nerve fibers (Patel, Y.A., Butera, R.J., 2015. Differential fiber-specific block of nerve conduction in mammalian peripheral nerves using kilohertz electrical stimulation. Journal of neurophysiology 113, 3923-3929). Is there evidence, that this device at these stimulation parameters really activates nerve fibers within the vagus nerve?

Response: Yes, there are strong evidence that VNS with following parameters: 5-kHz sine wave burst lasting for 1 millisecond, with such bursts repeated once every 40 milliseconds (25 Hz), generating a 24-V peak voltage and 60-mA peak output current, activates vagus nerve fibers in cluster headache patients (3), chronic migraine and epilepsy (5). Recently, a VNS study and review showed that 25 Hz produces significant cortical effects in the vagal afferent pathway (6).

The study conducted by Patel and colleagues (7) used high-frequency stimulation in both the vagus and the sciatic nerves in rats to stimulate fiber types or afferent/efferent traffic.

It has been shown that VNS inhibits spinal cord neurons below level C3 but excites neurons between C1 and C3 (8). Patel and Colleagues stimulate below C3 (sciatic nerve) with high-frequency stimulation. In contrast, we are stimulating above C3 with 5 kHz, and an amplitude of 60 mA, which has been shown to be effective.

We have now added following sentence on page 8 (Interventions). “Those parameters have been used to activate the vagal nerve in electrophysiological studies (9,10).”

Reviewer wrote: Pain Medication: Are the subjects allowed to increase or decrease their pain medication during the study period? It seems unethical to not allow the subjects to adjust their pain medication to the level of experienced pain. If the patients are allowed to change the pain medication (or the dose of the analgesics) during the study, these changes need to be monitored and analyzed.

Response: We agree with the reviewer, that medication changes need to be monitored and analyzed. In the study, changes in medication are reported by the patient in the diary. We have now included following sentence on page 6:

“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.”

Reviewer wrote: HRV Assessment: Is the device used to assess HRV validated against the TASK force monitor or any other established clinical device?

Response: Among others, CVT is a commonly used measure derived from power spectral analysis of heart rate variability (1). The CVT assessment method used in our study has been validated in 200 healthy subject by Farmer and colleagues (1), demonstrating an excellent reproducibility in healthy subjects over a period of 1 year. Additionally, the CVT assessment method has been validated on 42 diabetes patients, showing that CVT is a convenient, clinical method to assess the parasympathetic nervous system tone (11). So far, no studies have validated the CVT assessment method in CP patients, however, we anticipate that the reproducibility of the CVT assessment would be similar to diabetes patients.

Minor Comments:

Reviewer wrote: Avoid keywords that are already in the title

Response: We have now replaced some of the key words on page 2.

“Key words: Transcutaneous electric nerve stimulation; Viscera; Chronic Pain; Gastrointestinal disease; Clinical trials”

Reviewer wrote: Introduction, page 4, lines 89-90: Aims cannot be answered.

Response: We have now specified aim one (Page 4). “The primary clinical objective is to assess the effect of t-VNS on the daily pain experience documented in a pain diary in chronic pancreatitis patients.”

Response to reviewer #2

We appreciate the helpful comments, which we find relevant and constructive. The manuscript has now been carefully evaluated (changes highlighted in the manuscript) and all comments are addressed in the point-to-point response below.

General Comments:

Reviewer wrote: Based on the antinociceptive effect of the vagus nerve (VN) and VN stimulation (VNS), the authors aim to investigate the effect of non-invasive transcutaneous VNS (t-VNS) on clinical pain in patients with chronic pancreatitis (CP) by comparison to sham t-VNS. This is an interesting and original study, as stated by the authors, since there are no clinical trials on VNS in CP.

Response: Thank you very much for your kind remarks.

Reviewer wrote: Abstract: there is no data in the “Methods” section regarding the recording of vagal tone and plasmatic cytokines while it is detailed in the manuscript.

Response: We apologize and have now added cardiac vagal tone and blood sample testing in the abstract (Page 2).

“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.”

Reviewer wrote: The number of patients is rather small for me, as specified by the authors.

Response: We agree with reviewer that the sample size might be small in regards to the secondary outcomes. We have performed a statistical power analysis based on the primary outcomes (VAS scores –from daily pain diary), revealing that 16 CP patients in a cross-sectional study is sufficient to detect a difference. Thus, we are confident that the number of patients for the primary outcomes is sufficient. The power calculation (standard deviation) is based on data from a study with chronic pancreatitis patients, who received pregabalin treatment, which related to an improvement in clinical measures of the pain score(12).

We did not perform a statistical analysis for the explorative secondary outcomes such as cardiac vagal tone, and blood samples, etc., given that our primary goal is to detect significant reduction in VAS score after active treatment as well as to demonstrate an association to the secondary outcomes, i.e. cytokine changes, cardiac vagal tone changes, etc.

One page 14 (Discussion), we have now specified the sentence regarding low sample size: “Thirdly, the relatively low number of patients may hamper the results including the explorative secondary outcomes; however, we eliminate the inter-individual variability because of the cross-over design.”

Reviewer wrote: Why the authors stimulate both VN while most of the studies on VNS stimulate the left VN?

Response: This is a very good point. For invasive VNS stimulation, only left vagal nerve is stimulated by wrapping a fine wire electrode around the left cervical vagal nerve. Case reports suggest that the right vagus can be used in circumstances where approaching the left vagus is inadvisable. Since the right vagus innervates the sinoatrial node, stimulating on the right is best done with ECG monitoring. These recommendations are primarily for invasive VNS for instance in patients with epilepsy (13).

To date, there are no clear evidence or recommendations in regards to unilateral or bilateral stimulation with transcutaneous VNS. Hitherto, previous transcutaneous VNS stimulation studies using the GammaCore device have stimulated bilaterally (on both left and right vagal nerve) in chronic pain patients (9) and in immune-mediated inflammatory diseases (10). One possible explanation could be that, both left and right vagal nerves need to be stimulated to ensure that the input from the transcutaneous VNS to the nucleus tractus sollitarius in the brainstem is sufficient in order to project the afferent input to other regions of the central nervous system. It is uncertain whether unilateral stimulation with transcutaneous VNS is enough to modulate the brainstem, and higher CNS centers.

Currently, we do not know whether unilateral stimulation is better than bilateral stimulation, or maybe there are no difference between unilateral and bilateral stimulation. However, in order to stimulate sufficiently with transcutaneous VNS, which is a non-invasive approach, we have chosen to replicate the same stimulation approach as the previous transcutaneous VNS studies assessing the GammaCore device. Future studies will indeed be need to evaluate the correct stimulation approach and investigate whether there is any difference between unilateral and bilateral stimulation.

We have now added following sentence on page 7 (Method section): “Bilateral stimulation has shown to be effective in previous studies with GammaCore (9,10). ”

Reviewer wrote: The duration of the study (2 weeks) seems too short for me. Indeed, VNS is a slow acting therapy as demonstrated in the treatment of epilepsy. VNS is known to induce modifications of neuronal plasticity, which are supposed to take time. In addition, if the effect of t-VNS is delayed over time, when switching to sham treatment it could induce a bias of the results of sham stimulation.

Response: This is a valuable point and we agree with the reviewer that a carry-over effect could possibly exist in our study. To date, no other studies have conducted a cross-over study with the Gammacore device. Therefore, estimating the wash-out period has been challenging. We agree that several VNS studies have shown long-term effect with VNS in patients with epilepsy (14). VNS treatment in epilepsy patients have been conducted with both invasive and non-invasive VNS, therefore it is important to differentiate between invasive VNS treatment and non-invasive transcutaneous VNS treatment. Most of the long-term VNS studies have been conducted with invasive VNS.

Previous prospective, double-blind, placebo-controlled, randomized studies with transcutaneous VNS have demonstrated that Gamma-Core provided improved pain relief versus sham treatment at 30, 60 and 120 minutes in migraine patients (15). Therefore, we expect that two weeks are enough to modulate the processing of pain in the brain resulting in improved pain relief. However, we can discard that the effect of GammaCore may potentially be carried over for the second treatment with sham device.

Previous neuromodulation studies have used a two weeks wash-out to reset the effect of neuromodulation (16). Therefore, we expect that minimum two weeks wash-out might be sufficient to bring back the original pain symptoms. Ideally, it would be more powerful, if the washout period could be increased few weeks, however, increasing wash-out period results in extending of study period. Extending the study period, makes it difficult to retain the patients in the study.

Reviewer wrote: The authors did not specify which type of CP patients they will include in the study: alcoholic CP??

Response: This study include all types of CP patients. We have now added following on page 6: "All aetiological types of CP patients would be included (incl. alcohol, nicotine, hereditary, efferent duct factors, and immunological aetiologies)."

Reviewer wrote: The duration of CP and thus chronic pain is supposed to vary between patients and thus the central integration of pain and central sensitization is supposed to be patient-dependent and different between patients.

Response: This is correct. We are aware of the fact that the pain pattern is not the same for all patients i.e. CP patients could either have constant intermittent pain, constant pain, or constant pain with exacerbations. Therefore, the central sensitization will be patient-dependent as the reviewer pointed out. Indeed, the transcutaneous VNS treatment is patient-dependent, meaning that the patient decides the intensity of the stimulation. Some patients would use a low intensity while other patients will use a high intensity. In comparison to other drug studies providing the same dosage for all patients, transcutaneous VNS treatment, provide different intensity depending on the individual patient's pain experience.

We have now elucidated the dosage of the transcutaneous VNS treatment on page 8: "The intensity of the stimulation can vary from patient to patient. The intensity for stimulation is reached by increasing the stimulation to the maximum the patient can tolerate without excessive pain. Some patients can tolerate less than other patients depending on the pain level. Therefore the dosage of every stimulation is patient-dependent (17). "

Reviewer wrote: The analgesic treatments may interfere with VNS. To state that "patients must consider their pain as insufficiently treated with their prescribed analgesic treatment" is rather unclear. The authors need to comment on these points.

Response: We agree with the reviewer that the analgesic treatment may potentially interfere VNS treatment. The inclusion criteria "The patients must suffer from chronic abdominal pain characteristics for CP, meet the criteria for chronic pain (pain ≥ 3 days per week for at least 3 months) and must consider their pain as insufficiently treated with their prescribed analgesic treatment" is quite wide.

Since it is unethical to ask the patient to pause the medication, we asked them to continue their treatment. Indeed, patients will receive different treatment (i.e. paracetamol, NSAID, opioids) with different dosage. This leads to a heterogeneous patient group and makes it difficult to assess the sole effect of transcutaneous VNS treatment. On the other hand, the strength of this criteria is, that this study mimics the clinical world. The transcutaneous VNS treatment is aimed to be an add-on treatment, and not a new treatment that should completely provide pain relief in CP patients. Thus, it is a strength of the study to assess the effect of transcutaneous VNS treatment with patients on pain medication.

Finally, if we had specified the inclusion criteria too strictly (i.e., that patients have to be on opioid treatment and score minimum 5 on VAS scale), we would definitely have selection-bias since patient group would not represent CP population in general.

We have now added in the Discussion section following sentence on page 14: “Firstly, the patient group is very heterogeneous, they may suffer from co-morbidities and may receive other pharmacological therapies, which may bias the results and consequently makes it difficult to assess the isolated effect of the VNS treatment.”
Reviewer wrote: Pain is multidimensional and rather subjective. In particular, pain is a stress and stress increases pain and may induce anxiety, depression. Questionnaires regarding stress, depression, anxiety, coping strategies should be included in the trial.

Response: We agree that pain is multidimensional and therefore psychological parameters could have been included in this study. This study does not directly assess the psychological functioning, however we are assessing those parameters indirectly through quality of life questionnaire, C30, version 3.0 (QoLQ-C30) (18). Among others quality of life questionnaire assess a) role functioning, b) emotional functioning, c) cognitive functioning and d) social functioning.

Reviewer wrote: Specify if patients use to perform complementary & alternative medicines.

Response: This is a very important point, as also addressed by reviewer 1. We have now clarified that the patients are allowed to take additional/supplementary pain medication on page 6: “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

Reviewer wrote: What about the reproducibility of t-VNS in patients? I mean, do the patients will applicate correctly, along the VN, the stimulator. One can wonder that depending on the level of application of the device along the VN and laterally to the VN, results should be different.

Response: All patients will be trained by the researchers to use the stimulator correctly according to the manufactures’ protocol. As part of the training, the patient will be marked on the skin over the carotid artery. This mark will help the patient to place the device at the same position enabling the patient to place the device correctly. Although we are training all the patients, it is of course uncertain whether the patients will stimulate correctly at home. Thus, this is another limitation in the study which is added in the manuscript in the discussion part, Page 14. “Finally, although, all the patients will be trained to use the device correctly according to manufactures’ protocol, it is uncertain whether the patients will applicate the device correctly.”

Reviewer wrote: Compliance is difficult to appreciate in non-invasive VNS by comparison to invasive VNS. Reading the remaining doses does not mean that patients have applicated the device on the VN.

Response: Compliance is always as major problem when running clinical studies both in drugs studies and in medical device studies. In drug studies, the researcher can count the remaining pills in container, similarly, we are able to count the remaining doses left on the device on the display. The major difference regarding assessing the compliance between drugs study and medical device study is that the true compliance can be revealed in drugs studies by collecting blood samples while it cannot be performed in medical device. This is not only a specific limitation in our study, but remains a common challenge in clinical studies assessing the effect of medical device.

Reviewer wrote: The background regarding the antinociceptive effect of VNS should be improved.

Response We have now revised the background regarding the antinociceptive effect of VNS and have added following paragraph in the introduction, Page 4. “The exact mechanisms by which VNS modulates chronic pain is unclear, however it has been proposed that the analgesic effect is potentially mediated by vagal afferents that inhibit spinal nociceptive reflexes and transmission(8). 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(8,19). It has also been demonstrated that VNS inhibits spinal cord neurons below C3 but excites neurons between C1 and C3, suggesting that propriospinal neurons from high segments play an essential role in vagally mediated antinociception. Thus, VNS appears to induce neuromodulatory antinociception through peripheral and central, ascending and descending pathways (20).”

Reviewer wrote: A chapter regarding “expected results” is missing.

Response: We agree and have now added a chapter regarding expected results in the Discussion, Page 14. “Regarding expected outcome, we hypothesize that VNS will reduce the pain in CP patients and induce changes in pain associated brain networks as well in the autonomic, inflammatory parameters and in the sensory system. Also, we expect that the neuromodulation will improve the overall quality of life in CP patients. ”

Response to reviewer #3

We appreciate the helpful comments, which we find relevant and constructive. The manuscript has now been carefully evaluated (changes highlighted in the manuscript) and all comments are addressed in the point-to-point response below.

General comment:

Reviewer wrote: The protocol presented in the paper appears to be well written and sufficiently detailed.

Response: Thank you very much for your kind remarks.

Reviewer wrote: In the introduction the Authors state that t-VNS is FDA-approved for the preventive treatment of cluster headache and migraine. At state of art, in migraine t-VNS is approved only for the acute treatment. I suggest to update the introduction and the references to address this point

Response: This has been corrected on page 4. “Also, the non-pharmacological treatment is FDA-approved for the acute treatment in migraine patients(21).”

Reviewer wrote: Both in the "introduction" and in the "methods" the Authors define a "primary clinical objective" and a "primary experimental objective", anyway the power calculations is only defined for the clinical one. I suggest to remove the adjective "primary" from the experimental objective.

Response: It is correct that the power calculation is only based on the primary clinical objective. We have now removed “primary” from the experimental objective.

Reviewer wrote: In the "Inclusion criteria and exclusion criteria", Authors state that patiens must consider their pain as unsufficiently treated. Is this evalutaion only subjetive? How do you define it?

Response: Insufficiently treated means that the patients have underwent all available treatment approach, i.e. paracetamol, NSAID, opioids and adjuvant analgesics. If the patient still suffer from pain after having received the above mentioned treatment, the patient is considered as insufficiently treated. However, in the end, only the medical doctor decide/evaluate whether the patient is insufficiently treated and eligible to participate in the clinical trial.

Reviewer wrote: In the "Interventions section" I suggest to define the period prior to enrollment during which the pharmacological treatment / standard of care must be stable.

Response: We have now added following sentence in the “Intervention section”, page 7. “Prior to receiving the t-VNS treatment/sham treatment, the standard care must be stable.”

Reviewer wrote: The most important point to address concerns patients blinding. In line 193 of blinding section, Authors state that patients do not know that sham treatment is inactive. I think it could be unethical to not inform patients regards the real treatment modalities and the expected effectiveness.

Response: The patients will be informed that they will undergo two different interventions with two different devices. Also, they will be informed that the mechanisms differ between the two devices. We have now revised the sentence on page 9: “Particularly, patients will be informed that they have to undergo two different interventions with two different devices, and the purpose of this study is to investigate the most effective treatment.”

It can be discussed whether it is unethical to not inform the patient, that the sham treatment is inactive. Considering clinical equipoise, we assess that the pros weight up the cons, hence we decided not to inform the patients regarding sham treatment.

References

1. Farmer AD, Coen SJ, Kano M, Weltens N, Ly HG, Botha C, et al. Normal values and reproducibility of the real-time index of vagal tone in healthy humans: a multi-center study. Ann Gastroenterol Q Publ Hell Soc Gastroenterol. 2014;27(4):362–8.

2. Juel J, Brock C, Olesen SS, Madzak A, Farmer AD, Aziz Q, et al. Acute physiological and electrical accentuation of vagal tone has no effect on pain or gastrointestinal motility in chronic pancreatitis. J Pain Res. 2017;10:1347–55 PubMed .

3. Silberstein SD, Mechtler LL, Kudrow DB, Calhoun AH, McClure C, Saper JR, et al. Non???Invasive Vagus Nerve Stimulation for the ACute Treatment of Cluster Headache: Findings From the Randomized, Double-Blind, Sham-Controlled ACT1 Study. Headache. 2016;56(8):1317 PubMed –32.

4. H. B, S.P. F, J. K, J P. Blinding assessment in clinical trials: A review of statistical methods and a proposal of blinding assessment protocol. Clin Res Regul Aff. 2010;

5. Barbanti P, Grazzi L, Egeo G, Padovan AM, Liebler E, Bussone G. Non-invasive vagus nerve stimulation for acute treatment of high-frequency and chronic migraine: an open-label study. J

Headache Pain [Internet]. 2015;16(1):0–4. Available from: http://dx.doi.org/10.1186/s10194-015-0542-4

6. Badran BW, Dowdle LT, Mithoefer OJ, LaBate NT, Coatsworth J, Brown JC, et al. Neurophysiologic effects of transcutaneous auricular vagus nerve stimulation (taVNS) via electrical stimulation of the tragus: A concurrent taVNS/fMRI study and review. Brain Stimul [Internet]. 2018;11(3):492–500. Available from: https://doi.org/10.1016/j.brs.2017.12.009

7. Patel YA, Butera RJ. Differential fiber-specific block of nerve conduction in mammalian peripheral nerves using kilohertz electrical stimulation. J Neurophysiol. 2015;

8. Chakravarthy K, Chaudhry H, Williams K, Christo PJ. Review of the Uses of Vagal Nerve Stimulation in Chronic Pain Management. Vol. 19, Current Pain and Headache Reports. 2015.

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

10. Tarn J, Legg S, Mitchell S, Simon B, Ng WF. The Effects of Noninvasive Vagus Nerve Stimulation on Fatigue and Immune Responses in Patients With Primary Sjögren's Syndrome. Neuromodulation. 2018;2018.

11. Brock C, Jessen N, Brock B, Jakobsen PE, Hansen TK, Rantanen JM, et al. Cardiac vagal tone, a non-invasive measure of parasympathetic tone, is a clinically relevant tool in Type 1 diabetes mellitus. Diabet Med. 2017;34(10):1428 PubMed –34.

12. Trial C, Olesen SS, Bouwense SAW, Smith OHGW, Goor HVAN. CLINICAL — PANCREAS Pregabalin Reduces Pain in Patients With Chronic Pancreatitis in a. YGAST [Internet]. 2011;141(2):536–43. Available from: http://dx.doi.org/10.1053/j.gastro.2011.04.003

13. Johnson RL, Wilson CG. A review of vagus nerve stimulation as a therapeutic intervention. J Inflamm Res. 2018;11:203–13 PubMed .

14. Morris GL 3rd, Mueller WM. Long-term treatment with vagus nerve stimulation in patients with refractory epilepsy. The Vagus Nerve Stimulation Study Group E01-E05. Neurology. 1999 Nov;53(8):1731–5.

15. Tassorelli C, Grazzi L, Tommaso M De, Pierangeli G. Noninvasive vagus nerve stimulation as acute therapy for migraine The randomized PRESTO study. 2018;0.

16. Vitória A, Araújo L De, Ribeiro V, Barbosa N, Galdino GS, Fregni F, et al. Effects of high-frequency transcranial magnetic stimulation on functional performance in individuals with incomplete spinal cord injury : study protocol for a randomized controlled trial. 2017;1–11.

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

18. Fayers P. M. et al. EORTC QLQ-C30 Scoring Manual The EORTC QLQ-C30 Introduction. EORTC QLQ-C30 Scoring Man EORTC QLQ-C30 Introd [Internet]. 2001;30:1–67. Available from: http://www.eortc.be/qol/files/scmanualqlq-c30.pdf

19. Frangos E, Ellrich J, Komisaruk BR. Non-invasive access to the vagus nerve central projections via electrical stimulation of the external ear: FMRI evidence in humans. Brain Stimul. 2015;8(3):624–36.

20. Yuan H, Silberstein SD. Vagus Nerve and Vagus Nerve Stimulation, a Comprehensive Review: Part II. Headache. 2016;56(2):259–66.

21. Gaul C, Diener H-C, Silver N, Magis D, Reuter U, Andersson A, et al. Non-invasive vagus nerve stimulation for PREVention and Acute treatment of chronic cluster headache (PREVA): A randomised controlled study. Cephalalgia. 2016 May;36(6):534–46.

VERSION 2 – REVIEW

REVIEWER	Harald M. Stauss Burrell College of Osteopathic Medicine Las Cruces, NM USA
REVIEW RETURNED	06-Jun-2019

GENERAL COMMENTS	The authors have adequately addressed all my previous comments. Nice study! Can't wait to see the results.

REVIEWER	Bruno BONAZ Division of Gastroenterology, CHU Grenoble Alpes, 38043 Grenoble Cedex 09, France
REVIEW RETURNED	06-Jun-2019

GENERAL COMMENTS	The authors have adequately responded to my comments.

REVIEWER	Roberto De Icco 1) Headache Science Centre, IRCCS Mondino Foundation, Pavia, Italy 2) Deapartment of Brain and Behavioral Sciences, University of Pavia, Pavia, Italy
REVIEW RETURNED	17-Jun-2019

GENERAL COMMENTS	I have no more comment.