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Vagus Nerve Stimulation as a Novel Treatment for Systemic Lupus Erythematous: Study protocol for a randomized, parallel group, sham-controlled investigator initiated clinical trial, the SLE-VNS Study.

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TITLE PAGE

Title

Vagus Nerve Stimulation as a Novel Treatment for Systemic Lupus Erythematous: Study protocol for a randomized, parallel group, sham-controlled investigator initiated clinical trial, the SLE-VNS Study.

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ABSTRACT

Introduction:

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease. SLE is treated with immunosuppressants with suboptimal efficacy and high risk of serious side effects. SLE patients have increased risk of mortality, organ damage and debilitating treatment-resistant fatigue. Autonomic nervous system dysfunction (AD) is present in approximately half of the patients and may promote autoimmunity by weakening the vagally mediated anti-inflammatory reflex. Recent studies suggest that transcutaneous vagus nerve stimulation (tVNS) has few side effects and beneficial effects on fatigue, pain, disease activity and organ function. This study investigates whether adjuvant tVNS improves measures of fatigue (primary endpoint), AD, clinical disease activity, inflammation, pain, organ function and quality of life.

Hence, this study will contribute to the understanding of AD as a potentially important precursor of fatigue, disease activity, progression and complications in SLE, and how tVNS mechanistically may attenuate this. As adjuvant tVNS use may reduce the need for traditional immunosuppressive therapy, this trial may prompt a shift in the treatment of SLE and potentially other autoimmune disorders.

Methods and analysis:

Eighty-four SLE patients with fatigue and AD will be randomized 1:1 to active or sham-tVNS in this double-blinded parallel-group study. In Period 1 (1 week), participants will receive a 4-min tVNS 4 times daily and report on fatigue daily. After a 2-week pause, Period 2 (8 weeks) will entail tVNS twice daily and participants will report on fatigue, pain and disease activity weekly. Secondary endpoints will be assessed before and after each period and after one week in Period 2.

Ethics and dissemination:

The study is approved by the Danish Medical Research Ethical Committees (case no: 2120231) and results will be published in international per-reviewed journals.

Trial registration number: NCT05315739.

ARTICLE SUMMARY

Strengths and limitations of this study

- This is one of the first studies investigating the effects of transcutaneous vagus nerve stimulation (tVNS) in patients with autoimmune diseases using a randomized, double-blinded, sham-controlled design.
- Fatigue is reported as the most frequent, invalidating and burdensome disease manifestation of systemic lupus erythematosus (SLE), and thus chosen as a primary outcome.
- Compared to previous studies, we will include more and less selected patients, assess effects across the most relevant organ systems, conduct extensive baseline characterization and explore dose-response qualities of tVNS, and thus put tVNS into a clinical context.
- tVNS is performed by the patient at home, which limits verification of correct stimulation intensity, duration and anatomical location, but reflects real-life use.
- A cross-over design is stronger than a parallel study design, but the latter was chosen to ensure optimal blinding.

KEYWORDS
Rheumatology, neurophysiology, immunology

INTRODUCTION

Systemic lupus erythematosus (SLE) is a systemic chronic autoimmune disease with a heterogenous presentation that may lead to numerous organ manifestations, comorbidities and decreased quality of life.[1] The life expectancy of SLE patients in Denmark is reduced with 25 years compared with the background population,[2] and patients with comorbidities including nephritis, neuropsychiatric or cardiovascular diseases have the worst prognosis. For the uncomplicated patient, the 10-year cumulative pure medical costs are roughly 16,000 euro, but increase tenfold with organ damage.[3] Fatigue occurs in more than 80%[4 5] and is reported as the main barrier to maintaining employment in patients with SLE.[6] Fatigue and musculoskeletal pain are reported as the subjectively most burdensome symptom for SLE patients.[7] Consequently, SLE has marked impact on morbidity, mortality, healthcare costs and quality of life.

Immunosuppressants, the cornerstone of current care, can have multiple adverse effects, including diabetes, osteoporosis and opportunistic infections,[8 9] and may have only limited effect on controlling disease activity,[10] fatigue and other constitutional symptoms.[7] Thus, alternative treatments that can attenuate autoimmune inflammation and treatment resistant symptoms with few adverse effects are in demand.

Recent studies suggest that stimulating the autonomic nervous system holds this potential. Autonomic nervous system dysfunction (AD), occurs in a large proportion (54%) of Danish SLE patients and is characterized by impaired, especially, parasympathetic vagally mediated function.[11] AD further relates to a wide range of disease manifestations that are highly prevalent in SLE: Fatigue,[12] impaired quality of life,[11] pain,[13] inflammation,[14] as well as impaired vascular,[15 16] cardiac[17 18] and renal functions.[19] Increasing the parasympathetic vagus nerve activity by

transcutaneous vagus nerve stimulation (tVNS) may reverse such consequences of AD. tVNS has decreased fatigue induced in healthy humans[20] and in patients with inflammatory rheumatic diseases.[21 22] Further, tVNS has improved pain tolerance in healthy humans[23] and reduced pain related to cluster headache and migraine.[24 25] Additionally, vagus nerve stimulation has been shown to decrease inflammation in animals,[26 27] healthy humans[28] and patients with systemic autoimmune diseases,[21 29-32] which may be vagally mediated via the cholinergic anti-inflammatory reflex.[33] Cardiovascular organ dysfunction may be alleviated by tVNS, which can improve microcirculation[34] and reduce aortic stiffening[35] as well as improve cardiac function in rats[36] and human patients.[37] All together this suggest that tVNS may effectively reduce adverse manifestations of SLE.

In contrast to traditional immunosuppressive treatment, tVNS with intended device holds a good safety profile. To the best of our knowledge, no serious adverse events related to this tVNS device have been reported and the most common side effects typically resolve immediately after the stimulation and entail lip or facial drooping (11%), headache (8%), dizziness (3%) and application site discomfort (2.5%).[24 38-41]

Based on the above we aim to conduct a comprehensive clinical trial with the hypothesis, that adjuvant treatment with tVNS in addition to standard care in SLE patients improves patient reported fatigue. Further, we will investigate how tVNS influences other important SLE disease outcomes that reflect the systemic and heterogenic nature of SLE, including AD, disease activity, pain tolerability, as well as renal and cardiovascular functions.

METHODS AND ANALYSES

Study design and overview

The SLE-VNS study is a 1:1 randomized, parallel group, sham-controlled investigator initiated clinical trial. The study is expected to run from May 2022 to ultimo 2024 including data analyses. First participant first visit is expected to take place in May 2022, and last participant last visit in June 2023. The study will be conducted at the Copenhagen Research Center for Autoimmune Connective Tissue Diseases (COPEACT), Rigshospitalet, Copenhagen, Denmark. It is designed with a patient representative (SLE Europe) and in the framework of an ongoing study, investigating tVNS in diabetic patients with diabetic autonomic neuropathy.[42] The study is composed of two work packages (WP; Figure 1).

Work package I:

In WP-I, the participants will self-administer either bilateral active or sham tVNS at the cervical part of the vagus nerve 4 times daily for 7 days. The participants will report on fatigue (primary outcome) daily in a subject diary, and secondary outcomes will be assessed at baseline (Day 0) and Day 7.

Work package II:

After two weeks without intervention, all participants will proceed with their allocation into WP-II. tVNS will be self-administered bilaterally 2 times daily for 8 weeks. In weekly online surveys, participants will report on fatigue, musculoskeletal pain and disease activity, as described below. Secondary outcomes will be assessed at baseline (Day 0, WP-II), Day 7 and Week 8.

One week after cessation of the intervention in WP-II a follow-up safety visit with clinical blood samples and ECG will be conducted. Further, the participants will be asked whether they believe they received active or sham treatment.

Study participants

Eighty-four patients with SLE, diagnosed according to the internationally accepted disease classification criteria,[43] with signs of fatigue and AD (see inclusion criteria, Table 1) will be included.

Recruitment and enrollment:

Potential participants will be identified at the COPEACT and receive oral and written information about the trial from information screens and leaflets or their regular physician. Screening and inclusion of candidates will be performed by a medical doctor. Eligible participants will have signed the informed consent after meeting all the inclusion criteria and none of the exclusion criteria listed in Table 1.

Participants may be discontinued from the study if they are considered non-compliant, withdraw their consent or experience unacceptable adverse events. The discontinued participants will be replaced by new eligible participants in the same treatment arm (active/sham) to ensure sufficient study power.

Table 1 Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Age ≥ 18	Significant cardiovascular disease, including congestive heart failure, known severe coronary artery disease, or recent myocardial infarction (within 5 years) as assessed by a physician
SLE diagnosis* with disease duration of ≥1 year	Blood pressure < 100/60 or > 160/105
Stable disease and medication the past 28 days as defined by: 1) No change of immunosuppressing therapy 2) Receiving maximally 10 mg prednisone daily	Clinically significant bradycardia or tachycardia
Signs of fatigue: FACIT-Fatigue questionnaire-score ≤ 40	History of abnormal baseline ECG, including prolonged QTc-interval, or arrhythmia
Signs of autonomic dysfunction: One or more of the following: 1) AD-score ≥ 1 † 2) Electrochemical resistance < 50µS (hands) or < 70µS (feet) ‡ 3) COMPASS-31 questionnaire-score > 12	Previous surgery on the vagus nerve or abnormal cervical anatomy
Ability to read and understand Danish	Implanted or portable electro-mechanical medical devices, e.g. pacemaker, defibrillator, cochlear implant and infusion pump
Willingness and ability to comply with the scheduled visits, treatment plan, laboratory tests and other trial procedures	Metallic device such as a stent, bone plate or bone screw implanted at or near the neck
Signed and dated informed consent document	Receiving active laser treatment for proliferative retinopathy
	Active cancer or cancer in remission
	History of brain tumor, aneurysm, bleed, head trauma, clinically significant syncope or seizures
	Any clinical abnormalities that, in the opinion of the investigator, may increase the risk associated with trial participation or may interfere with the interpretation of the trial results
	Ongoing lactation, pregnancy, intended pregnancy (for both females and males) during the trial
	Participation in other clinical trials less than three months prior to inclusion, unless such a participation is judged to have no influence on the recordings

Table notes:
Abbreviations: FACIT= Functional Assessment of Chronic Illness Therapy; ECG = electrocardiography; AD = autonomic dysfunction; COMPASS = Composite Autonomic Symptoms Score.
*as per the internationally accepted disease classification criteria.
†Measured by the Vagus™ device (elaborated under *"Outcomes and experimental procedure").*
‡Measured by the SUDOSCAN device (elaborated under the sections *"Outcomes and experimental procedure"*).

Table 1 *Inclusion and exclusion criteria*

Baseline characterization:

Participant characteristics will be recorded at WP-I baseline to assess group similarity and allow for stratified responder analyses. The general characteristics will include age, sex, race, height, weight, education, employment status, medication affecting autonomic function, and former cardiovascular and other diseases. SLE characteristics will include items from the disease classification criteria,[43] disease activity score,[44] damage index[45] and immunosuppressive medication. Finally, biochemical and immunological evaluations will be performed as part of the SLE characterization, including autoantibodies against dsDNA, SSA, SSB, U1RNP, Smith antigen, cardiolipin, beta-2-glycoprotein, lupus anticoagulant and direct agglutinin test unless documented within the previous year.

Intervention

The active tVNS device:

tVNS will be carried out with the handheld, battery-powered gammaCore Sapphire device (electroCore, Inc., NJ, USA) that sends electrical signals through the skin and soft tissue of the neck to activate the vagus nerve. The device is a class IIa medical device and is CE marked (CE 571753) for: (a) acute and/or prophylactic treatment of certain primary headaches (migraine, cluster headache and hemicrania continua) and medication overuse headache; (b) treatment or prevention of symptoms of reactive airway disease; (c) adjunctive therapy to reduce the symptoms of certain anxiety and depression conditions; (d) adjunctive therapy in the prevention of partial onset and generalized seizures associated with epilepsy; and (e) adjunctive therapy to reduce the symptoms of gastric motility disorders and irritable bowel syndrome.

Stimulation with the device is provided through two steel contact electrodes covered with conductive gel (Sigma gel, Parker Laboratories, New Jersey, USA). When activated, the device produces a proprietary low-voltage electrical signal comprising a 5-kHz sine wave burst lasting for

1 millisecond. Bursts are repeated once every 40 ms (25 Hz), generating a 24 V peak voltage and 60 mA peak output current. Upon activation, the electrical current is transmitted for 120 seconds. The intensity of the stimulation is adjusted by the user in the range of 1–40 arbitrary units via the digital user interface.

Sham-device:

Sham tVNS will be administered by a sham device identical to the active device in appearance and application. The sham device can, however, not produce electrical stimulation upon activation but provide a light "vibrational sound" to mimic the active treatment.

Instruction:

The participants will be thoroughly instructed in the use of the device by research personnel, who is not otherwise involved in the study to minimize any risk of unblinding. Accordingly, the participants will be instructed to retain from sharing information about the sensation of the treatment to the study personnel. A Danish user guide and a subject diary will be handed out along with the device. The participants will be instructed to perform daily self-administered stimulations during the two WPs. During the initial instruction session, the participants will be instructed to position the device at the cervical course of the vagus nerve, anteriorly to the sternocleidomastoid muscles and laterally to the carotid arteries. The correct placement will be marked with a permanent marker on the skin and the participants will be encouraged to refresh the markings throughout the trial and take a picture of the location. The participants will receive their first treatment during the instruction session to ensure correct use.

Interventional stimulation:

During WP-I, participants will perform 4 stimulation doses daily (every 6 hours), and during WP-II, only 2 stimulation doses daily (every 12 hours). Each stimulation dose consists of bilateral tVNS: 120 seconds to each vagus nerve. The participants will be instructed to use the highest tolerable stimulation intensity and note the intensity and time of each stimulation in the subject diary.

The tVNS will be applied as an add-on treatment to the participant's standard of care immunosuppressing medication. If clinically indicated, this medication can be changed during the trial, and these changes will be recorded.

Outcomes and experimental procedures

The outcomes and methods of assessment are summarized in Table 2 and described in detail below.

Table 2 Outcomes, methods and timepoints of assessment

Outcomes	Methods of assessment	Timepoint WP-I	Timepoint WP-II
Patient reported outcomes			
Fatigue (primary outcome)	FACIT-Fatigue questionnaire	Daily	Weekly
Autonomic symptoms	COMPASS-31 questionnaire	Baseline, Day 7	Baseline, Day 7, Week 8
SLE disease activity	The SLAQ and PtGA questionnaires	Baseline, Day 7	Weekly
Pain	Subjective pain on visual analog scale	Baseline, Day 7	Weekly
Quality of life	SF-12 questionnaire	Baseline, Day 7	Baseline, Day 7, Week 8
Autonomic nervous system function			
Resting autonomic function	5-min resting HRV and cardiac vagal tone 5-min resting blood pressure and heart rate Stimulation of sweat glands	Baseline, Day 7	Baseline, Day 7, Week 8

Cardiovascular autonomic reflex function	Four cardiovascular reflex tests and response in changes to heart rate and blood pressure	Baseline, Day 7	Baseline, Day 7, Week 8
Continuous autonomic function	Holter HRV monitoring	Continuously during WP-I	Continuously first week of WP-II
SLE disease activity indices			
SLE disease activity	SLEDAI-2K, SRI-50, SLE-DAS, PGA, DAS-28 clinical disease evaluations	Baseline, Day 7	Baseline, Day 7, Week 8
Treatment (tertiary outcome)	Medication history	Retrospective change from inclusion to WP-I until three months after WP-II.	
Organ function			
Pain tolerability	Cold pressor test, conditioned pain modulation	Baseline, Day 7	Baseline, Day 7, Week 8
Cardiac function	Echocardiography	Baseline, Day 7	Baseline, Day 7, Week 8
Vascular function	Capillaroscopy and arterial stiffness	Baseline, Day 7	Baseline, Day 7, Week 8
Biochemical function			
SLE routine status	Routine assessment of hematological, serological, and urinary markers	Baseline, Day 7	Baseline, Day 7, Week 8
SLE inflammatory status	Multiplex plasma cytokines, whole blood expression analyses, flow cytometry, whole blood stimulation assays	Baseline, Day 7	Baseline, Day 7, Week 8
Renal function	eGFR and urine albumin- and protein /creatinine-ratio, spot-urine	Baseline, Day 7	Baseline, Day 7, Week 8
Metabolic control	Plasma lipid and glucose profiles	Baseline, Day 7	Baseline, Day 7, Week 8

Table notes:
WP = Work Package; FACIT = Functional Assessment of Chronic Illness Therapy; COMPASS = Composite Autonomic Symptoms Score; SLE = systemic lupus erythematosus; SLAQ = Systemic Lupus Activity Questionnaire; PtGA = Patient Global Assessment; SF = Short Form; HRV = Heart rate variability, SLEDAI-2K = SLE Disease Activity Index 2000; SRI = SLEDAI Responder Index; SLE-DAS = SLE Disease Activity Score; PGA = Physician Global Assessment; DAS-28 = Disease Activity Score; eGFR = estimated glomerular filtration rate.

Table 2 *Outcomes, methods and timepoints of assessment*

Primary outcome:

The Functional Assessment of Chronic Illness Therapy Fatigue (FACIT-F) scale is a validated 13-item questionnaire that assesses patient-perceived fatigue and its impact upon daily activities and function over the past 7 days.[46] It has been used in numerous clinical SLE trials, has superior internal consistency and higher sensitivity compared to other fatigue measures[47 48] and is, thus, included as the primary outcome measure of fatigue.

Other patient-reported outcomes:

The Composite Autonomic Symptoms Score (COMPASS)-31 questionnaire will be applied to provide a quantitative measure of the participants self-reported AD symptoms.[49] The participants self-reported SLE disease activity will be evaluated with the Systemic Lupus Activity Questionnaire (SLAQ)[50] and the Patient Global Assessment (PtGA).[51] Further, the participants will assess the average musculoskeletal pain on an 11-point visual analog scale.[52] Quality of life will be evaluated with the validated 12-item short form (SF-12) questionnaire, derived from the original SF-36,[53] and a physical and mental component score of patient-reported health-related quality of life will be calculated.

Autonomic nervous system function:

The visit-based tests of autonomic nervous system function will be undertaken in the morning in a quiet room according to recommended protocol[54] where smoking, food and caffeine intake are restricted prior to testing.

Resting autonomic function will be assessed in four ways: 1) a 5-minute resting heart rate variability (HRV) will be measured with the handheld ECG Vagus™-device (Medicus Engineering,

Aarhus, Denmark)[55]; (2) 5-minute resting cardiac vagal tone will be measured with the non-invasive ECG eMotion Faros-device (Mega ElectronicsLtd, Kuopio, Finland)[56]; 3) after the 5-minute rest, blood pressure and heart rate will be measured with standard equipment; and 4) stimulated sweat secretion will be measured as the electrochemical reaction mediated by chloride ions after stimulation of sweat glands in hands and feet with the non-invasive SUDOSCAN-device (Impeto Medical, California, San Diego, USA).[57]

Cardiovascular autonomic reflexes will be assessed in 2 ways: 1) by 3 consecutive heart rate-based cardiovascular reflex tests with the Vagus™-device, in which the ratio of the maximal and minimal beat-to-beat intervals in relation to standing, deep breathing and the Valsalva maneuver are compared with age-dependent cut-off levels to assess the degree of AD: no, early (1 abnormal) and manifest (>1 abnormal test) dysfunction;[58] and 2) by assessment of orthostatic blood pressure changes with the participant standing for 5 minutes after supine rest and blood pressure measurements each minute.

Continuous autonomic function will be assessed with a small patch sensor Holter device (ePatch®, BioTelemetry Technology ApS, Hørsholm, DK) that records a 3-lead ECG for 7 consecutive days.[59] Participants will press a button on the device just prior to the tVNS, leaving a location mark in the data set that allows for HRV analyses in relation to the tVNS. Time and frequency domain HRV parameters will be calculated based on the ePatch- and Vagus™-measurements.[60]

SLE disease activity:

Disease activity will be evaluated by clinical and laboratory examination according to tree different activity scores (SLEDAI-2K: *SLE Disease Activity Index-2000*, SRI-50: *SLEDAI Responder Index-*

50% and SLE-DAS: *SLE-Disease Activity Score*). The SLEDAI-2K[44] is most commonly used for activity assessment, whereas SRI-50 accounts for clinically significant improvements between visits,[61] and SLE-DAS is suggested to have improved sensitivity to change and specificity compared with the SLEDAI-2K.[62] Furthermore, the physician's judgement of overall disease activity will be scored in the *Physician Global Assessment* (PGA)[63] by answering "How do you rate your patient's current disease activity?" with mild=1 to 3=most active disease imaginable. The physician-assessed number of painful and swollen joints according to the Disease Activity Score-28[64] will be evaluated. Finally, based on the medication history, any changes to the patient's regular SLE medication will be noted throughout and until 3 months after the study.

Pain tolerability:

The tolerance to sensory pain stimuli will be assessed with bone and muscle pressure with a handheld pressure algometer (Type 2, Somedic Production AB, Sweden) and a circulating ice-chilled water (2°C) bath. At first, the algometer will apply pressure (30kPA/s) to the tibia and quadriceps muscle. Thereafter, the hand will be immersed into the water for 120 seconds or until the pain becomes intolerable. Pain intensity will be rated regularly by the visual analogue scale during the immersion. Immediately after the immersion, the quadriceps muscle pressure will be reapplied, which allows for quantification of the conditioned pain modulation capacity.[65]

Organ function:

A transthoracic echocardiographic ultrasound examination (LOGIQ S8, GE Electronic) will be performed in order to assess cardiac geometry, ventricular mass, diastolic and systolic function.[66] Arterial stiffness will be assessed with pulse wave velocity measured by ECG traced pulse-wave doppler ultrasound at the carotid and external iliac artery.[67] Further, microvascular morphology will

be assessed by in-vivo nailfold video capillaroscopy with the Dino-Lite digital microscope (Vodskov, Denmark), revealing both the architecture of capillary rows and fine details of each vessel.[68] To characterize renal function, urine and blood samples will be analyzed for eGFR and urinary protein-creatinine ratio.

Biochemical and immunological function:

Routine: SLE biochemical status based on plasma and serum routine analyses will be performed to assess changes relevant to disease activity and other disease properties.

Experimental: To asses immunological function, the following will be measured: a) plasma cytokines reflecting inflammatory activation and inhibition, b) interferon-regulated gene expression (nCounter platform, NanoString Technologies, Seattle, WA), c) immune cell population distribution in whole blood (fluorescence-activated cell sorting), and d) functional immune cell stimulation (TruCulture®). To characterize the effects on metabolic control, plasma lipid and glucose profiles will be performed.

Randomization and blinding

Included participants will be provided with a unique randomization ID number. The collaborative site at Aalborg University Hospital will be responsible for the block-randomization (8 participants) with www.randomization.com. The randomization list will be kept at Aalborg Hospital, and only sealed envelopes containing the treatment allocation for each participant will be kept at a secure location at the COPEACT for individual unblinding in case of medical emergencies. Hence, all personnel involved in the study and participants will be blinded to the randomization. Following the last participant's last visit, a blinded data set divided into treatment "A" and "B" will be prepared for all outcomes to allow for blinded data analyses.

Adverse events

The participants will be instructed to report on adverse events at every visit and to contact the research personnel during WP-I and -II if adverse events arise. All adverse events will be recorded in the case report form (CRF). A physician investigator will assess all adverse events for causality with tVNS. Study personnel must immediately report any serious adverse event or serious adverse device effect to the primary investigator. All device effects will be reported to the manufacturer yearly and any serious adverse events within 7 days. Additionally, all adverse events and -effects will be reported to the Danish medical research ethical authority after the study end. Based on occurrence of serious adverse events, the primary investigator will be able to terminate the study. The participants will be covered by the regular patient insurance during their participation in the trial.

Data collection and data management

Data will be collected by experienced research personnel trained in good clinical practice (GCP) and entered to electronic CRFs using RedCAP Electronic Data Capture Tool pertaining to the given approval by the Danish Data Protection Agency (P-2022-114). Data from physical questionnaires and participant diaries will be entered manually to the electronic CRF by two different researchers to limit errors. Digital source data from e.g. image-based or autonomic outcomes will be saved on a secure drive with the participant identification number and analyzed blinded after trial end. Blood and urine samples will be labelled and stored in a secure research biobank for analyzation after trial end and stored for a maximum of 10 years. All other experimental data will be entered directly into the CRFs. Digitalized data will be backed up and stored for 5 years under the responsibility of the principal investigator, whereas physical CRFs with source material will be kept at a secure location for 5 years.

Data analysis

The primary outcome will be analyzed by intention-to-treat approach, meaning that all randomized participants will be included in their initially assigned study arm regardless of adherence to study protocol. Changes in the primary outcome measure will be compared between the two groups by Student`s t-test. Secondary endpoints will be analyzed by per-protocol approach by general linear modeling of repeated measures and application of relevant post-hoc analyses or Fisher's exact test as appropriate. The potential effect of differences in baseline values and possible unblinding will be investigated by appropriate adjustments in general linear models or stratified analyses.

For all analyses, $P \leq 0.05$ will be considered statistically significant. The applied statistical program will be SPSS statistics (version 25, IBM Corporation).

Sample size calculation

This study is powered to detect a minimal clinically important difference of 5.9 points on the FACIT-Fatigue scale[69] between the active and sham tVNS treated groups after 1 (WP-I) or 8 weeks (WP-II) weeks of stimulation. Based on a mean±SD baseline score of 20±8.0,[70] 29 participants per group are required with the use of the intended significance level to provide a statistical power of 80%. With allowance of a 30% dropout rate, we aim to include 42 participants in each arm.

Monitoring

Internal monitoring will be conducted weekly to ensure that the protocol, national regulations and GCP standards are followed. The monitor will review source documents and medical records to confirm CRF-recorded data and will monitor all signed informed consent documents and adverse events logs. Quality assurance audits by relevant regulatory authorities may be performed.

Patient and public involvement

The study outcomes were discussed and chosen in collaboration with a SLE patient representative. Instead of choosing an objective measure as primary outcome, we chose patient reported fatigue as the primary objective of the study, as it is highly prevalent and burdensome in SLE and an objective measure may not correlate with patient evaluation and satisfaction with the treatment.

After study completion, the participants will be informed on their study allocation (active/sham), and study results will be disseminated to relevant patient associations. No public involvement was included in the design phase of the study.

Ethics and dissemination

The study protocol has been approved by the Danish Medical Research Ethical Committees (case no: 2120231). The study will be performed in accordance with this published protocol and the registration at ClinicalTrials.gov, the principles of GCP (DS/EN ISO 14155:2020), the guidelines of the revised Helsinki declaration and applicable local regulatory requirements and laws.

All publication rights belong to the principal investigator. Positive as well as negative and inconclusive trial results will be published in international peer-reviewed journals. A primary author will be subscribed according to the Vancouver system.

DISCUSSION

This study was designed to provide novel substantial evidence on the effect of tVNS on fatigue in SLE. The design further allows for a detailed and comprehensive description of effects on other disease manifestations relevant to SLE patients.

In other patient populations, tVNS has ameliorated manifestations frequently observed in SLE. Unfortunately, only few of the studies have been systematically controlled, and until recently, the implications of tVNS treatment of SLE patients remained undescribed. Interestingly, a recent randomized, double-blinded, sham-controlled pilot study of 18 SLE patients showed attenuating effects on pain, fatigue and number of swollen joints following four days of 5-min auricular tVNS.[70] However, the study only included few and highly selected participants with high levels of musculoskeletal pain and disease activity and followed the participants for 12 days. Further, the study did not find effects on other markers of inflammation and disease activity. We speculate that power and follow-up length may influence these results. Hence, we aim to complete a comprehensive study that could account for this.

The current study holds the overall strength that it aims to put tVNS into a clinical context. This will be done by a) including participants that represent the majority of SLE patients, as fatigue and AD are common in SLE; b) conducting extensive baseline characterization that will enable identification of markers related to possible tVNS responders; and c) providing extended follow-up and assessment of dose-response qualities of tVNS, which should give insights to dynamic of tVNS effects. All together, these factors could help facilitate clinical implementation if tVNS is found effective. Supplementary to the primary outcome, this study will also investigate the effects of tVNS across the most relevant organ systems implicated in SLE. This will give insights to the prospect of using tVNS as an alternative to the current standard treatment with immunosuppressants. Further, this may enable a better understanding of the diverse clinical picture presented by SLE patients and the pathophysiological mechanisms of fatigue, AD and inflammatory activity, which hitherto is poorly described.

The study does hold some limitations. We will not be able to verify whether each active stimulation is performed correctly, as the treatment will be self-administered at home. Therefore, participants will undergo a thorough introduction and perform the first stimulation under supervision, including emphasis on the correct device position by marking it on their skin, and every stimulation will be logged in diaries. Also, there is a risk of some participants guessing if they receive sham treatment based on missing signs of muscle and skin nerve activation. To quantify the latter, subjects will be asked about this after completion of the study. The chosen sham-method was, however, judged the best possible comparator. To optimize the blinding and overall study quality, the treatment will be tested in a parallel-group design, the tVNS participant instruction will be performed by a person not otherwise engaged in the study in a similar manner regardless of allocated treatment-arm, and participants will be instructed to retain from sharing information about the sensation of the treatment to study personnel. As for randomization method, the time for the effects of tVNS to fade should be considered but has not previously been investigated. In the SLE pilot study, the effects of tVNS on fatigue and pain remained 7 days after the intervention.[70] Therefore, randomizing the order of the WPs could be advantageous. However, as the study is conducted in the framework of another study to allow for comparison with effects of tVNS in diabetic patients, we chose the current study design.

With this study we aim to provide novel clinical evidence about the effects of tVNS on fatigue and other important clinical and paraclinical manifestations of SLE. This study may contribute to the introduction of a safe and effective treatment of SLE as an alternative or supplement to the current standard of care immunosuppression. Such treatments would constitute a paradigmatic shift in the care of patients with SLE and other chronic inflammatory diseases.

DATA STATEMENT

Within the limitations of the national regulations on data sharing and after the publication of trial results, the data generated can be provided in anonymized form upon reasonable request from researchers who provide a methodological sound proposal.

AUTHOR CONTRIBUTIONS

Amanda Hempel Zinglersen: Conceptualization, methodology, validation, formal analysis, investigation, writing – original draft, writing – review & editing, visualization, supervision, project administration, funding acquisition. *Ida Lynghøj Drange*: Investigation, writing – original draft, writing – review & editing, visualization. *Katrine Aagaard Myhr:* Methodology, writing – review & editing, project administration. *Andreas Fuchs:* Methodology, writing – review & editing, supervision, project administration, funding acquisition. *Mogens Pfeiffer-Jensen:* Conceptualization, methodology, validation, resources, writing – review & editing, supervision, funding acquisition. *Christina Brock:* Conceptualization, methodology, validation, resources, writing – review & editing, supervision, project administration, funding acquisition. *Søren Jacobsen:* Conceptualization, methodology, formal analysis, resources, writing – review & editing, visualization, supervision, project administration, funding acquisition.

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interpretation of data, writing of the report or decision to submit the subsequent article(s) for publication.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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FIGURE LEGENDS

Figure 1 Overview of the systemic lupus erythematosus (SLE)-vagus nerve stimulation (VNS)

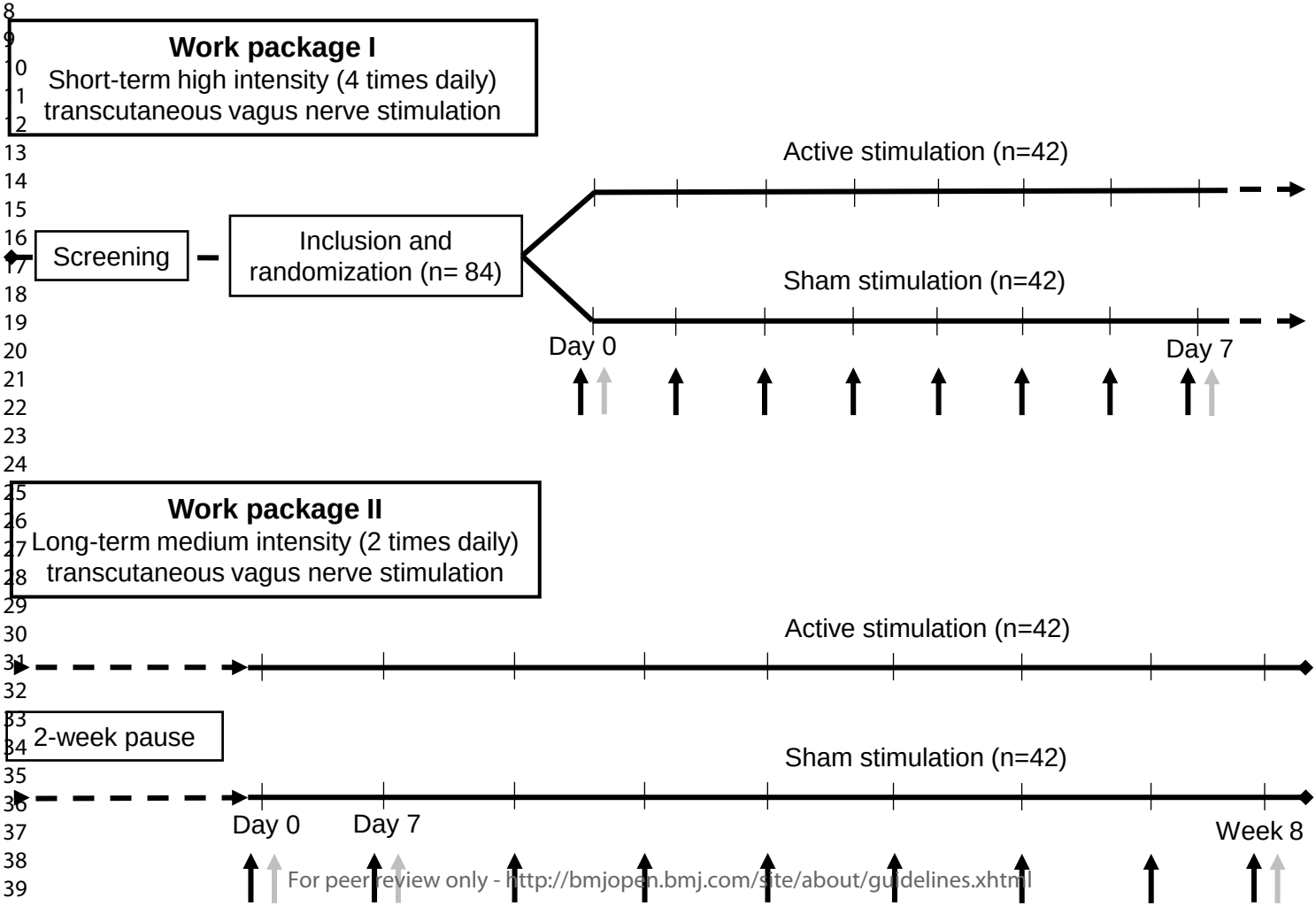
study

PRIMARY AIM

Patient-reported level of fatigue

SECONDARY AIMS

- Patient-reported pain, disease activity and quality of life
- Autonomic nervous system function
- SLE disease activity and inflammation
- Pain tolerability
- Cardiovascular and kidney function



Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1

Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	
Protocol version	#3	Date and version identifier	
Funding	#4	Sources and types of financial, material, and other support	22
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	22
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	NA
Roles and responsibilities: sponsor and funder	#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	NA
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and	NA

		other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	4, 13-16
Objectives	#7	Specific objectives or hypotheses	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, non-inferiority, exploratory)	6
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6

Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7-8
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9-11
Interventions: modifications	#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
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	10
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	11
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	11-16

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 1
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	18
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	7
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	16
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque,	16

		sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	16
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	16
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	16
Methods: Data collection, management, and analysis			
Data collection plan	#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	17

Data collection plan: retention	#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	17
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	17
Statistics: outcomes	#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	18
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	18
Statistics: analysis population and missing data	#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)	18
Methods: Monitoring			
Data monitoring: formal committee	#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	18

		details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	
Data monitoring: interim analysis	#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	NA
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	17
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	18
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	19
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)	NA

Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	7
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
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	17
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	23
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	22
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	17
Dissemination policy: trial results	#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	19

Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	19
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	22
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	NA
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	NA

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Vagus Nerve Stimulation as a Novel Treatment for Systemic Lupus Erythematous: Study protocol for a randomized, parallel group, sham-controlled investigator initiated clinical trial, the SLE-VNS Study.

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TITLE PAGE

Title

Vagus Nerve Stimulation as a Novel Treatment for Systemic Lupus Erythematous: Study protocol for a randomized, parallel group, sham-controlled investigator initiated clinical trial, the SLE-VNS Study.

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ABSTRACT

Introduction:

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease. SLE is treated with immunosuppressants with suboptimal efficacy and high risk of serious side effects. SLE patients have increased risk of mortality, organ damage and debilitating treatment-resistant fatigue. Autonomic nervous system dysfunction (AD) is present in approximately half of the patients and may promote autoimmunity by weakening the vagally mediated anti-inflammatory reflex. Recent studies suggest that transcutaneous vagus nerve stimulation (tVNS) has few side effects and beneficial effects on fatigue, pain, disease activity and organ function. This study investigates whether adjuvant tVNS improves measures of fatigue (primary endpoint), AD, clinical disease activity, inflammation, pain, organ function and quality of life.

Hence, this study will contribute to the understanding of AD as a potentially important precursor of fatigue, disease activity, progression and complications in SLE, and how tVNS mechanistically may attenuate this. As adjuvant tVNS use may reduce the need for traditional immunosuppressive therapy, this trial may prompt a shift in the treatment of SLE and potentially other autoimmune disorders.

Methods and analysis:

Eighty-four SLE patients with fatigue and AD will be randomized 1:1 to active or sham-tVNS in this double-blinded parallel-group study. In Period 1 (1 week), participants will receive a 4-min tVNS 4 times daily and report on fatigue daily. After a 2-week pause, Period 2 (8 weeks) will entail tVNS twice daily and participants will report on fatigue, pain and disease activity weekly. Secondary endpoints will be assessed before and after each period and after one week in Period 2.

Ethics and dissemination:

The study is approved by the Danish Medical Research Ethical Committees (case no: 2120231) and results will be published in international per-reviewed journals.

Trial registration number: NCT05315739.

ARTICLE SUMMARY

Strengths and limitations of this study

- This is one of the first studies investigating the effects of transcutaneous vagus nerve stimulation (tVNS) in patients with autoimmune diseases using a randomized, double-blinded, sham-controlled design.
- Fatigue is reported as the most frequent, invalidating and burdensome disease manifestation of systemic lupus erythematosus (SLE), and thus chosen as a primary outcome.
- Compared to previous studies, we will include more and less selected patients, assess effects across the most relevant organ systems, conduct extensive baseline characterization and explore dose-response qualities of tVNS, and thus put tVNS into a clinical context.
- tVNS is performed by the patient at home, which limits verification of correct stimulation intensity, duration and anatomical location, but reflects real-life use.
- A cross-over design is stronger than a parallel study design, but the latter was chosen to ensure optimal blinding.

KEYWORDS
Rheumatology, neurophysiology, immunology

INTRODUCTION

Systemic lupus erythematosus (SLE) is a systemic chronic autoimmune disease with a heterogenous presentation that may lead to numerous organ manifestations, comorbidities and decreased quality of life.[1] The life expectancy of SLE patients in Denmark is reduced with 25 years compared with the background population,[2] and patients with comorbidities including nephritis, neuropsychiatric or cardiovascular diseases have the worst prognosis. For the uncomplicated patient, the 10-year cumulative pure medical costs are roughly 16,000 euro, but increase tenfold with organ damage.[3] Fatigue occurs in more than 80%[4 5] and is reported as the main barrier to maintaining employment in patients with SLE.[6] Fatigue and musculoskeletal pain are reported as the subjectively most burdensome symptom for SLE patients.[7] Consequently, SLE has marked impact on morbidity, mortality, healthcare costs and quality of life.

Immunosuppressants, the cornerstone of current care, can have multiple adverse effects, including diabetes, osteoporosis and opportunistic infections,[8 9] and may have only limited effect on controlling disease activity,[10] fatigue and other constitutional symptoms.[7] Thus, alternative treatments that can attenuate autoimmune inflammation and treatment resistant symptoms with few adverse effects are in demand.

Recent studies suggest that stimulating the autonomic nervous system holds this potential. Autonomic nervous system dysfunction (AD), occurs in a large proportion (54%) of Danish SLE patients and is characterized by impaired, especially, parasympathetic vagally mediated function.[11] AD further relates to a wide range of disease manifestations that are highly prevalent in SLE: Fatigue,[12] impaired quality of life,[11] pain,[13] inflammation,[14] as well as impaired vascular,[15 16] cardiac[17 18] and renal functions.[19] Increasing the parasympathetic vagus nerve activity by

transcutaneous vagus nerve stimulation (tVNS) may reverse such consequences of AD. tVNS has decreased fatigue induced in healthy humans[20] and in patients with inflammatory rheumatic diseases.[21 22] Further, tVNS has improved pain tolerance in healthy humans[23] and reduced pain related to cluster headache and migraine.[24 25] Additionally, vagus nerve stimulation has been shown to decrease inflammation in animals,[26 27] healthy humans[28] and patients with systemic autoimmune diseases,[21 29-32] which may be vagally mediated via the cholinergic anti-inflammatory reflex.[33] Cardiovascular organ dysfunction may be alleviated by tVNS, which can improve microcirculation[34] and reduce aortic stiffening[35] as well as improve cardiac function in rats[36] and human patients.[37] All together this suggest that tVNS may effectively reduce adverse manifestations of SLE.

In contrast to traditional immunosuppressive treatment, tVNS with intended device holds a good safety profile. To the best of our knowledge, no serious adverse events related to this tVNS device have been reported and the most common side effects typically resolve immediately after the stimulation and entail lip or facial drooping (11%), headache (8%), dizziness (3%) and application site discomfort (2.5%).[24 38-41]

Based on the above we aim to conduct a comprehensive clinical trial with the hypothesis, that adjuvant treatment with tVNS in addition to standard care in SLE patients improves patient reported fatigue (primary outcome). Further, we will investigate how tVNS influences other important SLE disease outcomes that reflect the systemic and heterogenic nature of SLE, including AD, disease activity, pain tolerability, as well as renal and cardiovascular functions (secondary outcomes).

METHODS AND ANALYSES

Study design and overview

The SLE-VNS study is a 1:1 randomized, parallel group, sham-controlled investigator initiated clinical trial. The study is expected to run from May 2022 to ultimo 2024 including data analyses. First participant first visit is expected to take place in May 2022, and last participant last visit in June 2023. The study will be conducted at the Copenhagen Research Center for Autoimmune Connective Tissue Diseases (COPEACT), Rigshospitalet, Copenhagen, Denmark. It is designed with a patient representative (SLE Europe) and in the framework of an ongoing study, investigating tVNS in diabetic patients with diabetic autonomic neuropathy.[42] The study is composed of two work packages (WP; Figure 1).

Work package I:

In WP-I, the participants will self-administer either bilateral active or sham tVNS at the cervical part of the vagus nerve 4 times daily for 7 days. The participants will report on fatigue daily in a subject diary, and all secondary outcomes will be assessed at baseline (Day 0) and Day 7 (Figure 1; Table 1).

Work package II:

After two weeks without intervention, all participants will proceed with their allocation into WP-II. tVNS will be self-administered bilaterally 2 times daily for 8 weeks. In weekly online surveys, participants will report on fatigue, musculoskeletal pain and disease activity, as described below. Other secondary outcomes will be assessed at baseline (Day 0, WP-II), Day 7 and Week 8 (Figure 1; Table 1). After all assessments at the final week 8-visit, the participants will be asked whether they believe they received active or sham treatment.

A safety visit is conducted one week after cessation of the intervention in WP-II including blood samples and ECG not related to the outcomes of the study.

Table 1 Primary and secondary outcomes, methods and timepoints of assessment

Outcomes	Methods of assessment	Timepoint WP-I	Timepoint WP-II
Patient reported outcomes			
Fatigue (primary outcome)	FACIT-Fatigue questionnaire	Daily	Weekly
Autonomic symptoms	COMPASS-31 questionnaire	Baseline, Day 7	Baseline, Day 7, Week 8
SLE disease activity	The SLAQ and PtGA questionnaires	Baseline, Day 7	Weekly
Pain	Subjective pain on visual analog scale	Baseline, Day 7	Weekly
Quality of life	SF-12 questionnaire	Baseline, Day 7	Baseline, Day 7, Week 8
Autonomic nervous system function			
Resting autonomic function	5-min resting HRV and cardiac vagal tone 5-min resting blood pressure and heart rate Stimulation of sweat glands	Baseline, Day 7	Baseline, Day 7, Week 8
Cardiovascular autonomic reflex function	Four cardiovascular reflex tests and response in changes to heart rate and blood pressure	Baseline, Day 7	Baseline, Day 7, Week 8
Continuous autonomic function	Holter HRV monitoring	Continuously during WP-I	Continuously first week of WP-II
SLE disease activity indices			
SLE disease activity	SLEDAI-2K, SRI-50, SLE-DAS, PGA, DAS-28 clinical disease evaluations	Baseline, Day 7	Baseline, Day 7, Week 8
Treatment	Medication history	Retrospective change from inclusion to WP-I until three months after WP-II.	
Organ function			
Pain tolerability	Cold pressor test, conditioned pain modulation	Baseline, Day 7	Baseline, Day 7, Week 8
Cardiac function	Echocardiography	Baseline, Day 7	Baseline, Day 7, Week 8
Vascular function	Capillaroscopy and arterial stiffness	Baseline, Day 7	Baseline, Day 7, Week 8
Biochemical function			

SLE routine status	Routine assessment of hematological, serological, and urinary markers	Baseline, Day 7	Baseline, Day 7, Week 8
SLE inflammatory status	Multiplex plasma cytokines, whole blood expression analyses, flow cytometry, whole blood stimulation assays	Baseline, Day 7	Baseline, Day 7, Week 8
Renal function	eGFR and urine albumin- and protein /creatinine-ratio, spot-urine	Baseline, Day 7	Baseline, Day 7, Week 8
Metabolic control	Plasma lipid and glucose profiles	Baseline, Day 7	Baseline, Day 7, Week 8

Table notes:
WP = Work Package; FACIT = Functional Assessment of Chronic Illness Therapy; COMPASS = Composite Autonomic Symptoms Score; SLE = systemic lupus erythematosus; SLAQ = Systemic Lupus Activity Questionnaire; PtGA = Patient Global Assessment; SF = Short Form; HRV = Heart rate variability, SLEDAI-2K = SLE Disease Activity Index 2000; SRI = SLEDAI Responder Index; SLE-DAS = SLE Disease Activity Score; PGA = Physician Global Assessment; DAS-28 = Disease Activity Score; eGFR = estimated glomerular filtration rate.

Table 1 *Outcomes, methods and timepoints of assessment*

Study participants

Eighty-four patients with SLE, diagnosed according to the internationally accepted disease classification criteria,[43] with signs of fatigue and AD (see inclusion criteria, Table 2) will be included.

Recruitment and enrollment:

Potential participants will be identified at the COPEACT and receive oral and written information about the trial from information screens and leaflets or their regular physician. Screening and inclusion of candidates will be performed by a medical doctor. Eligible participants will have signed the informed consent after meeting all the inclusion criteria and none of the exclusion criteria listed in Table 2.

Participants may be discontinued from the study if they are considered non-compliant, withdraw their consent or experience unacceptable adverse events. The discontinued participants will be

replaced by new eligible participants in the same treatment arm (active/sham) to ensure sufficient study power.

Table 2 Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Age ≥ 18	Significant cardiovascular disease, including congestive heart failure, known severe coronary artery disease, or recent myocardial infarction (within 5 years) as assessed by a physician
SLE diagnosis* with disease duration of ≥1 year	Blood pressure < 100/60 or > 160/105
Stable disease and medication the past 28 days as defined by: 1) No change of immunosuppressing therapy 2) Receiving maximally 10 mg prednisone daily	Clinically significant bradycardia or tachycardia
Signs of fatigue: FACIT-Fatigue questionnaire-score ≤ 40	History of abnormal baseline ECG, including prolonged QTc-interval, or arrhythmia
Signs of autonomic dysfunction: One or more of the following: 1) AD-score ≥ 1 † 2) Electrochemical resistance < 50µS (hands) or < 70µS (feet) ‡ 3) COMPASS-31 questionnaire-score > 12	Previous surgery on the vagus nerve or abnormal cervical anatomy
Ability to read and understand Danish	Implanted or portable electro-mechanical medical devices, e.g. pacemaker, defibrillator, cochlear implant and infusion pump
Willingness and ability to comply with the scheduled visits, treatment plan, laboratory tests and other trial procedures	Metallic device such as a stent, bone plate or bone screw implanted at or near the neck
Signed and dated informed consent document	Receiving active laser treatment for proliferative retinopathy
	Active cancer or cancer in remission
	History of brain tumor, aneurysm, bleed, head trauma, clinically significant syncope or seizures
	Any clinical abnormalities that, in the opinion of the investigator, may increase the risk associated with trial participation or may interfere with the interpretation of the trial results
	Ongoing lactation, pregnancy, intended pregnancy (for both females and males) during the trial
	Participation in other clinical trials less than three months prior to inclusion, unless such a participation is judged to have no influence on the recordings

Table notes:
Abbreviations: FACIT= Functional Assessment of Chronic Illness Therapy; ECG = electrocardiography; AD = autonomic dysfunction; COMPASS = Composite Autonomic Symptoms Score.
*as per the internationally accepted disease classification criteria.
†Measured by the Vagus™ device (elaborated under *"Outcomes and experimental procedure").*
‡Measured by the SUDOSCAN device (elaborated under the sections *"Outcomes and experimental procedure"*).

Table 2 *Inclusion and exclusion criteria*

Baseline characterization:

Participant characteristics will be recorded at WP-I baseline to assess group similarity and allow for stratified responder analyses. The general characteristics will include age, sex, race, height, weight, education, employment status, medication affecting autonomic function, and former cardiovascular and other diseases. SLE characteristics will include items from the disease classification criteria,[43] disease activity score,[44] damage index[45] and immunosuppressive medication such as antimalarials, corticosteroids and synthetic and biological disease modifying antirheumatic drugs (DMARDs). Finally, biochemical and immunological evaluations will be performed as part of the SLE characterization, including autoantibodies against dsDNA, SSA, SSB, U1RNP, Smith antigen, cardiolipin, beta-2-glycoprotein, lupus anticoagulant and direct agglutinin test unless documented within the previous year.

Intervention

The active tVNS device:

tVNS will be carried out with the handheld, battery-powered gammaCore Sapphire device (electroCore, Inc., NJ, USA) that sends electrical signals through the skin and soft tissue of the neck to activate the vagus nerve. The device is a class IIa medical device and is CE marked (CE 571753) for: (a) acute and/or prophylactic treatment of certain primary headaches (migraine, cluster headache and hemicrania continua) and medication overuse headache; (b) treatment or prevention of symptoms of reactive airway disease; (c) adjunctive therapy to reduce the symptoms of certain anxiety and depression conditions; (d) adjunctive therapy in the prevention of partial onset and generalized seizures associated with epilepsy; and (e) adjunctive therapy to reduce the symptoms of gastric motility disorders and irritable bowel syndrome.

Stimulation with the device is provided through two steel contact electrodes covered with conductive gel (Sigma gel, Parker Laboratories, New Jersey, USA). When activated, the device produces a proprietary low-voltage electrical signal comprising a 5-kHz sine wave burst lasting for 1 millisecond. Bursts are repeated once every 40 ms (25 Hz), generating a 24 V peak voltage and 60 mA peak output current. Upon activation, the electrical current is transmitted for 120 seconds. The intensity of the stimulation is adjusted by the user in the range of 1–40 arbitrary units via the digital user interface.

Sham-device:

Sham tVNS will be administered by a sham device identical to the active device in appearance and application. The sham device can, however, not produce electrical stimulation upon activation but provide a light "vibrational sound" to mimic the active treatment.

Instruction:

The participants will be thoroughly instructed in the use of the device by research personnel, who is not otherwise involved in the study to minimize any risk of unblinding. Accordingly, the participants will be instructed to retain from sharing information about the sensation of the treatment to the study personnel. A Danish user guide and a subject diary will be handed out along with the device. The participants will be instructed to perform daily self-administered stimulations during the two WPs. During the initial instruction session, the participants will be instructed to position the device at the cervical course of the vagus nerve, anteriorly to the sternocleidomastoid muscles and laterally to the carotid arteries. The correct placement will be marked with a permanent marker on the skin and the participants will be encouraged to refresh the markings throughout the

trial and take a picture of the location. The participants will receive their first treatment during the instruction session to ensure correct use.

Interventional stimulation:

During WP-I, participants will perform 4 stimulation doses daily (every 6 hours), and during WP-II, only 2 stimulation doses daily (every 12 hours). Each stimulation dose consists of bilateral tVNS: 120 seconds to each vagus nerve. The participants will be instructed to use the highest tolerable stimulation intensity and note the intensity and time of each stimulation in the subject diary.

The tVNS will be applied as an add-on treatment to the participant's standard of care immunosuppressing medication. If clinically indicated, this medication can be changed during the trial, and these changes will be recorded.

Outcomes and experimental procedures

The outcomes and methods of assessment are summarized in Table 1 and described in detail below.

Primary outcome:

The Functional Assessment of Chronic Illness Therapy Fatigue (FACIT-F) scale is a validated 13-item questionnaire that assesses patient-perceived fatigue and its impact upon daily activities and function over the past 7 days.[46] It has been used in numerous clinical SLE trials, has superior internal consistency and higher sensitivity compared to other fatigue measures[47 48] and is, thus, included as the primary outcome measure of fatigue.

Other patient-reported outcomes:

The Composite Autonomic Symptoms Score (COMPASS)-31 questionnaire will be applied to provide a quantitative measure of the participants self-reported AD symptoms.[49] The participants self-reported SLE disease activity will be evaluated with the Systemic Lupus Activity Questionnaire (SLAQ)[50] and the Patient Global Assessment (PtGA).[51] Further, the participants will assess the average musculoskeletal pain on an 11-point visual analog scale.[52] Quality of life will be evaluated with the validated 12-item short form (SF-12) questionnaire, derived from the original SF-36,[53] and a physical and mental component score of patient-reported health-related quality of life will be calculated.

Autonomic nervous system function:

The visit-based tests of autonomic nervous system function will be undertaken in the morning in a quiet room according to recommended protocol[54] where smoking, food and caffeine intake are restricted prior to testing.

Resting autonomic function will be assessed in four ways: 1) a 5-minute resting heart rate variability (HRV) will be measured with the handheld ECG Vagus™-device (Medicus Engineering, Aarhus, Denmark)[55]; (2) 5-minute resting cardiac vagal tone will be measured with the non-invasive ECG eMotion Faros-device (Mega ElectronicsLtd, Kuopio, Finland)[56]; 3) after the 5-minute rest, blood pressure and heart rate will be measured with standard equipment; and 4) stimulated sweat secretion will be measured as the electrochemical reaction mediated by chloride ions after stimulation of sweat glands in hands and feet with the non-invasive SUDOSCAN-device (Impeto Medical, California, San Diego, USA).[57]

Cardiovascular autonomic reflexes will be assessed in 2 ways: 1) by 3 consecutive heart rate-based cardiovascular reflex tests with the Vagus™-device, in which the ratio of the maximal and minimal beat-to-beat intervals in relation to standing, deep breathing and the Valsalva maneuver are compared with age-dependent cut-off levels to assess the degree of AD: no, early (1 abnormal) and manifest (>1 abnormal test) dysfunction;[58] and 2) by assessment of orthostatic blood pressure changes with the participant standing for 5 minutes after supine rest and blood pressure measurements each minute.

Continuous autonomic function will be assessed with a small patch sensor Holter device (ePatch®, BioTelemetry Technology ApS, Hørsholm, DK) that records a 3-lead ECG for 7 consecutive days.[59] Participants will press a button on the device just prior to the tVNS, leaving a location mark in the data set that allows for HRV analyses in relation to the tVNS. Time and frequency domain HRV parameters will be calculated based on the ePatch- and Vagus™-measurements.[60]

SLE disease activity:

Disease activity will be evaluated by clinical and laboratory examination according to tree different activity scores (SLEDAI-2K: *SLE Disease Activity Index-2000*, SRI-50: *SLEDAI Responder Index-50%* and SLE-DAS: *SLE-Disease Activity Score*). The SLEDAI-2K[44] is most commonly used for activity assessment, whereas SRI-50 accounts for clinically significant improvements between visits,[61] and SLE-DAS is suggested to have improved sensitivity to change and specificity compared with the SLEDAI-2K.[62] Furthermore, the physician's judgement of overall disease activity will be scored in the *Physician Global Assessment* (PGA)[63] by answering "How do you rate your patient's current disease activity?" with mild=1 to 3=most active disease imaginable. The physician-assessed number of painful and swollen joints according to the Disease Activity Score-

28[64] will be evaluated. Finally, based on the medication history, any changes to the patient's regular SLE medication will be noted throughout and until 3 months after the study. Anti-inflammatory medication will be grouped into the following groups: Antimalarials, glucocorticoids, synthetic- and biologic DMARDs. Changes will be analyzed based on introduction, termination and dosage of drugs during the course of the study.

Pain tolerability:

The tolerance to sensory pain stimuli will be assessed with bone and muscle pressure with a handheld pressure algometer (Type 2, Somedic Production AB, Sweden) and a circulating ice-chilled water (2°C) bath. At first, the algometer will apply pressure (30kPA/s) to the tibia and quadriceps muscle. Thereafter, the hand will be immersed into the water for 120 seconds or until the pain becomes intolerable. Pain intensity will be rated regularly by the visual analogue scale during the immersion. Immediately after the immersion, the quadriceps muscle pressure will be reapplied, which allows for quantification of the conditioned pain modulation capacity.[65]

Organ function:

A transthoracic echocardiographic ultrasound examination (LOGIQ S8, GE Electronic) will be performed in order to assess cardiac geometry, ventricular mass, diastolic and systolic function.[66] Arterial stiffness will be assessed with pulse wave velocity measured by ECG traced pulse-wave doppler ultrasound at the carotid and external iliac artery.[67] Further, microvascular morphology will be assessed by in-vivo nailfold video capillaroscopy with the Dino-Lite digital microscope (Vodskov, Denmark), revealing both the architecture of capillary rows and fine details of each vessel.[68] To characterize renal function, urine and blood samples will be analyzed for eGFR and urinary protein-creatinine ratio.

Biochemical and immunological function:

Routine: SLE biochemical status based on plasma and serum routine analyses will be performed to assess changes relevant to disease activity and other disease properties.

Experimental: To asses immunological function, the following will be measured: a) plasma cytokines reflecting inflammatory activation and inhibition, b) interferon-regulated gene expression (nCounter platform, NanoString Technologies, Seattle, WA), c) immune cell population distribution in whole blood (fluorescence-activated cell sorting), and d) functional immune cell stimulation (TruCulture®). To characterize the effects on metabolic control, plasma lipid and glucose profiles will be performed.

Randomization and blinding

Included participants will be provided with a unique randomization ID number. The collaborative site at Aalborg University Hospital will be responsible for the block-randomization (8 participants) with www.randomization.com. The randomization list will be kept at Aalborg Hospital, and only sealed envelopes containing the treatment allocation for each participant will be kept at a secure location at the COPEACT for individual unblinding in case of medical emergencies. Hence, all personnel involved in the study and participants will be blinded to the randomization. Following the last participant's last visit, a blinded data set divided into treatment "A" and "B" will be prepared for all outcomes to allow for blinded data analyses.

Adverse events

The participants will be instructed to report on adverse events at every visit and to contact the research personnel during WP-I and -II if adverse events arise. All adverse events will be recorded in the case report form (CRF). A physician investigator will assess all adverse events for causality with tVNS. Study personnel must immediately report any serious adverse event or serious adverse

device effect to the primary investigator. All device effects will be reported to the manufacturer yearly and any serious adverse events within 7 days. Additionally, all adverse events and -effects will be reported to the Danish medical research ethical authority after the study end. Based on occurrence of serious adverse events, the primary investigator will be able to terminate the study. The participants will be covered by the regular patient insurance during their participation in the trial.

Data collection and data management

Data will be collected by experienced research personnel trained in good clinical practice (GCP) and entered to electronic CRFs using RedCAP Electronic Data Capture Tool pertaining to the given approval by the Danish Data Protection Agency (P-2022-114). Data from physical questionnaires and participant diaries will be entered manually to the electronic CRF by two different researchers to limit errors. Digital source data from e.g. image-based or autonomic outcomes will be saved on a secure drive with the participant identification number and analyzed blinded after trial end. Blood and urine samples will be labelled and stored in a secure research biobank for analyzation after trial end and stored for a maximum of 10 years. All other experimental data will be entered directly into the CRFs. Digitalized data will be backed up and stored for 5 years under the responsibility of the principal investigator, whereas physical CRFs with source material will be kept at a secure location for 5 years.

Data analysis

The primary outcome will be analyzed by intention-to-treat approach, meaning that all randomized participants will be included in their initially assigned study arm regardless of adherence to study protocol. Changes in the primary outcome measure will be compared between the two groups by

Student`s t-test. Secondary endpoints will be analyzed by per-protocol approach by general linear modeling of repeated measures and application of relevant post-hoc analyses or Fisher's exact test as appropriate. The potential effect of differences in baseline values and possible unblinding will be investigated by appropriate adjustments in general linear models or stratified analyses.

For all analyses, $P \leq 0.05$ will be considered statistically significant. The applied statistical program will be SPSS statistics (version 25, IBM Corporation).

Sample size calculation

This study is powered to detect a minimal clinically important difference of 5.9 points on the FACIT-Fatigue scale[69] between the active and sham tVNS treated groups after 1 (WP-I) or 8 weeks (WP-II) weeks of stimulation. Based on a mean±SD baseline score of 20±8.0,[70] 29 participants per group are required with the use of the intended significance level to provide a statistical power of 80%. With allowance of a 30% dropout rate, we aim to include 42 participants in each arm.

Monitoring

Internal monitoring will be conducted weekly to ensure that the protocol, national regulations and GCP standards are followed. The monitor will review source documents and medical records to confirm CRF-recorded data and will monitor all signed informed consent documents and adverse events logs. Quality assurance audits by relevant regulatory authorities may be performed.

Patient and public involvement

The study outcomes were discussed and chosen in collaboration with a SLE patient representative. Instead of choosing an objective measure as primary outcome, we chose patient reported fatigue as

the primary objective of the study, as it is highly prevalent and burdensome in SLE and an objective measure may not correlate with patient evaluation and satisfaction with the treatment.

After study completion, the participants will be informed on their study allocation (active/sham), and study results will be disseminated to relevant patient associations. No public involvement was included in the design phase of the study.

Ethics and dissemination

The study protocol has been approved by the Danish Medical Research Ethical Committees (case no: 2120231). The study will be performed in accordance with this published protocol and the registration at ClinicalTrials.gov, the principles of GCP (DS/EN ISO 14155:2020), the guidelines of the revised Helsinki declaration and applicable local regulatory requirements and laws.

All publication rights belong to the principal investigator. Positive as well as negative and inconclusive trial results will be published in international peer-reviewed journals. A primary author will be subscribed according to the Vancouver system.

DISCUSSION

This study was designed to provide novel substantial evidence on the effect of tVNS on fatigue in SLE. The design further allows for a detailed and comprehensive description of effects on other disease manifestations relevant to SLE patients.

In other patient populations, tVNS has ameliorated manifestations frequently observed in SLE. Unfortunately, only few of the studies have been systematically controlled, and until recently, the implications of tVNS treatment of SLE patients remained undescribed. Interestingly, a recent randomized, double-blinded, sham-controlled pilot study of 18 SLE patients showed attenuating

effects on pain, fatigue and number of swollen joints following four days of 5-min auricular tVNS.[70] However, the study only included few and highly selected participants with high levels of musculoskeletal pain and disease activity and followed the participants for 12 days. Further, the study did not find effects on other markers of inflammation and disease activity. We speculate that power and follow-up length may influence these results. Hence, we aim to complete a comprehensive study that could account for this.

The current study holds the overall strength that it aims to put tVNS into a clinical context. This will be done by a) including participants that represent the majority of SLE patients, as fatigue and AD are common in SLE; b) conducting extensive baseline characterization that will enable identification of markers related to possible tVNS responders; and c) providing extended follow-up and assessment of dose-response qualities of tVNS, which should give insights to dynamic of tVNS effects. All together, these factors could help facilitate clinical implementation if tVNS is found effective. Supplementary to the primary outcome, this study will also investigate the effects of tVNS across the most relevant organ systems implicated in SLE. This will give insights to the prospect of using tVNS as an alternative to the current standard treatment with immunosuppressants. Further, this may enable a better understanding of the diverse clinical picture presented by SLE patients and the pathophysiological mechanisms of fatigue, AD and inflammatory activity, which hitherto is poorly described.

The study does hold some limitations. We will not be able to verify whether each active stimulation is performed correctly, as the treatment will be self-administered at home. Therefore, participants will undergo a thorough introduction and perform the first stimulation under supervision, including emphasis on the correct device position by marking it on their skin, and every stimulation will be logged in diaries. Also, there is a risk of some participants guessing if they receive sham treatment

based on missing signs of muscle and skin nerve activation. To quantify the latter, subjects will be asked about this after completion of the study. The chosen sham-method was, however, judged the best possible comparator. To optimize the blinding and overall study quality, the treatment will be tested in a parallel-group design, the tVNS participant instruction will be performed by a person not otherwise engaged in the study in a similar manner regardless of allocated treatment-arm, and participants will be instructed to retain from sharing information about the sensation of the treatment to study personnel. As for randomization method, the time for the effects of tVNS to fade should be considered but has not previously been investigated. In the SLE pilot study, the effects of tVNS on fatigue and pain remained 7 days after the intervention.[70] Therefore, randomizing the order of the WPs could be advantageous. However, as the study is conducted in the framework of another study to allow for comparison with effects of tVNS in diabetic patients, we chose the current study design.

With this study we aim to provide novel clinical evidence about the effects of tVNS on fatigue and other important clinical and paraclinical manifestations of SLE. This study may contribute to the introduction of a safe and effective treatment of SLE as an alternative or supplement to the current standard of care immunosuppression. Such treatments would constitute a paradigmatic shift in the care of patients with SLE and other chronic inflammatory diseases.

DATA STATEMENT

Within the limitations of the national regulations on data sharing and after the publication of trial results, the data generated can be provided in anonymized form upon reasonable request from researchers who provide a methodological sound proposal.

AUTHOR CONTRIBUTIONS

Amanda Hempel Zinglersen: Conceptualization, methodology, validation, formal analysis, investigation, writing – original draft, writing – review & editing, visualization, supervision, project administration, funding acquisition. *Ida Lynghøj Drange*: Investigation, writing – original draft, writing – review & editing, visualization. *Katrine Aagaard Myhr:* Methodology, writing – review & editing, project administration. *Andreas Fuchs:* Methodology, writing – review & editing, supervision, project administration, funding acquisition. *Mogens Pfeiffer-Jensen:* Conceptualization, methodology, validation, resources, writing – review & editing, supervision, funding acquisition. *Christina Brock:* Conceptualization, methodology, validation, resources, writing – review & editing, supervision, project administration, funding acquisition. *Søren Jacobsen:* Conceptualization, methodology, formal analysis, resources, writing – review & editing, visualization, supervision, project administration, funding acquisition.

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DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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FIGURE LEGENDS

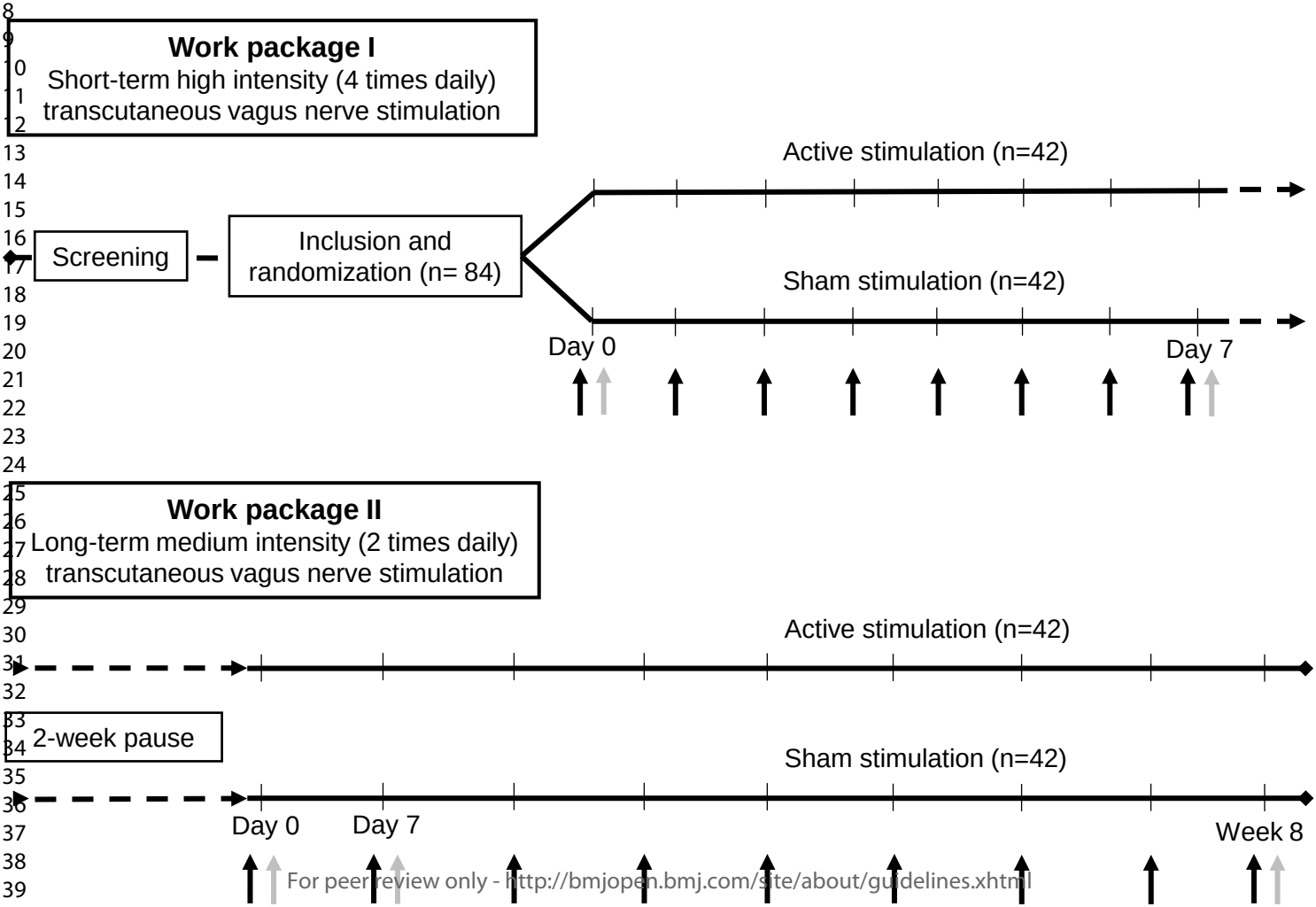
Figure 1 Overview of the systemic lupus erythematosus (SLE)-vagus nerve stimulation (VNS) study

PRIMARY AIM

Patient-reported level of fatigue

SECONDARY AIMS

- Patient-reported pain, disease activity and quality of life
- Autonomic nervous system function
- SLE disease activity and inflammation
- Pain tolerability
- Cardiovascular and kidney function



Reporting checklist for protocol of a clinical trial.

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In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1

Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	
Protocol version	#3	Date and version identifier	
Funding	#4	Sources and types of financial, material, and other support	22
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	22
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	NA
Roles and responsibilities: sponsor and funder	#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	NA
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and	NA

		other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	4, 13-16
Objectives	#7	Specific objectives or hypotheses	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, non-inferiority, exploratory)	6
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6

Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7-8
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9-11
Interventions: modifications	#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
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	10
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	11
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	11-16

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 1
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	18
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	7
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	16
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque,	16

		sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	16
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	16
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	16
Methods: Data collection, management, and analysis			
Data collection plan	#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	17

Data collection plan: retention	#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	17
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	17
Statistics: outcomes	#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	18
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	18
Statistics: analysis population and missing data	#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)	18
Methods: Monitoring			
Data monitoring: formal committee	#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	18

		details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	
Data monitoring: interim analysis	#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	NA
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	17
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	18
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	19
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)	NA

Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	7
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
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	17
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	23
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	22
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	17
Dissemination policy: trial results	#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	19

Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	19
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	22

Appendices

Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	NA
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	NA