ORIGINAL RESEARCH

Transcutaneous auricular branch vagal nerve stimulation as a non-invasive add-on therapeutic approach for pain in systemic sclerosis

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ABSTRACT

Objective Systemic sclerosis (SSc) is an autoimmune disease with health-related quality of life (HRQoL) high impairment. Pain is of paramount importance to be targeted by therapeutic approaches. Our study aim was to perform an add-on device-based non-invasive neuromodulatory treatment through transcutaneous auricular vagal nerve stimulation (tVNS) in patients with SSc, assessing its effects on pain as primary endpoint and on inflammation, cardiovascular autonomic control and HRQoL.

Methods Thirty-two patients with SSc were enrolled based on reported pain assessed through Numeric Rating Scale (NRS). Twenty-one (80%) with limited cutaneous SSc completed a randomised, cross-over, patient-blind trial, in which interventional and active control were used in random order for 4 weeks, interspersed with 4 weeks washout. NRS, Patient-Reported Outcomes Measurement Information System-29 (PROMIS-29) Item4 for pain interference, heart rate variability (HRV), serum cytokines and HRQoL questionnaires (Health Assessment Questionnaire, Patient Health Questionnaire-9, University of California, Los Angeles Gastrointestinal Tract, Pittsburgh Sleep Quality Index) were assessed at baseline, at T1 (after 1 month of tVNS or active control), at T2 (after washout) and at T3 (after 1 month of active control or tVNS). T-test for paired data and Wilcoxon signed-rank test for non-normally distributed parameters were performed to compare the effect of tVNS and active control.

Results NRS pain was significantly reduced by tVNS and not by active control (Mean±SD: −27.7%±21.3% vs −7.7%±26.3%, p=0.002). Interleukin-6 was downregulated in tVNS versus active control (p=0.029). No significant differences were observed in tVNS versus active control for PROMIS-29 Item4, QoL scales and HRV with both spectral and symbolic analyses.

Conclusion tVNS demonstrated to be a safe and non-invasive add-on tool to reduce pain in SSc.

INTRODUCTION

Systemic sclerosis (SSc) is a systemic autoimmune disease characterised by microvascular disfunction, autoantibody production and widespread fibrosis of skin and internal organs. Due to its complexity, SSc is one of the systemic autoimmune diseases with the highest quality-of-life impairment, chronic pain, dramatic progressive organ damage1,2 and high standardised mortality ratio (3.5). Pain affects about 83% of patients with SSc, mostly due to digital ulcers and inflammatory joint involvement.3,4 Pain affects routine daily activities and is related to the development of sleep disturbances and depressive mood.5

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT?

⇒ Systemic sclerosis (SSc) is one of the systemic autoimmune diseases with the most severe impact on quality of life, among several causes, chronic pain. The transcutaneous auricular vagal nerve stimulation (tVNS) was proved to reduce pain in other autoimmune diseases, such as rheumatoid arthritis and systemic lupus erythematosus.

WHAT DOES THIS STUDY ADD?

⇒ This is the first randomised, controlled, cross-over, patient-blind trial to perform non-invasive neuro-modulatory treatment through tVNS in patients with SSc.

⇒ tVNS demonstrated to be a safe and non-invasive add-on tool to reduce chronic pain in patients with SSc.

⇒ We observed a decrease of interleukin-6 serum levels after tVNS use, further investigations are needed.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This study adds important information on analgesic effects of tVNS in autoimmune diseases and confirms its high safety profile.
Recent clinical trials proposed a new non-invasive neuromodulation technique, the transcutaneous auricular vagal nerve stimulation (tVNS), to reduce inflammatory pain in autoimmune diseases, such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), showing promising results.\(^8\)\(^-\)\(^1^1\) VNS is a non-pharmacological treatment already used for decades in the treatment of refractory epilepsy, uncontrolled migraine and depression.\(^1^2\)\(^-\)\(^1^4\) tVNS positive impact on pain and inflammation could be related to its effects on the parasympathetic branch of the autonomic nervous system (ANS).\(^1^5\) Interestingly, evidence shows that ANS contributes to the control of both the innate and adaptive immunity preserving the homeostasis of the immune responses.\(^1^6\) Namely, the parasympathetic branch has an immunosuppressive and regulatory effect, known as ‘the cholinergic anti-inflammatory pathway’,\(^1^7\) mediated by the \(\alpha_7\) subunit of the nicotinic acetylcholine receptor (\(\alpha_7nAChR\)) on macrophages and T cells. When ANS balance is lost, due to a sympathetic-mediated beta-adrenergic activation, a state of immune over-reaction together with an excess of inflammation predominates, as demonstrated in sepsis preclinical mice models, in chronic autoimmune diseases\(^1^8\)\(^-\)\(^1^9\) and in COVID-19.\(^2^0\)

A cardiovascular sympatho-vagal imbalance, namely a predominant sympathetic and a reduced parasympathetic modulation, was reported in several autoimmune diseases including inflammatory bowel diseases, RA, SLE and SSc as well, especially in those patients with more severe fibrosis and long disease duration.\(^2^1\)\(^-\)\(^2^3\)

Based on the above premises, here we aimed to apply tVNS as a non-invasive device-based treatment in patients with SSc to assess its effect on pain and, as secondary outcomes, on inflammation, cardiovascular autonomic control and health-related quality of life (HRQoL) measures.

**MATERIALS AND METHODS**

**Population**

For the present randomised, controlled cross-over trial, we enrolled 32 consecutively adult patients with SSc with moderate-to-severe chronic pain from the Scleroderma Unit of Internal Medicine, Immunology and Allergology Department (Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, Milan, Italy) between March 2019 and January 2022. All patients fulfilled the 2013 American College of Rheumatology/European league against rheumatism (ACR/EULAR) classification criteria for SSc.\(^2^4\) Patients included were reporting chronic pain (Numeric Rating Scale score (NRS) \(\geq 6\)) with at least one type of SSc-related pain (eg, joint pain or pain from active ulcers) within the month prior to enrolment. The absence of a stable sinus rhythm on the ECG, ongoing therapy with beta-blocker drugs, presence of clinically significant rheumatic diseases other than SSc, recent hospitalisation (<6 months), active infection, vagotomy, presence of implantable devices (cochlear implants, deep brain stimulators, implanted vagal stimulators, cardiac pacemakers) and pregnancy were all exclusion criteria. We did not consider a limit to disease duration nor to the amount of limited cutaneous SSc (lcSSc) or diffuse cutaneous SSc (dcSSc) to be included in the study.

**Study procedures**

A randomised, controlled, AB×BA cross-over trial was performed. Patients were randomly assigned with a 1:1 ratio to the interventional arm with tVNS (25 Hz, arm A) on the cymba concha or to the active control group (1 Hz, arm B) on the cymba concha for 4 not consecutive hours/day for 4 weeks. Each group shifted to the opposite arm after a washout period of 4 weeks. The study was conducted in single-blind for the patients.

Baseline assessment (T0) included the assessment of pain during the last week through NRS, 10 min ECG and respiratory activity recording during resting conditions, blood sample collection and the administration of questionnaires for the evaluation of five domains, such as pain interference, functional disability, gastrointestinal symptoms, sleep quality and depressive symptoms. In addition to T0 assessment, three other experimental evaluation sessions (T1, T2, T3) were performed after the first 4 weeks of treatment, after the washout period and after the last 4 weeks of treatment, respectively (figure 1).

**Figure 1** Study design. Participants were enrolled in a randomised, controlled, AB×BA cross-over study. tVNS, transcutaneous auricular vagal nerve stimulation.
**Stimulation**

The stimulation parameters included a duty cycle of 30s on and 30s off in order to not irritate the skin and a pulse width of 250 μs. The stimulation frequency was 25 Hz for real stimulation and 1 Hz for active control. The device was portable and patients performed the tVNS or the active control for 4 non-consecutive hours/day for 4 weeks at home. Two titan electrodes located in a structure similar to an earphone were to be placed on the cymba conchae of the left ear (real tVNS), an area 100% innervated by the auricular branch of the vagus nerve. Electrode pads were used with electrode contact spray to facilitate the electrical conduction as well as the patient comfort. The intensity range of the device spread from 0.2 mA to 5 mA. The stimulation intensity was set by each patient based on the personal minimum intensity required to perceive electrical stimulation described as a pricking or tingling sensation on the skin without discomfort. The active control with 1 Hz stimulation was necessary to ensure blinding due to the cross-over design of the study. As a matter of fact, the other parameters were maintained in order to elicit the same sensation evoked by real tVNS (eg, tingling sensation).

This type of device allowed close monitoring of patient compliance. As a matter of fact, it was possible to access the history of the stimulations performed on the last day, on the last week and on the last month. The device recorded the percentage of effective stimulation, evaluated through the correct contact between the electrode and the skin, performed with respect to the 4 hours per day as per protocol. A compliance threshold of 85% of total stimulation time was set for inclusion in the final analysis.

**Pain assessment**

The pain perceived in the last week was assessed at each time point (T0–T3) through the NRS. Patients were asked to rate the pain experienced in the last week using whole numbers on a scale from 0 to 10, where 0 represents ‘no pain’ and 10 represents ‘the worst pain possible’. A reduction of approximately two points or a reduction of approximately 30% of the NRS score from the baseline value represents a clinically important difference. The interference of pain with daily activities was assessed of approximately 30% of the NRS score from the baseline value.26 A reduction of approximately 2 points or a reduction of approximately 30% of the NRS score from the baseline value represents a clinically important difference.26 The interference of pain with daily activities was assessed of approximately 30% of the NRS score from the baseline value.

The HRQoL was investigated through four main domains and corresponding questionnaires: functional disability, gastrointestinal symptoms, depressive symptoms and sleep quality. Functional disability was quantified through the Health Assessment Questionnaire (HAQ). The score range is between 0, indicating no functional disability, and 3, severe functional disability.27 The presence and severity of gastrointestinal symptoms were assessed through the University of California at Los Angeles Scleroderma Clinical Trial Consortium GIT 2.0 instrument (UCLA GIT 2.0). The UCLA GIT 2.0 questionnaire contains 34 items, organised into seven subscales: reflux, distention/bloating, diarrhoea, faecal soiling, constipation, emotional well-being and social functioning.28 The total UCLA GIT 2.0 score is calculated by averaging all subscales, except the one for constipation, and ranges from 0 to 2.83, higher scores indicating more severe symptomatology and worse HRQoL.29 The presence of depressive symptoms was assessed through the Patient Health Questionnaire-9 (PHQ-9). The score ranges from 0 to 27 and a PHQ-9 score ≥10 has a sensitivity of 88% and a specificity of 88% for major depression.30 The sleep quality was evaluated using the questionnaire Pittsburgh Sleep Quality Index (PSQI). We considered the global score of the test, which has a possible range of 0–21 points.31 A global score higher than 5 is considered as an indicator of relevant sleep disturbances.

**Cardiovascular autonomic control assessment**

ECG and respiratory recordings were performed at rest, in supine position with spontaneous breathing, for 10 min at each time point (T0–T3). All participants were informed to avoid taking food and caffeine in the 2 hours preceding the recording session. All measurements were performed in a quiet temperature-controlled room (between 20°C and 24°C). ECG (lead II) and respiratory activity were recorded using an ad hoc telemetric system device (LAB3, Marazza, Monza, Italy).

The heart rate variability (HRV) analysis was performed off-line to evaluate the cardiovascular autonomic modulation. Linear spectral analysis and non-linear symbolic analysis were performed through a specific software (Heart Scope II, AMPS, ITA) on the R-R time series derived from the ECG signals. One segment of 250±50 beats at rest without artefacts was selected from the R-R time series. The autoregressive model was applied to identify the spectral power in the low-frequency band (LF, bounded between 0.04 and 0.15 Hz), an index of sympathetic modulation and baroceptive activity, and in the high-frequency band (HF, bounded between 0.15 and 0.40 Hz), an index of parasympathetic modulation which is synchronous with respiratory activity in case of normal free breathing (9–24 breaths/min).32–34 The LF and HF components were expressed in normalised units (LFnu and HFnu) to represent the relative amount of each component compared with the total power of the HRV spectrum.33 The algorithm also calculates the LF/HF ratio, which is considered an index of the sympathovagal balance.35 36

The symbolic analysis was performed on the same segments in order to evaluate non-reciprocal changes of sympathetic and parasympathetic modulation.37 38 The R-R time series was converted into three beats patterns: (1) 0V, patterns with no variation, all three symbols are equal; (2) 2LV, patterns with two like variations, all symbols are different from the previous one and they are
in ascending or descending order and (3) 2UV, patterns with two unlike variations, all symbols are different from the previous one but not in a consequent order. The percentage of the patterns 0V is a marker of cardiac sympathetic modulation and 2UV or 2LV are markers of cardiac vagal modulation.40

**Plasma biomarkers analysis**

The plasmatic concentrations (pg/mL) of interleukin (IL)-6, IL-10, IL-1β, interferon-γ, tumour necrosis factor-α (TNF-α), tumour necrosis factor receptor 1, brain-derived neurotrophic factor, triggering receptor expressed on myeloid cells 1, triggering receptor expressed on myeloid cells 2, neurofilament light chain were quantified using the Simple Plex Ella (Ella) (ProteinSimple, Bio-Techne, Minneapolis, Minnesota, USA), an automated immunoassay platform that allows the rapid quantitation of analytes from a single disposable microfluidic cartridge.

**Statistical analysis**

Data were analysed using SPSS Statistics V.27 (IBM, Armonk, New York, USA). The Shapiro-Wilk test was performed to evaluate the normal distribution of the data. Results were expressed as absolute frequency, relative frequency, means and SD or median and IQR. The two-way Analysis of Variance for repeated measure was performed to compare the two baseline periods (T0 and T2) considering Time (T0 vs T2) as within subject factor and treatment schedule assigned by randomisation (A×B or B×A) as between subject factor. To compare the effect of tVNS and active control, the relative percent change (Δrel%) post-treatment with tVNS or with active control (T1 or T3) from baseline (T0 or T2) of NRS score, questionnaire scores and HRV indices was calculated [Δrel%=(post-baseline)/baseline*100]. The Student’s t-test for paired data was performed to compare the effect of tVNS and of active control on normally distributed parameters. The Wilcoxon signed-rank test was used to compare the effect of tVNS and of active control on non-normally distributed parameters. The χ² test was performed to evaluate differences between the number of patients who experienced a clinically significant reduction in pain after tVNS and the number of patients who experienced a clinically significant reduction in pain after active control. A p value <0.05 was considered statistically significant.

**RESULTS**

A total of 35 eligible consecutive patients were identified within our centre. Two patients refused to participate, and one patient did not tolerate tVNS even at the

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*Figure 2*  Flowchart of patient enrolment. HRV, heart rate variability.
Table 1  Baseline demographic and clinical description of the enrolled patients

<table>
<thead>
<tr>
<th>Features</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean years±SD</td>
<td>58±11</td>
</tr>
<tr>
<td>Gender, Female, n (%)</td>
<td>18 (86)</td>
</tr>
<tr>
<td>Ethnicity, Caucasian, n (%)</td>
<td>20 (95)</td>
</tr>
<tr>
<td>ANA, n (%)</td>
<td>19 (90)</td>
</tr>
<tr>
<td>ACA, n (%)</td>
<td>9 (43)</td>
</tr>
<tr>
<td>Anti-ScI70, n (%)</td>
<td>7 (33)</td>
</tr>
<tr>
<td>Disease duration, mean±SD (years)</td>
<td>17±7</td>
</tr>
<tr>
<td>lcSSc, n (%)</td>
<td>19 (90)</td>
</tr>
<tr>
<td>dcSSc, n (%)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>FVC %, mean±SD (years)</td>
<td>98±27</td>
</tr>
<tr>
<td>DLCO %, mean±SD (years)</td>
<td>65±17</td>
</tr>
<tr>
<td>ILD, n (%)</td>
<td>8 (38)</td>
</tr>
<tr>
<td>EF (%), mean±SD</td>
<td>63±4</td>
</tr>
<tr>
<td>PAPs (mm Hg), mean±SD</td>
<td>26±7</td>
</tr>
<tr>
<td>Upper GI, n (%)</td>
<td>18 (86)</td>
</tr>
<tr>
<td>Immunosuppressants, n (%)</td>
<td>6 (29)</td>
</tr>
<tr>
<td>Low dose aspirin, n (%)</td>
<td>16 (76)</td>
</tr>
<tr>
<td>CCB, n (%)</td>
<td>16 (76)</td>
</tr>
<tr>
<td>Iloprost, n (%)</td>
<td>18 (86)</td>
</tr>
<tr>
<td>Prednisone, n (%)</td>
<td>8 (38)</td>
</tr>
</tbody>
</table>

ACA, anti-centromere antibodies; ANA, anti-nuclear antibodies; Anti-ScI70, anti-topoisomerase I antibodies; CCB, calcium channel blocker assumption; dcSSc, diffuse cutaneous SSC; DLCO, diffusing capacity for carbon monoxide; EF, ejection fraction; FVC, forced vital capacity; GI, gastrointestinal involvement; ILD, interstitial lung disease; lcSSc, limited cutaneous SSC; PAPs, systolic pulmonary arterial pressure on echocardiography; SSC, systemic sclerosis.

Effects of tVNS

tVNS resulted in a significant reduction of chronic pain levels from baseline than active control (figure 3, mean±SD: −27.7%±21.3% vs −7.7%±26.3%, p=0.002). Moreover, tVNS achieved a clinically significant reduction in chronic pain with a ΔNRS of two points (mean±SD: −2.0±1.6). This result was not reached by the active control (mean±SD: −0.5±1.8). Thirteen patients achieved a pain reduction of at least two NRS points after tVNS, while only five patients achieved a pain reduction of at least two NRS points after active control (p=0.029).

As reported in table 2, tVNS did not determine significant effects on the other aspects of HRQoL. In particular, there was no difference between the relative changes after tVNS and after active control in pain interference with daily activities (assessed through PROMIS-29 Item 4a), functional disability, gastrointestinal symptoms, depressive symptoms and sleep quality.

The relative changes of HRV indexes from baseline (T0 or T2) determined by tVNS and active control did not show significant differences as reported in table 3. Finally, tVNS determined a significant reduction of plasma IL-6 levels, not present after active control (median −17.1%, IQR (−33.6%; 16.2%) vs median 39.6%, IQR (−10.1%; 287.7%)). The other inflammatory parameters did not show any significant differences between the post-tVNS and post-active control changes, as shown in table 4.
DISCUSSION

The major finding of this randomised cross-over clinical trial is that non-invasive tVNS was able to significantly reduce chronic pain in patients affected by SSc. Moreover, a reduction in IL-6 levels post-tVNS treatment was observed.

Similar findings had already been reported in patients with SLE and RA\textsuperscript{10,11,41,42}. Indeed, in a recent randomised, double-blind, controlled trial, Aranow et al showed a reduction of pain and plasma levels of substance P, a neurotransmitter associated with inflammation and pain, in patients with SLE treated with tVNS for four consecutive days.\textsuperscript{10} Moreover, tVNS was well tolerated and no significant side effects were reported. At difference, authors did not find a direct effect on serum inflammatory cytokines, nor a sustained tVNS effect over time, maybe due to the short period of treatment.\textsuperscript{10} Furthermore, another study conducted on patients with RA with high disease activity, showed effects of VNS, applied transcutaneously to the cervical vagus nerve, in reducing both pain and inflammatory biomarkers, thus supporting an anti-inflammatory effect of tVNS.\textsuperscript{41} Other studies using different VNS type of electrodes and stimulation pattern showed a decrease of inflammation in patients with RA and IBD,\textsuperscript{42,43} and a recent study\textsuperscript{41} reported a reduction of inflammatory biomarkers and fatigue in patients with Sjogren’s Syndrome.

We analysed serum levels of several cytokines finding a significant decrease of IL-6 levels following tVNS, while no decrease was observed after active control. Interestingly, IL-6 is an acute phase response inducer and its serum levels in SSc correlate with acute phase proteins, such as TNF-\(\alpha\) and high sensitive C reactive protein.\textsuperscript{44} IL-6 role in SSc is well documented, promoting a T-helper 2 driven fibrosis at lung and skin level.\textsuperscript{45} Preclinical studies showed that the vagus nerve directly influences inflammation through the cholinergic anti-inflammatory pathway suppressing macrophage production of TNF-\(\alpha\) and IL-6 through the \(\alpha 7\) subunit of nAChR.\textsuperscript{46} Certain systemic pro-inflammatory cytokines such as IL-1\(\beta\), IL-6 and TNF-\(\alpha\) are involved in the process of peripheral pain sensitisation\textsuperscript{47} and it could be speculated that the reduction in chronic pain might be due to a downregulation

Table 2

<table>
<thead>
<tr>
<th>Index</th>
<th>tVNS</th>
<th>Active control</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRS (%)</td>
<td>−27.7±21.3</td>
<td>−7.7±26.3</td>
<td>0.002</td>
</tr>
<tr>
<td>PROMIS item 4a (%)</td>
<td>−15.7 (−69.4; 17.1)</td>
<td>3.3 (−60.2; 47.5)</td>
<td>0.252</td>
</tr>
<tr>
<td>UCLA GIT 2.0 (%)</td>
<td>3.9±87.6</td>
<td>2.6±83.7</td>
<td>0.919</td>
</tr>
<tr>
<td>HAQ (%)</td>
<td>−7.70 (−37.6; 10.4)</td>
<td>−11.1 (−25.0; 28.6)</td>
<td>0.678</td>
</tr>
<tr>
<td>PHQ-9 (%)</td>
<td>−17.3±55.2</td>
<td>−7.4±45.9</td>
<td>0.566</td>
</tr>
<tr>
<td>PSQI (%)</td>
<td>3.6±46.6</td>
<td>21.2±52.8</td>
<td>0.223</td>
</tr>
</tbody>
</table>

Significant p values <0.05 are shown in bold. UCLA GIT, University of California, Los Angeles Gastrointestinal Tract; HAQ, Health Assessment Questionnaire; HRQoL, health-related quality of life; NRS, Numeric Rating Scale; PHQ-9, Patient Health Questionnaire-9; PROMIS, Patient-Reported Outcomes Measurement Information System; PSQI, Pittsburgh Sleep Quality Index; tVNS, transcutaneous auricular vagal nerve stimulation.

Figure 3  Relative mean percent change of chronic pain assessed by NRS score after active control and tVNS; *p<0.05. NRS, Numeric Rating Scale; tVNS, transcutaneous auricular vagal nerve stimulation.
of the sensitising inflammatory stimulus. However, other than IL-6, the several inflammatory markers tested in the present study did not show a significant serum level reduction following tVNS, thus further larger and mechnistic studies are needed to support an anti-inflammatory effect of tVNS in patients. Alternatively, the analgesic effect of tVNS could also be consequent to a direct effect on brainstem autonomic regulatory pathways. The stimulation of the auricular branch of vagus nerve can modulate the activity of nucleus tractus solitarius which has main afferent projections to the locus coeruleus that regulate pain threshold.\(^48\)\(^49\)

Pain, especially chronic one, is known to be related to a decrease of HRQoL and to sleep disturbances in SSc.\(^7\) Furthermore, the gastrointestinal (GI) tract is affected in about 90% of patients with SSc and in physiologic conditions ANS balances the GI motility and its normal stress response.\(^50\)\(^51\) However, while in the present study we observed a direct tVNS effect on pain measured through the NRS, no difference between effective and active control was observed relating to HRQoL and sleep quality scales, nor GI symptoms as assessed through the UCLA GIT 2.0. In addition, cardiovascular autonomic modulation, assessed by HRV indexes, did not show differences after stimulation. These results could be due to a longer treatment duration needed to assess substantial clinical change in these three domains also, it has to be acknowledged that the high disease duration (mean of 17 years) of the patients enrolled represent a higher disease associated organ damage more difficult to reverse thus consequently with difficulties in improving HRQoL. Cardiovascular autonomic alterations are known to be present in patients with SSc, and they seem to be associated with clinical features such as disease duration,

### Table 3
Comparison between relative changes from baseline (T0 or T2) after tVNS and after active control of HRV indexes

<table>
<thead>
<tr>
<th>Index</th>
<th>tVNS, mean±SD or median (IQR)</th>
<th>Active control, mean±SD or median (IQR)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (%)</td>
<td>0.6±10.3</td>
<td>0.3±12.2</td>
<td>0.950</td>
</tr>
<tr>
<td>TP (%)</td>
<td>40.9±131.5</td>
<td>17.0±113.2</td>
<td>0.612</td>
</tr>
<tr>
<td>LFnu (%)</td>
<td>8.9 (−9.9; 78.5)</td>
<td>7.7 (−18.4; 91.3)</td>
<td>0.891</td>
</tr>
<tr>
<td>HFnu (%)</td>
<td>−2.2 (−46.1; 126.5)</td>
<td>−34.9 (−69.9; 40.8)</td>
<td>0.515</td>
</tr>
<tr>
<td>LF/HF (%)</td>
<td>19.6 (−64.5; 543.4)</td>
<td>97.69 (−46.0; 468.75)</td>
<td>0.523</td>
</tr>
<tr>
<td>0V (%)</td>
<td>18.7 (−21.2; 86.5)</td>
<td>25.1 (−38.6; 53.8)</td>
<td>0.352</td>
</tr>
<tr>
<td>2LV (%)</td>
<td>−17.7 (−43.1; 38.3)</td>
<td>−10.4 (−46.3; 157.3)</td>
<td>0.671</td>
</tr>
<tr>
<td>2UV (%)</td>
<td>−18.3 (−42.6; 15.2)</td>
<td>3.6 (−12.4; 46.6)</td>
<td>0.196</td>
</tr>
</tbody>
</table>

HF, high-frequency band; HR, heart rate; HRV, heart rate variability; LF, low-frequency band; 2LV%, patterns with two like variations; nu, normalised units; tVNS, transcutaneous auricular vagal nerve stimulation; 2UV%, patterns with two unlike variations; 0V%, patterns with no variations.

### Table 4
Comparison between relative changes from baseline (T0 or T2) after tVNS and after active control of inflammatory profile

<table>
<thead>
<tr>
<th>Index</th>
<th>tVNS, mean±SD or median (IQR)</th>
<th>Active control, mean±SD or median (IQR)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 (%)</td>
<td>−17.1 (−33.6; 16.2)</td>
<td>39.6 (−10.1; 287.7)</td>
<td>0.029</td>
</tr>
<tr>
<td>IL-10 (%)</td>
<td>30.4±53.6</td>
<td>9.6±30.5</td>
<td>0.148</td>
</tr>
<tr>
<td>IL-1β (%)</td>
<td>21.0±203.0</td>
<td>63.8±178.4</td>
<td>0.357</td>
</tr>
<tr>
<td>IFN-γ (%)</td>
<td>32.8±100.5</td>
<td>43.8±77.6</td>
<td>0.294</td>
</tr>
<tr>
<td>TNF-α (%)</td>
<td>2.2±13.4</td>
<td>0.3±168</td>
<td>0.846</td>
</tr>
<tr>
<td>TNFR1 (%)</td>
<td>−1.8±16.0</td>
<td>11.4±28.2</td>
<td>0.121</td>
</tr>
<tr>
<td>BDNF (%)</td>
<td>−0.3±34.9</td>
<td>−5.0±25.2</td>
<td>0.657</td>
</tr>
<tr>
<td>TREM1 (%)</td>
<td>3.6±23.8</td>
<td>−7.0±17.7</td>
<td>0.149</td>
</tr>
<tr>
<td>TREM2 (%)</td>
<td>7.3±25.6</td>
<td>10.5±32.5</td>
<td>0.684</td>
</tr>
<tr>
<td>NfL (%)</td>
<td>6.1±25.1</td>
<td>12.5±41.5</td>
<td>0.585</td>
</tr>
</tbody>
</table>

Significant p values<0.05 are shown in bold.
BDNF, brain-derived neurotrophic factor; IFN-γ, interferon-γ; IL, interleukin; NfL, neurofilament light chain; TNFR1, tumour necrosis factor receptor 1; TNF-α, tumour necrosis factor-α; TREM1, triggering receptor expressed on myeloid cells 1; TREM2, triggering receptor expressed on myeloid cells 2; tVNS, transcutaneous auricular vagal nerve stimulation.
skin involvement and anti-topoisomerase autoantibody positivity. Due to the long disease history and disease severity of the present cohort, it is possible to hypothesise that the duration of tVNS treatment was not enough to induce a change in cardiovascular autonomic control. Further studies with a longer and chronic tVNS application are needed to evaluate the possibility of restoring the sympathovaginal balance.

Our study showed as a strength the cross-over trial design, which ensured solidity of the statistical results. We demonstrated also that tVNS is a safe non-invasive therapeutical option, since no adverse event has been observed during interventional stimulation. Also, to the best of our knowledge, this was the first study applying a neuromodulation technique to treat chronic pain in SSC.

We acknowledge several limitations for the present study. First, a limited sample size, mainly due to patients’ dropouts related to the intercurrent pandemic situation was an important limit. Second, the majority of SSC included in the trial had a lcSSc subset with a long disease duration and further studies including higher numbers of dcSSc and a shorter disease duration have to be performed. Third, tVNS is still lacking of a gold standard pattern of stimulation, in terms of time length of the treatment and stimulation parameters, thus results should not be extrapolated to different stimulation patterns.

CONCLUSION
In conclusion, our data support the use of tVNS as a non-invasive tool for the treatment of chronic pain in patients with SSC, as well as in other systemic autoimmune diseases. The tVNS effect on IL-6 observed in the present SSC cohort is worthy to be further investigated on larger cohorts.

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Contributors AC, CB, BA and LB analysed the data. CB and AC wrote and revised the manuscript. AC, CB, ADT and MT worked on the clinical database. AC, CB, BA, MM, CS and GDR collected data and assisted with the analysis. AC, ADT, MT, CS and GDR performed cardiovascular autonomic control assessment. AC, CB, NM and ET elaborated the conception and study design. All authors of the present manuscript contributed to the general conception of the work and to the analysis and interpretation of data. All authors revised critically the manuscript and approve the final version, guaranteeing accuracy and integrity of any part of the work. NMIs the guarantor of the study with full responsibility for the work and the conduct of the study.

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