Assessment of safety and feasibility of non-invasive vagus nerve stimulation for treatment of acute stroke

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ABSTRACT

Background: Non-invasive vagus nerve stimulation (nVNS) using a hand-held stimulator placed on the neck is an FDA-approved treatment for primary headache disorders. The safety of nVNS is unknown in stroke patients.

Objective: To assess the safety and feasibility of nVNS for the acute treatment of stroke.

Methods: TR-VENUS (clinicaltrials.gov identifier NCT03733431) was a randomized, sham-controlled, open-label, multicenter trial conducted in patients with acute ischemic stroke (IS) or intracerebral hemorrhage (ICH). Patients were randomly assigned to standard-dose nVNS, high-dose nVNS, or sham stimulation. The primary endpoint was a composite safety outcome defined as bradycardia or reduction in mean arterial blood pressure during treatment or progression of neurological deficit or death within 24 h of treatment. The feasibility endpoints were the proportion of eligible subjects receiving nVNS within 6 h of symptom onset and the proportion completing all pre-specified treatment doses. Efficacy assessments included infarct growth from baseline to 24 h after treatment.

Results: Sixty-nine patients (61 IS, 8 ICH) completed the study. The composite safety outcome was achieved in 32.0% in sham and 47.7% in nVNS group (p = 0.203). Treatment was initiated in all but two randomized patients. All dosed subjects received 100% of pre-specified stimulations. A non-significant reduction in infarct growth was observed in the high-dose nVNS group (184.2% in sham vs. 63.3% in high-dose nVNS; p = 0.109).

Conclusions: The results of this study suggest that nVNS may be safe and feasible in the setting of acute stroke. These findings support further development of nVNS as a potential treatment for acute ischemic stroke.

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1. Introduction

Stroke is a major cause of death and disability. Each year, 12 million people worldwide suffer from a stroke and 6.5 million lives are lost as a result of it [1]. Approximately 4 out of every 5 strokes are ischemic strokes. Currently available treatments for acute...
ischemic stroke are all reperfusion/recanalization based. Despite its proven efficacy, reperfusion/recanalization treatment can only be offered to a small fraction of the patients with stroke owing to the narrow time-window and stringent selection criteria for eligibility. Furthermore, reperfusion/recanalization treatment is only moderately effective; of those who are amenable to reperfusion/recanalization therapies, only around one-half can achieve an independent functional outcome \[2,3\]. Overall, stroke continues to be a significant public health challenge worldwide.

Vagus nerve stimulation (VNS) via an implantable stimulator is a US Food and Drug Administration (FDA) approved treatment for drug-resistant epilepsy and depression. Recent evidence suggests that VNS may also be protective against ischemic brain injury. Electrical stimulation of the vagus nerve using a pair of electrodes placed directly over the nerve fiber for a period of 1 h reduces infarct volume by up to 56% when initiated within 3 h after middle cerebral artery occlusion (MCAO) in rats \[4–6\]. The neuroprotective effect of VNS is consistent across different models of MCAO in both healthy and spontaneously hypertensive rats \[4–13\].

Although direct VNS via an implantable stimulator is protective in animal models of cerebral ischemia, the need for surgical implantation makes it an impractical means of treatment of acute stroke in humans. Recently, non-invasive stimulation of the vagus nerve via a handheld nerve stimulator placed on the skin overlying the vagus nerve in the carotid triangle has become possible. Published data indicate that non-invasive VNS (nVNS) provides ischemic neuroprotection with up to 46% reduction in infarct size after 2-h transient MCAO in rats \[11–13\]. The neuroprotective effect is retained when nVNS is applied up to 4 h after the induction of ischemia, suggesting that nVNS could be feasible for early treatment of stroke in humans \[11\]. nVNS is an FDA-approved treatment for primary headache including the acute and preventative treatment of migraine and cluster headache and has been used for this purpose since 2017. While the safety of nVNS is established in primary headache disorders, stroke patients are older, more often suffer from cardiac and other comorbidities, and are more prone to develop hemodynamic perturbations. The non-invasive Transcutaneous cervical Vagus nErve stimulatioN as a treatment for acute Stroke (TR-VENUS) study aimed to determine the safety and feasibility of nVNS when delivered immediately upon imaging-confirmed diagnosis of acute stroke within 6 h of symptom onset.

2. Materials and methods

2.1. Trial design

TR-VENUS (ClinicalTrials.gov identifier: NCT03733431) was an investigator-initiated, randomized, sham-controlled, open-label, multicenter trial assessing the safety and feasibility of nVNS for the acute treatment of stroke. The study was conducted at 9 academic centers from May 10, 2019, through December 31, 2020.

The trial was approved by the ethics committees of the coordinating site (Hacettepe University, Ankara, Turkey) and the Turkish Ministry of Health, run by an independent contract research organization, conducted in compliance with the guidelines and regulations of the FDA, European Medicines Agency, and Turkish Ministry of Health on Clinical Trials (2014), and performed in accordance with the International Council for Harmonisation Good Clinical Practice E6 (R2, 2016) and the Declaration of Helsinki. All subjects or family members provided written informed consent before participating in the trial.

2.2. Trial population

Subjects (≥18 years of age) diagnosed with acute ischemic stroke (IS) or intracerebral hemorrhage (ICH), who were admitted within the first 6 h after stroke onset, constituted the study population. Inclusion and exclusion criteria are provided in Table 1. All subjects, regardless of treatment group assignment, received standard care for acute stroke.

2.3. Trial treatments and procedures

The study was conducted in 2 phases (Fig. 1). In phase-1, IS patients were randomly assigned to receive standard-dose nVNS or sham using a 2:1 randomization schedule. The planned sample size for phase-1 was 30. At the end of phase-1, an interim safety analysis was performed. The stopping rule at this analysis was the observation of a composite primary safety endpoint incidence that was 25% higher in the nVNS group than in sham stimulation group. An independent data safety monitoring board evaluated the interim safety data and recommended continuing to phase-2 of the study. In phase-2, 40 additional patients with IS or ICH were planned to be randomized to high-dose nVNS or sham using 2:1 randomization schedule. Subjects were stratified by baseline stroke severity (<12 or ≥12 on the National Institutes of Health Stroke Scale [NIHSS]) before being randomly assigned to nVNS or sham treatment using an interactive web-based system.

nVNS was applied using a commercially available hand-held stimulator (gammaCore®, electroCore, Inc., USA). gammaCore® is a pre-programmed, push-button stimulator that was designed for self-administration by patients and requires a minimal level of training for application (Supplemental Fig. 1). Stimulation was delivered by coating the stainless-steel discs with conductive gel and positioning the device below the mandibular angle medial to the sternocleidomastoid muscle and lateral to the larynx and applying gentle pressure to increase the proximity between the stimulation electrodes and the vagus nerve (Supplemental Fig. 2). Stimulation was given to the left vagus nerve unless contraindicated for a documented reason such as known near occlusive stenosis or bruit over the left internal carotid artery and local infection, rash, or prior surgery on the left side of the neck. The sham stimulation was applied along the lateral border of the sternocleidomastoid muscle on the left side to avoid mechanical stimulation of the vagus nerve in the carotid triangle (Supplemental Fig. 2).

The nVNS signal consists of 5-kHz sine waves repeated at a frequency of 25 Hz, with stimulation intensity ranging from 0 to 24 V. As per the device prospectus, each stimulation was 2 min long. The stimulation intensity was adjusted by the investigator using a scale of 0 (no stimulation) to 40 (maximum stimulation), with the intensity being titrated for each patient by gradually increasing the intensity up to the point when pain or lip droop on the ipsilateral site was experienced. In subjects with impaired cooperation, the investigator adjusted the dose to an intensity just below that which caused lip droop. Lip droop has been used as a surrogate marker for vagus nerve activation in prior studies of nVNS \[14,15\] and is considered to indicate that the stimulation has exceeded the threshold for activation of the afferent vagal nerve fibers and spread to the somatic efferent nerve fibers innervating the superficial neck muscles responsible for the lip droop \[16,17\]. Sham treatment was delivered using an identical device that produced a buzzing sound but no electrical stimulation.

All subjects had a baseline assessment with cerebral computed tomography (CT). Magnetic resonance imaging (MRI) was obtained in a subset of patients before the initiation of study treatment in selected study sites. Follow-up imaging was CT in subjects with ICH.
Heart rate (HR) and blood pressure were closely monitored immediately before and at 2 and 5 min after initiation of each stimulation and 30 min after completion of all assigned stimulations. The NIHSS score was recorded at 30 min after completion of study treatment and at 24 h. The modified Rankin Scale was used to assess the degree of disability 90 days posttreatment.

2.4. Outcomes and study objectives

All study endpoints are listed in Table 2. The primary objective was to assess the safety of nVNS for the acute treatment of stroke. The primary safety endpoint was a composite outcome that included the occurrence of any of the following: bradycardia (HR \( \leq 50 \) bpm) during treatment period, \( \geq 20 \) mmHg reduction in mean arterial blood pressure (MAP) during treatment period, neurologic worsening (\( \geq 4 \)-point increase in NIHSS score) within 24 h after initiation of therapy, or death within 24 h after the initiation of therapy. Treatment period was defined as the period starting from immediately prior to the first stimulation to 30 min after the completion of the last stimulation.

A predefined subgroup analysis in the subset with baseline MRI examined the effect of treatment on infarct growth (Table 2). Absolute infarct growth was defined as the difference between lesion volume at 24 h and at baseline on diffusion weighted imaging (DWI). Relative infarct growth was defined as \( \left( \frac{24\text{-h volume}}{\text{baseline volume}} \right) \times 100 \). Both absolute and relative infarct growth assessments were repeated in the subgroup with clinical diffusion mismatch (CDM) as defined by baseline ischemic lesion volume \( \leq 25 \) cc and NIHSS score \( \geq 8 \) [18]. In the subset with ICH, hematoma volume on neuroimaging at baseline and 24 h were calculated using CT. All volumes were measured using a semiautomated lesion outlining software (3D-Slicer version 4.1; http://www.slicer.org) by an investigator who was blinded to treatment assignment [19].

All participants were blinded to treatment groups. Assessments for hemodynamic and other safety variables during treatment period were performed by unblinded investigators who

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**Table 1**

| Inclusion criteria | Exclusion criteria |
|--------------------|-------------------|
| 1. Men or women aged \( \geq 18 \) years who have been admitted to neurologic intensive care or stroke units with ischemic or hemorrhagic stroke |
| 2. Symptom onset time within 6 h or with unknown time of onset and no evidence of acute ischemia on FLAIR imaging |
| 3. Patients who have given written informed consent before undertaking any study-related procedure |
| 1. Pre-stroke disability \( \geq 2 \) according to modified Rankin Scale |
| 2. Admission NIHSS score \( \leq 4 \) or \( > 30 \) |
| 3. Admission NIHSS score item 1a \( \geq 2 \) |
| 4. Early dramatic neurological improvement (NIHSS score improvement \( \geq 8 \)) prior to study randomization suggesting resolution of signs/symptoms of stroke |
| 5. Classical lacunar syndrome |
| 6. Known severe (\( > 90 \% \) stenosis) bilateral carotid artery disease, carotid hypersensitivity, and/or history of bilateral carotid endarterectomy or neck surgery involving the region of carotid triangle |
| 7. Low blood pressure (SBP \( \leq 100 \) mm Hg or DBP \( \leq 60 \) mm Hg) or bradycardia (HR \( \leq 60 \) bpm) at admission and/or high blood pressure (SBP \( > 220 \) mm Hg or DBP \( > 130 \) mm Hg) despite initial line of treatment |
| 8. Conditions/medications that would interfere with study treatment* |
| 9. Conditions/circumstances that could interfere with the conduct of the study* |

* Full exclusion criteria can be found in Supplemental Table 1.

DBP indicates diastolic blood pressure; FLAIR, fluid attenuated inversion recovery; HR, heart rate; NIHSS, National Institutes of Health Stroke Scale; SBP, systolic blood pressure.
administered the treatment. Follow-up neurological examinations, imaging endpoints, and secondary safety parameters were assessed by blinded investigators who were not involved in the administration of treatment.

2.5. Statistical analyses

Primary composite safety outcome, and secondary safety outcomes were assessed in the intent-to-treat (ITT) set that consisted of all subjects in the randomized cohort who received at least one stimulation of the assigned treatment. Feasibility analysis was performed in the randomized set that included all enrolled subjects for whom a randomization number was assigned. Efficacy analysis was conducted in the set that included all randomized and dosed patients with IS.

Because this was the first study of nVNS in acute stroke, the anticipated harm/effect size was unknown. Hence, the sample size was not based on statistical considerations. The planned total sample size for this study was 70. Descriptive statistics were used to summarize patient demographics, vital signs, and medical history data by treatment group. The safety endpoints of bradycardia and reduction in MAP were analyzed using generalized linear regression models to account for multiple measures per subject (i.e., multiple stimulations) and multiple assessments per stimulation. Change in MAP was calculated at 2- and 5-min after each stimulation in comparison to the baseline immediately prior to the respective stimulation and at 30 min after the completion of all stimulations in comparison to the treatment baseline. Treatment baseline denoted immediately prior to the first stimulation in sham and standard-dose nVNS and prior to the eighth stimulation in high-dose nVNS. Additionally, mean MAP and mean change in MAP across the treatment period were analyzed using general linear models accounting for within-subject correlation. Results were presented as least-squares mean proportions/least-squares means and corresponding 95% confidence intervals (CIs). $p$ values were from the resulting $F$ tests. McNemar’s test was used to compare change in MAP in the high-dose nVNS group between first and second stimulation epochs. Comparisons between the sham and nVNS groups were conducted using Chi-square or Fisher’s exact tests, as appropriate. Radiologic efficacy data in subjects with IS were summarized using median and interquartile ranges (IQRs).

Clinical efficacy outcomes in the IS cohort were presented as n/N (%). Inverse probability weighting (IPW) was performed as necessary to adjust for covariates. For continuous variables comparing sham to nVNS, data were analyzed using SAS® 9.4 (SAS Institute Inc.). No alpha adjustment for multiple comparisons was performed. A $p$ value < 0.05 was considered statistically significant.

3. Results

The randomization set included 71 subjects (Fig. 2). Two subjects who were randomized did not receive the assigned treatment; one developed severe hypotension necessitating vasopressor therapy after randomization and one was diagnosed to have aortic dissection after randomization necessitating emergency surgery. Hence, the ITT population consisted of 69 subjects (61 IS and 8 ICH) who received sham ($n = 25$), standard-dose nVNS ($n = 19$), or high-dose nVNS ($n = 25$). Per protocol set included 68 subjects after exclusion of one subject in the sham group who was subsequently determined to have nonconvulsive status epilepticus mimicking stroke. Patient demographics and baseline characteristics were not different among groups (Table 3).

Ninety-seven percent of all randomized subjects received their first stimulation less than 6 h from stroke onset or last seen well. One-hundred-percent of the ITT population received all prespecified treatment stimulations per protocol. The incidence of composite primary safety endpoint was similar across the 3 treatment arms (Table 4). No deaths, acute coronary syndrome, symptomatic ICH, or stimulation site reactions were recorded. Two patients (8%) in the sham, 2 (11%) in the standard-dose nVNS ($p = 0.100$; standard-dose vs. sham), and 1 (4%) in the high-dose nVNS ($p = 1.00$; high-dose vs. sham) group developed bradycardia following one or more stimulations. Average MAP during the entire treatment period was 94.8 mmHg in the sham, 98.8 mmHg in the standard-dose VNS ($p = 0.231$; standard-dose vs. sham), and
**Fig. 2.** Consort Flow Diagram (ICH indicates intracerebral hemorrhage; mRS, modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scale; nVNS, non-invasive vagus nerve stimulation).

**Table 3**
Demographics and patient characteristics (ITT population).

|                                | Sham (n = 25) | nVNS (n = 44) | Standard-dose nVNS (n = 19) | High-dose nVNS (n = 25) |
|--------------------------------|---------------|---------------|-----------------------------|-------------------------|
| **Age (y), mean (SD)**         | 71 (11)       | 71 (14)       | 74 (13)                     | 68 (13)                 |
| **Female, n (%)**              | 10 (40)       | 17 (39)       | 8 (42)                      | 9 (36)                  |
| **NIHSS score at admission, median (IQR)** | 15 (7, 17) | 13 (10, 15)  | 13 (9, 15)                  | 12 (10, 15)             |
| **Baseline mRS 0, n (%)**      | 23 (92)       | 38 (86)       | 15 (79)                     | 23 (92)                 |
| **Baseline mRS 1, n (%)**      | 2 (8)         | 6 (14)        | 4 (21)                      | 2 (8)                   |
| **Stroke type**                |               |               |                             |                         |
| Ischemic stroke, n (%)         | 24 (96)       | 37 (84)       | 19 (100)                    | 18 (72)                 |
| ICH, n (%)                     | 1 (4)         | 7 (16)        | 0 (0)                       | 7 (28)                  |
| **Vitals at admission**        |               |               |                             |                         |
| Heart rate (bpm), mean (SD)    | 81 (18)       | 84 (20)       | 86 (21)                     | 83 (19)                 |
| Systolic blood pressure (mm Hg), mean (SD) | 154 (28) | 156 (23)   | 150 (20)                    | 161 (25)                |
| Diastolic blood pressure (mm Hg), mean (SD) | 86 (12) | 90 (16)   | 93 (18)                     | 87 (14)                 |
| Mean blood pressure (mm Hg), mean (SD) | 108 (16) | 112 (15) | 112 (17)                    | 112 (14)                |
| **Medical history**            |               |               |                             |                         |
| Hypertension, n (%)            | 19 (76)       | 33 (75)       | 15 (79)                     | 18 (72)                 |
| Diabetes Mellitus, n (%)       | 9 (36)        | 10 (23)       | 3 (16)                      | 7 (28)                  |
| Hyperlipidemia, n (%)          | 3 (12)        | 10 (23)       | 0 (0)                       | 10 (40)                 |
| Coronary heart disease, n (%)  | 9 (36)        | 17 (39)       | 6 (32)                      | 11 (44)                 |
| Congestive heart failure, n (%)| 4 (16)        | 1 (2)         | 0 (0)                       | 1 (4)                   |
| Atrial fibrillation, n (%)     | 9 (36)        | 14 (32)       | 8 (42)                      | 6 (24)                  |
| Rheumatic heart disease, n (%) | 0 (0)         | 1 (2)         | 0 (0)                       | 1 (4)                   |
| Prior transient ischemic attack, n (%) | 1 (4)  | 1 (2)       | 0 (0)                       | 1 (4)                   |
| Prior stroke, n (%)            | 3 (12)        | 6 (14)        | 4 (21)                      | 2 (8)                   |
| Current smoker, n (%)          | 3 (12)        | 6 (14)        | 3 (16)                      | 3 (12)                  |
| Current alcohol use, n (%)     | 2 (8)         | 2 (5)         | 1 (5)                       | 1 (4)                   |
| **Standard of care acute ischemic stroke treatments** | | | | |
| Intravenous thrombolysis, n (%) | 11 (46) | 17 (46) | 11 (58) | 6 (33) |
| Mechanical thrombectomy, n (%) | 6 (25) | 18 (49) | 12 (63) | 6 (33) |
| **Time to treatment**          |               |               |                             |                         |
| Time from witnessed symptoms to administration of the first dose of nVNS, median (IQR) | 4.0 (3.5, 4.7) | 4.3 (3.5, 5.5) | 5.1 (3.4, 5.5) | 4.2 (3.6, 5.3) |
| Time from patient last seen well to administration of the first dose of nVNS, median (IQR) | 4.5 (3.7, 5.3) | 5.0 (3.8, 5.7) | 5.2 (3.9, 5.8) | 4.4 (3.8, 5.5) |

ICH indicates intracerebral hemorrhage; IQR, interquartile range; ITT, intent-to-treat; mRS, modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scale; nVNS, non-invasive vagus nerve stimulation.

Chi-square test or Fisher’s exact test, as appropriate, for categorical variables and t-test or Wilcoxon rank-sum test, as appropriate, for continuous variables comparing sham to total nVNS, sham to standard-dose nVNS, and sham to high-dose nVNS.

Analyses restricted to ischemic stroke patients.
103.6 mmHg in the high-dose VNS groups (p = 0.006; high-dose vs. sham). Four (16%) subjects in the sham, 3 (16%) in the standard-dose nVNS (p = 1.000; standard-dose vs. sham), and 12 (48%) in the high-dose nVNS group (p = 0.032; high-dose vs. sham) experienced ≥20 mmHg reduction in MAP after one or more stimulations. The least squares mean proportion for stimulations associated with MAP reduction was 11% for sham, 11% for standard-dose nVNS (p = 0.985 standard-dose vs. sham), and 2.6% for high-dose nVNS (p = 0.128; high-dose vs. sham). The proportion of stimulations associated with MAP reduction within epochs 1 and 2 in the high-dose nVNS group were 32% and 24%, respectively (p = 0.527). Mean change in MAP from baseline to end of treatment was 0.38 mmHg (95% CI -0.97, 1.72) in the sham group, 0.25 mmHg (95% CI -1.30, 1.61) in the standard dose group, and −0.38 mmHg (95% CI -1.06, 0.51) in the high-dose group (p = 0.711; adjusted for baseline MAP). Peri-stimulation MAP reductions were not associated with any clinical worsening or other adverse events.

Clinical efficacy endpoints were similar between sham and nVNS groups in the IS population (Table 4). Relative ischemic lesion growth was 63% in nVNS and 184% in the sham group (p = 0.109). There was a trend towards reduction in absolute (p = 0.097) and significant reduction in relative (p = 0.005) ischemic growth in the high-dose nVNS group compared to sham in the subgroup with CDM (Supplemental Table 2). In the ICH cohort, the median baseline and 24-h hematoma volumes were 6.9 (4.5–10.8) mL and 5.7 (4.6–12.6) mL, respectively. No subject experienced >30% hematoma growth or clinical deterioration by 24 h post-ICH (Supplemental Table 3).
4. Discussion

This study demonstrates that nVNS is feasible, and potentially safe for treating acute IS. Our findings are consistent with accumulated human experience with nVNS in primary headache disorders [20–24] and implantable VNS in epilepsy and depression. To date, several thousand patients have received implanted VNS since its approval, providing nearly continuous stimulation with no serious safety concerns. nVNS is currently approved for acute treatment and prevention of cluster headache and migraine, and has emergency use authorization for respiratory distress in patients with COVID–19. Our results with nVNS do not indicate a heightened risk for significant side effects in a population that is typically older and more frequently suffer from comorbidities than patients with other approved indications.

Key safety endpoints in the present study included bradycardia and reduction in MAP. Indiscriminant stimulation of fibers in the vagal bundle could potentially cause bradycardia, hypotension, and bronchoconstriction. It has been suggested that nVNS primarily activates the low threshold afferent A-fibers vs. the high threshold efferent C-fibers and, hence, there is minimal risk of adverse cardiac or systemic parasympathetic effects [25]. Our data indicates that nVNS is not associated with reduction in the heart rate or average MAP during the treatment period, or neurological worsening or death within 24 h after initiation of therapy. We observed more incidents of peri-stimulation MAP reductions in the high-dose group. Such MAP reductions were, however, brief, not observed across all stimulations, and not associated with any adverse clinical or tissue outcomes. Similar proportions of stimulations associated with MAP reductions occurred during the first and second 1-h treatment periods in the high-dose nVNS group, suggesting that the total dose, the number of stimulations, overall treatment duration, and extended manipulation of the carotid triangle region were not the likely reasons for the observed MAP reductions. As aforementioned, high-dose nVNS did not cause reduction in average MAP over the entire course of treatment relative to treatment baseline suggesting that discrete events of brief reductions in MAP observed in this study did not result in a compromised hemodynamic state during the hyperacute phase of stroke.

Although the ICH cohort in this study was too small to derive any firm conclusions, we did not observe any important safety signal or increased hematoma growth following nVNS in subjects with ICH. This is in accordance with the animal data where nVNS did not cause increased hemorrhage volume on post-mortem brain samples obtained 24 h after the induction of ICH in both blood injection- and collagenase injection-induced ICH models in rats [26].

nVNS requires positioning of the stimulation electrodes over the skin in the carotid triangle by applying gentle pressure to increase the proximity between the stimulation electrodes and the vagus nerve. Repeated manipulation and stimulation of this area could cause arterial embolism in patients with underlying carotid artery disease. We observed no increase in the number of spatially distinct acute ischemic lesions on follow-up DWI in the overall nVNS-treated population as well as in those with ipsilateral carotid stenosis (>50%). This finding suggests that nVNS, when applied as described in the present study, does not pose a significant risk of raising arterial embolism.

Several mechanisms by which VNS can exert ischemic neuroprotection have been proposed; nVNS augments GABAergic inhibitory pathways [27], reduces blood–brain barrier permeability [13], and suppresses ischemia-induced microglial activation, particularly of the pro-inflammatory M1 phenotype, which is associated with reduced levels of inflammatory cytokines in preclinical models of stroke [10,11,28]. In addition, activation of vagal afferents by nVNS reduces cortical spreading depression and peri-infarction depolarizing waves [12,29,30]. In the subgroup with CDM, we found a reduction in relative infarct growth with high-dose nVNS, providing proof-of-concept that nVNS could be efficacious in the presence of salvageable brain tissue. We acknowledge that, despite positive efficacy signals, this study was not powered to test efficacy outcomes, and therefore, caution should be exercised when interpreting the efficacy data.

As an early-stage study, there are several limitations. The lack of imaging data for all subjects limits our ability to define the level of efficacy. In addition, limited number of ICH patients were enrolled, requiring confirmation of our conclusions regarding this population in a larger dataset. Although we observed an efficacy signal on neuroimaging, long-term efficacy on clinical and radiological metrics will require additional larger studies. Future studies using endpoints that better define the amount of salvageable brain tissue are also needed to evaluate the neuroprotective potential of nVNS.

5. Conclusions

This study supports safety, feasibility, and potential efficacy of nVNS in ischemic and hemorrhagic stroke. nVNS is a simple, non-invasive treatment developed for self-administration by patients for the treatment and prevention of primary headache disorders. If proven safe and effective in stroke, nVNS could be administered by the frontline medical personnel early after stroke onset even before ischemia and hemorrhage distinction is made by neuroimaging.

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Informed consent

Written informed consent was obtained from the patient or their proxies for being enrolled into the study.

Ethical approval

The study was approved by the ethics committee of Hacettepe University and the Turkish Ministry of Health (record number: KA-180098).

CRediT authorship contribution statement

Ethem Murat Arsava: Conceptualization, Methodology, Formal analysis, Investigation, Writing — original draft, Funding acquisition. Mehmet Akif Topcuoglu: Conceptualization, Methodology, Formal analysis, Investigation, Writing — review & editing, Funding acquisition. Ilknur Ay: Conceptualization, Methodology, Writing — review & editing. Atilla Ozcan Ozdemir: Investigation, Writing — review & editing. Ibrahim Levent Gungor: Investigation, Writing — review & editing. Canan Topay Işıkay: Investigation, Writing — review & editing. Bijen Nazli: Investigation, Writing — review & editing. Hasan Huseyin Kozak: Investigation, Writing — review & editing. Serefur Ozturk: Investigation, Writing — review & editing. Babur Dora: Investigation, Writing — review & editing. Hakan Ay: Conceptualization, Methodology, Formal analysis, Writing — original draft, Funding acquisition. Ali Unal: Writing — review & editing.
Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Ethem Murat Arsava Dr Arsava receives honoraria from Fresenius Kabi, Bayer AG, Daiichi-Sankyo, Pfizer, Sanofi, Abbott, and Nutricia. He serves on the advisory boards of Abbott, Daiichi-Sankyo, Bayer AG, Pfizer, Fresenius Kabi, and Nutricia. Mehmet Akif Topcuoglu Dr Topcuoglu receives honoraria from Fresenius Kabi, Daiichi-Sankyo, Sanofi, and Abbott. He serves on the advisory boards for Abbott, Fresenius Kabi, Daiichi-Sankyo, and Pfizer. Hakan Ay Hakan Ay is an employee of Takeda Pharmaceutical Company and holds an academic appointment at the Massachusetts General Hospital, Harvard Medical School. The remaining authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brs.2022.10.012.

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