RESEARCH ARTICLE DOI: 10.5935/0946-5448.20240032

International Tinnitus Journal. 2024;28(2):216-222

Exploring the Novel Impact of Vagal Nerve Stimulation on Pupillometry Measures in Tinnitus Patients

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ABSTRACT

Background: Tinnitus patients may suffer from autonomic symptoms, which can be measured with pupillometry. Tinnitus treatment with vagal nerve stimulation can improve the complaints, and this improvement could be evinced by pupillometry measures.

Objectives: This study aimed to assess the reliability of portable infrared pupillometry, investigate the impact of vagal nerve stimulation on pupillometry measures, and identify predictors of this impact using a comprehensive research methodology.

Methods: Forty-nine tinnitus patients were investigated with a portable infrared pupillometer, and an X-ray of the cervical spine was performed. The pupillometry was compared with a second measure after treatment with vagal nerve stimulation. Multiple linear regression models were used to examine the association between cervical spine measurements, hearing loss at 2 kHz, and maximum constriction difference before and after treatment with vagal nerve stimulation. Logistic regression was performed to study the association between vagal nerve stimulation and sex, hearing loss at 2 kHz, and cervical spine measurements.

Results: Inter-observer reliability was excellent for the baseline pupil diameter and moderate to good for the maximum constriction amplitude. The baseline pupil diameter and the maximum constriction amplitude increased after vagal nerve stimulation in patients with and decreased in patients without effect after treatment. Multiple regression shows a significant positive effect of hearing loss at 2 kHz and intervertebral disc space between the 6th and 7th cervical vertebra and a significant negative impact of the size of anterior osteophyte 5th cervical vertebra on the baseline pupil diameter difference between positive and negative therapeutic effects of vagal nerve stimulation. Logistic regression shows a significant positive effect of the male sex and of hearing loss at 2 kHz and a significant negative impact of the size of anterior osteophyte 5th cervical vertebra on the therapeutic effect of vagal nerve stimulation.

Conclusion: Our study affirms vagal nerve stimulation's potential to positively impact pupillometry measures. The excellent inter-observer reliability for the baseline pupil diameter and the significant increase in pupil diameter and maximum constriction velocity after vagal nerve stimulation provides reassurance and strong encouragement for further research.

Keywords: Vagal nerve stimulation, Pupillometry, Reliability, Tinnitus, Cervical spine, Autonomic nervous system.

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Paper submitted on September 24, 2024; and Accepted on October 04, 2024

INTRODUCTION

Tinnitus has a long-term impact on patients' well-being, and effective therapeutic options are sparse[1]. A promising method for treating chronic tinnitus is vagal nerve stimulation (VNS)[2]. The principle of VNS is the reversal of pathological neuroplastic changes of the auditory cortex in patients with tinnitus toward physiological neural activity and synchronicity[3]. Cranial nerves are unique neuromodulation targets providing high specificity and non-minimally-invasive access to deep brain regions[4]. VNS drives neural activity in Locus Coeruleus (LC), the principal source of noradrenergic modulation for the central nervous system[5]. Noradrenergic modulation provides a specific inhibitory signal to the auditory system, leading to less tinnitus percept[6].

Pupillometry can be utilized as diagnostic tool for tinnitus patients[7]. Baseline pupil diameter, maximum constriction amplitude, and maximum constriction velocity were significantly reduced in tinnitus patients, and this might indicate parasympathetic dysfunction. The approach to therapeutic VNS might benefit from a biomarker with the potential to reveal the activation of the LC to optimize clinical benefits. Therefore, we want to find out if pupillometry represents a biomarker for the successful outcome of VNS. Our study also aims to determine the reliability of pupillometry measurements and to detect the different predictor variables for the pupillometry measurements.

METHODS

Design

This retrospective study comprises all tinnitus patients who underwent pupillometry in Pain Clinic De Bilt between October 2023 and June 2024 (n = 49). The Ethics Committee United (Nieuwegein, the Netherlands) acknowledged this study (W24,188, September 11th 2024).

Data Assessment

The information obtained included clinical information and data of the audiogram, of the cervical spine radiographs, and of the quantitative pupillometry.

Radiograph of the neck

The way the radiographs of the cervical spine are measured is illustrated **(Figure 1)**.

Quantitative Pupillometry

Pupillometry were executed using an automated pupillometer (NeuroLight Algiscan, ID-MED, Marseille, France). Two assessments before therapy, and one assessment after therapy were performed for each eye of the patient. The following parameters were obtained: Baseline Pupil Diameter (BPD) (mm), Latency of Constriction (LC) (msec), pupillary constriction rate (i.e., the difference between baseline and post-stimulation pupil size, expressed as % of constriction from the baseline value) (PCR), Maximum Constriction Amplitude (MCA) (mm), and Maximal Constriction Velocity (MCV) (mm/sec).

Pulsed radiofrequency of the auricular branch of the vagal nerve

An anesthesiologist executed the treatment without sedation. After decontamination, a 22-gauge, 60 mm-long needle with a 5 mm active tip was positioned percutaneously at the inner tragus. Then, pulsed radiofrequency was applied at 42 or 55 V, 2 Hz, and 10 milliseconds for 10 minutes. Patients were re-evaluated seven weeks post-treatment.

Statistics

Statistical analysis was performed using IBM Corp. Released in 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp. The Intraclass Correlation Coefficient (ICC) with 95% confidence intervals was used to compute the inter-observer reliability. As a measure of test-retest agreement for each measure of pupillometry, the Standard Error of Measurement (SEM) was calculated. Using the SEM, the Minimal Detectable Changes (MDC) of the five pupillometry measures was calculated. Mean pupillometry values between patients with and without treatment were compared using unpaired t-tests. Multiple linear regressions analyzed the relationship

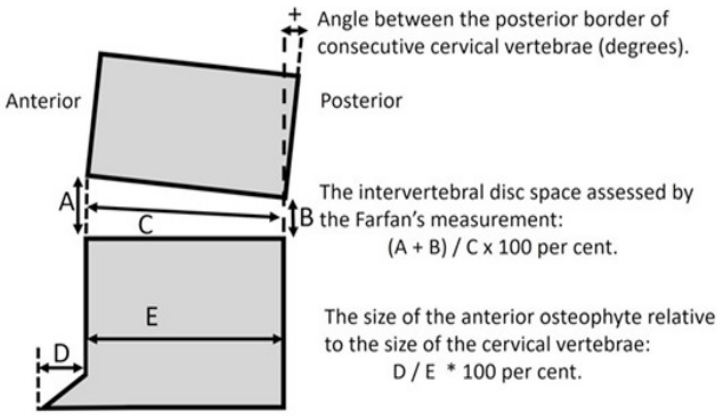

Figure 1: Measurements of the radiographs of the cervical spine.

between baseline pupil diameter difference between positive and negative therapeutic effects and several independent variables. Logistic regression was conducted to study the relationship between therapy and several predictors.

RESULTS

The details of the patients are specified **(Table 1)**. Reliability was calculated from repeated pupillometry measurements **(Table 2)**. Reliability refers to the reproducibility of a measurement. The ICC indicates the inter-observer reliability. A two-way random effect model and absolute agreement were selected. The BPD and MCV measurements showed excellent reliability, and the MCA, PCR, and LC showed moderate to good reliability. The SEM and MDC for BPD, MCA, and MCV were low, and for PCR and LC were high.

Mean pupillometry values between patients with and without effect after treatment were compared using unpaired t-tests **(Table 3)**. Significant lower values were found in patients without treatment effect for BPD and MCA.

Effect size measures of responsiveness were indicated in the form of the effect size index (ES) for the pupillometry measurements **(Table 4)**. The ES scores were calculated by dividing the mean difference scores by the standard deviation of the pupillometry measures before treatment. Cohen has suggested that an effect of 0.20 or less represents a small change, 0.50 means a moderate change, and 0.80 represents a large change[8]. The pupillometry ES values showed a predominantly small to moderate change. Patients with the therapeutic effect of the VNS had a small increase in the BPD and MCA, indicating a small positive sympathetic and parasympathetic effect of VNS **(Figure 2)**. Patients with no impact of VNS on their tinnitus had a small to moderate decrease of the BPD and MCA, indicating a small to moderate reduction of sympathetic and parasympathetic activity following VNS.

The relationship between BPD (mm) difference between positive and negative therapeutic effects and the size of anterior osteophytes, intervertebral disc space, and angle between the posterior border of consecutive vertebrae, sex, age, BMI, and treatment effect was estimated using

Table 1: Patient characteristics and pupillometry values.

Characteristics	Number of patients	%	Mean	SD
Male	19	39		
Female	30	61		
Age (years)			55	12
Body Mass Index			26	4.8
Positive therapeutic effect	22	45		
Negative therapeutic effect	27	55		
Baseline pupil diameter (BPD) (mm)			3.8	0.8
Maximum constriction amplitude (MCA) (mm)			1.1	0.38
Pupillary constriction rate (PCR) (%)			28.5	6.2
Maximum constriction velocity (MCV) (mm/sec)			3.2	1.09
Latency constriction (LC) (msec)			241.1	37.9

SD=standard deviation

Table 2: Interrater reliability of measurements of pupillometry.

Variable	N	ICC	CI(95%)	SEM	MDC
BPD (mm)	49	0.871	0.77 - 0.93	0.28	0.78
MCA (mm)	49	0.67	0.47 - 0.80	0.23	0.63
PCR (%)	49	0.624	0.41 - 0.78	4.12	11.43
MCV (mm/sec)	49	0.768	0.62 - 0.87	0.58	1.6
LC (msec)	49	0.66	0.46 - 0.80	23.8	65.98

ICC: intraclass correlation coefficient, single rater, two-way random, absolute agreement; CI: confidence interval; SEM: standard error of measurement; MDC: minimal detectable change; BPD: baseline pupil diameter; MCA: maximum constriction amplitude; PCR: pupillary constriction rate; MCV: maximal constriction velocity; LC: latency of constriction.

Table 3: Comparison of differences between pretreatment pupillometry values and posttreatment values in patients with and without effect after treatment.

	Effect (SD) (N=22)	No effect (SD) (N=27)	Difference (95% CI)	P value
BPD (mm)	0.08 (0.43)	-0.36 (0.57)	-0.43 (-0.73 to -0.13)	0.005
MCA (mm)	0.04 (0.30)	-0.23 (0.47)	-0.27 (-0.50 to -0.03)	0.026
PCR (%)	-0.09 (4.45)	-3.74 (9.29)	-3.65 (-7.74 to 0.44)	0.079
MCV (mm/sec)	0.08 (0.55)	--0.27 (0.92)	-0.35 (-0.78 to 0.08)	0.108
LC (msec)	-11.89 (46.28)	-6.26 (70.06)	5.63 (-29.39 to 40.64)	0.748

SD: standard deviation; CI: confidence interval; BPD: baseline pupil diameter; MCA: maximum constriction amplitude; PCR: pupillary constriction rate; MCV: maximal constriction velocity; LC: latency of constriction

Table 4: Effect size index from pupillometry measures.

Variable	N	ES
BPD	49	0.2
MCA	49	0.29
PCR	49	0.34
MCV	49	0.1
LC	49	0.23

ES: effect size index; BPD: baseline pupil diameter; MCA: maximum constriction amplitude; PCR: pupillary constriction rate; MCV: maximal constriction velocity; LC: latency of constriction

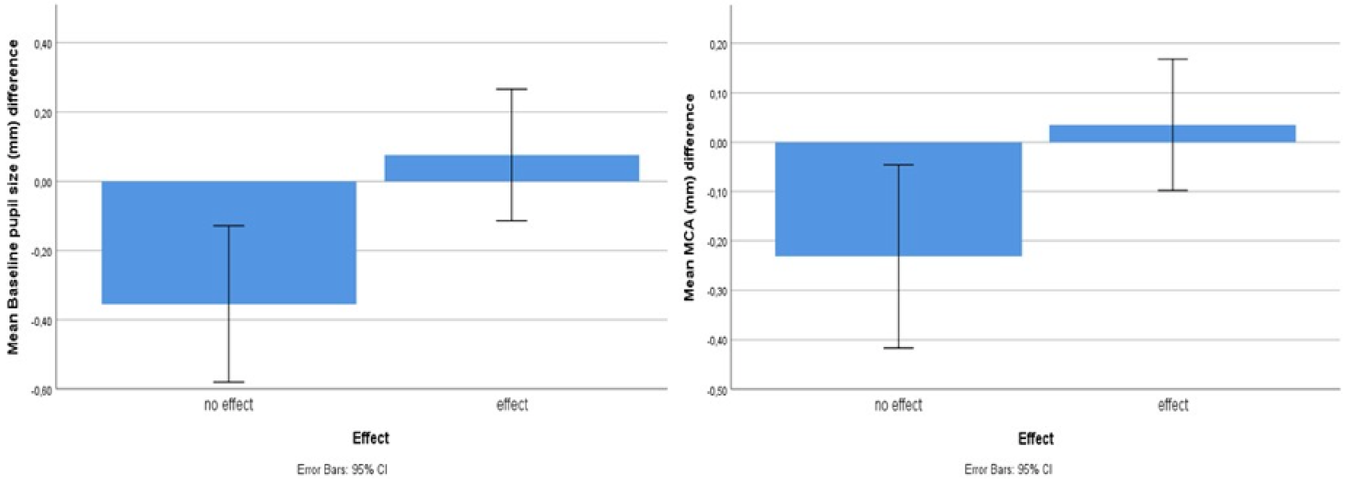

Figure 2: Simple bar charts for mean difference baseline pupil size (mm) and Maximum Constriction Amplitude (MCA) (mm) by effect.

Table 5: Multiple regression shows the effects of intervertebral disc space between the sixth and seventh cervical vertebra, as well as the impact of treatment on baseline pupil diameter (mm) difference between positive and negative therapeutic effects.

Variable	Coefficient	95% CI	Overall P value
Intervertebral disc space between the sixth and seventh cervical vertebra	0.028	0.012, 0.043	0.001
Effect of treatment	0.315	0.033, 0.596	0.029

CI: confidence interval

Table 6: Multiple regression shows the effects of intervertebral disc space between the sixth and seventh cervical vertebra and hearing loss at 2 kHz, as well as the size of anterior osteophyte 5th cervical vertebra on baseline pupil diameter (mm) difference between positive and negative therapeutic effects.

Variable	Coefficient	95% CI	Overall P value
Hearing loss at 2 kHz	0.01	0.003, 0.017	0.006
Size of anterior osteophyte 5th cervical vertebra	-0.022	-0.041, -0.003	0.021
Intervertebral disc space between the sixth and seventh cervical vertebra	0.031	0.014, 0.047	0.001

CI: confidence interval.

multiple regression. Non-significant variables were removed one by one, removing the most significant P value first until all remaining variables in the model were significant **(Table 5)**. The proportion of variance in the dependent variable that the independent variables can account for is 0.364. Better results of VNS are associated with an increase in intervertebral disc space height between the sixth and seventh cervical vertebra.

For multiple linear regression, 20 subjects per independent variable are recommended. Analyses with fewer subjects should be interpreted with greater caution. You should have at least ten outcomes for each independent variable in the model for multiple logistic regression. The relationship between BPD (mm) difference between positive and negative therapeutic effects and the size of anterior osteophytes, intervertebral disc space, and angle between the posterior border of consecutive vertebrae, sex, age, BMI and hearing loss (dB) at 2 kHz were estimated using multiple regression. Non-significant variables were removed one by one, removing the most significant P value until all remaining variables in the model were significant **(Table 6)**. The proportion of variance in the dependent variable that the independent variables can account for is 0.443. The size of the anterior osteophyte of the 5th cervical vertebra decreases the effect of VNS on baseline pupil diameter. Hearing loss at 2 kHz and the intervertebral disc height at C6-C7 increases the effect of VNS on BPD.

Table 7 shows a logistic regression showing the relation between a positive therapeutical effect and the combined effect of sex, hearing loss at 2 kHz, and the size of anterior osteophyte five **(Table 7)**. The Nagelkerke R^2 was 0.714.

The independent variables can account for 71.4% of the variation in therapeutic effect. The overall predictive accuracy is 95.6%. Male gender and hearing loss at 2 kHz increase the chance of a positive outcome of VNS in patients with tinnitus. However, the size of the anterior osteophyte of the 5th cervical vertebra decreases the possibility of a positive outcome effect of VNS.

DISCUSSION

This study used a portable infrared pupillometer to examine 49 tinnitus patients treated with VNS. Twenty-two patients showed a positive effect, and 27 had a negative impact on their tinnitus. We conclude that VNS can reduce the perception of tinnitus, and pupillometry can be used as a biomarker for the effect of VNS in tinnitus. Especially BPD and MCA, measured with pupillometry, are reliable measures associated with the outcome of VNS in patients with tinnitus. Be aware that male gender and hearing loss at 2 kHz increases the chance of a positive result of VNS in patients with tinnitus. Still, the size of the anterior osteophyte of the 5th cervical vertebra has the opposite effect on the chance of a positive outcome effect of VNS.

The vagal nerve (VN) is a mixed nerve with 20% efferent and 80% afferent fibers and is a significant component of the parasympathetic nervous system[9]. The afferent fibers of the VN converge onto two primary nuclei of the brainstem: the nucleus of the solitary tract (NTS) and the spinal trigeminal nucleus[5,9,10]. These nuclei, in turn, project mono- or polysynaptically to a vast network of areas in the central nervous system. The VN primarily projects to the NTS, a critical structure coordinating autonomic and visceral activities[10]. The NTS simultaneously coordinates autonomic information to the nucleus paragigantocellularis (PGi), the central nucleus of the amygdala (CNA), and the C1 adrenergic neurons in the rostral ventrolateral medulla **(Figure 3)**[11-14].

The PGi provides the main excitatory inputs to the LC but also, in a minority of cells, inhibits the LC[11,15]. The PGi carries much of the somatosensory and mechanoreceptor input from the skeletomotor system and auditory input from the cochlear nucleus, the inferior colliculus, and the auditory cortex[16]. The CNA may help to distinguish between ‘physiological’ and ‘psychological’ stressors[17]. The amygdala and LC state actively determine which sensory signals are selected for processing in sensory brain regions, and this process is also involved in the pathogenesis of tinnitus[18]. The C1 adrenergic neurons, activated by somatic and visceral stresses, target various groups of noradrenergic neurons, including the locus coeruleus and the intermediolateral cell column. C1 neurons have outputs to both sympathetic and parasympathetic effectors[14].

The LC’s importance in controlling autonomic function results from both direct pathways to the spinal cord

Table 7: Adjusted odds ratios for effects of sex and hearing loss at 2 kHz and the size of anterior osteophyte four on therapeutic effect.

Variable	Odds ratio	95% CI	P value
Sex (male)	1133.57	10.98, 117037.66	0.003
Hearing loss at 2 kHz	1.21	1.07,1.39	0.004
Size anterior osteophyte 5th cervical vertebra	0.643	0.48, 0.87	0.004

CI: confidence interval.

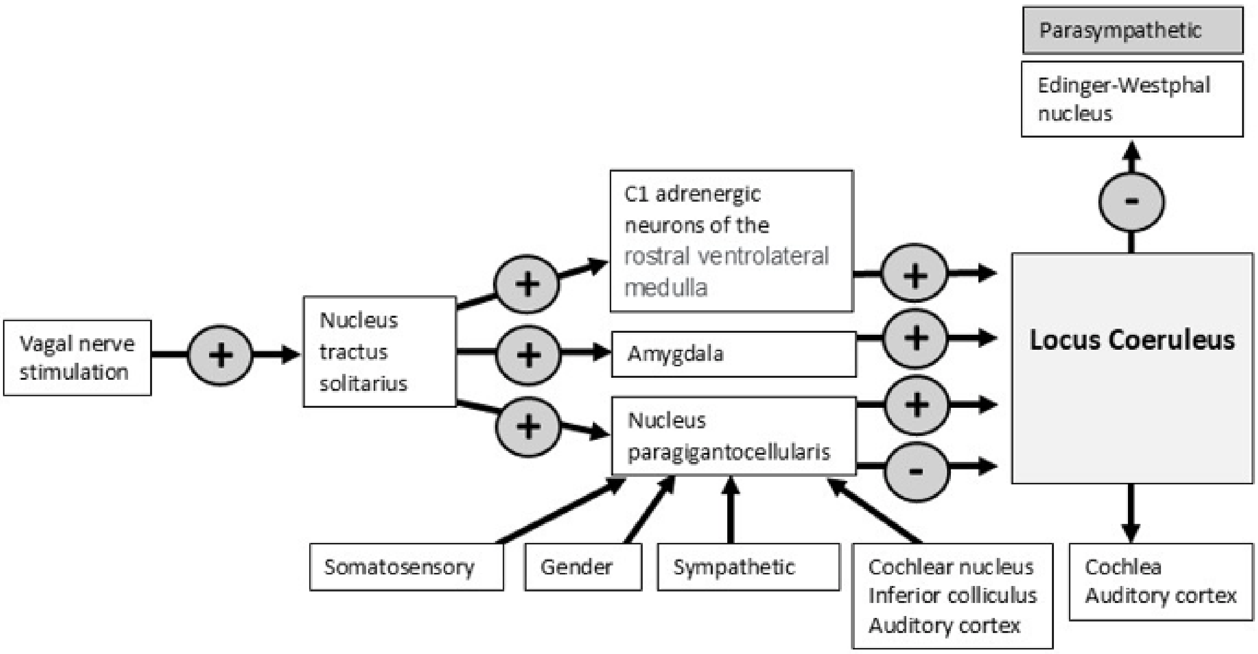

Figure 3: Theoretical model for vagal nerve stimulation in tinnitus patients.

and projections to autonomic nuclei, including the Edinger-Westphal nucleus[19,20]. LC activation bring about an increase in sympathetic activity and a decrease in parasympathetic activity via these projections. Locus coeruleus NA neurons also project to the cochlear nucleus and to the auditory cortex, inhibiting the spontaneous firing of the neurons[6]. Hyperactivity of the LC can explain the findings during pupillometry of tinnitus patients[7].

Patients with the therapeutic effect of the VNS had a slight increase in the BPD and MCA, indicating a small positive sympathetic and parasympathetic effect of VNS. Patients with no impact of VNS on their tinnitus had a slight to moderate decrease in the BPD and MCA, indicating a slight to moderate reduction in sympathetic and parasympathetic activity following VNS. One can say the therapeutic effect of VNS is associated with reducing the activity of the LC, and there is no impact of VNS with the failure to diminish the hyperactivity of the LC. Therefore, the inhibitory pathway of the PGi might play an essential role in the therapeutic effect of VNS for tinnitus.

Pupillometry may help select parameters to optimize VNS for clinical applications. Decreased activity in the LC in response to VNS likely represents the mechanistic link between stimulation current and good therapeutic effect[21]. Our study concludes that pupillometry can be used as a biomarker to assess the impact of VNS on tinnitus. Especially BPD and MCA, measured with pupillometry, are reliable measures associated with the outcome of VNS in patients with tinnitus.

In our study, male gender and hearing loss at 2 kHz increase the chance of a positive outcome of VNS in patients with tinnitus. Still, the size of the anterior osteophyte of the 5th cervical vertebra reduces the effects of VNS in patients with tinnitus. It is already known that optimal autonomic changes induced by VNS depend on stimulating parameters and gender[22]. The inferior colliculus shows a significant female-biased sex difference in the relative number of cells projecting to the PGi[23]. Changes in hormone levels may modulate the excitability of PGi afferents. Large anterior spurs of cervical vertebrae can stimulate the sympathetic, vagal, and glossopharyngeal nerves, resulting in parasympathetic nervous system dysfunction[2]. The PGi in the reticular formation receives direct inputs from sympathetic neurons and auditory input via the inferior colliculus[13,24]. Acoustic stimuli increased the firing of the neurons of the LC[25]. Hence, it seems that PGi acts as a relay station and that less auditory information and less sympathetic activation of the PGi might improve the outcome of VNS. Thus, stimulus parameters, sex difference, hearing loss at 2 kHz, and the size of the anterior osteophyte of the 5th cervical vertebra should be considered, and the optimal dose in the clinical application of VNS to ensure that patients receive the maximum possible benefit from the treatment.

Our findings should be interpreted in light of several limitations inherent in our study. The retrospective study design and the total number of patients involved are significant limitations. A prospective study with a more substantial number of patients is recommended.

CONCLUSION

Vagal nerve stimulation can reduce the perception of tinnitus, and pupillometry can be used as a biomarker for the effect of VNS in tinnitus. Especially baseline pupil diameter and maximum contraction amplitude, measured with pupillometry, are reliable measures associated with the outcome of VNS in patients with tinnitus. Male gender and hearing loss at 2 kHz increase the chance of a positive outcome of VNS in patients with tinnitus. Still, the size of the anterior osteophyte of the 5th cervical vertebra has the opposite effect on the chance of a positive outcome effect of VNS.

CONFLICTS OF INTEREST AND SOURCE OF FUNDING

The authors declare no conflict of interest.

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