RESEARCH ARTICLE

Acute right-sided transcutaneous vagus nerve stimulation improves cardio-vagal baroreflex gain in patients with chronic heart failure

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Abstract

Purpose The aim of this paper is to investigate the acute effects of short-term transcutaneous vagus nerve stimulation (tVNS) on cardio-vagal baroreflex gain and heart rate variability in patients with chronic heart failure (CHF).

Methods A total of 16 adults with CHF and left ventricular ejection fraction (LVEF) < 50% in sinus rhythm were enrolled (65 ± 8 years, 63% men, LVEF 40 ± 5%, 88% on beta-blockers, 50% on quadruple CHF therapy). Over a single experimental session, after a 10-min baseline recording, each patient underwent two trials of 10-min tVNS (Parasym Device, 200 μs, 30 Hz, 1 mA below discomfort threshold) at either the right or left tragus in a randomized order, separated by a 10-min recovery.

Results Compared with baseline, tVNS did not affect heart rate, blood pressure, and respiratory rate ($p > 0.05$), and no patients complained of discomfort or any adverse effect. Right-sided tVNS was associated with a significant increase in cardio-vagal baroreflex gain (from 5.6 ± 3.1 to 7.5 ± 3.8 ms/mmHg, Δ 1.9 ± 1.6 ms/mmHg, $p < 0.001$), while no change was observed with left-sided tVNS (Δ 0.5 ± 2.0 ms/mmHg, $p = 0.914$). These findings were independent of stimulation-side order (excluding any carry-over effect) and consistent across sex, LVEF category, and HF etiology subgroups (p-value for interaction > 0.05).

Conclusions Acute right-sided tVNS increases cardio-vagal baroreflex gain in patients with CHF and LVEF < 50%, with no tolerability concerns.

Keywords Transcutaneous vagus nerve stimulation · tVNS · Tragus · Baroreflex · Heart rate variability · Neuromodulation · Heart failure

Abbreviations

BMI	Body mass index
BP	Blood pressure
CHF	Chronic heart failure
HF	High frequency
HFmrEF	Heart failure with mildly reduced ejection fraction
HFpEF	Heart failure with preserved ejection fraction
HFrEF	Heart failure with reduced ejection fraction
HRV	Heart rate variability
LF	Low frequency
LVEF	Left ventricular ejection fraction
RMSSD	Root mean square of successive differences
SD	Standard deviation
SDNN	Standard deviation of all normal-to-normal intervals
tVNS	Transcutaneous vagus nerve stimulation

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Introduction

Autonomic imbalance, characterized by increased sympathetic activity and vagal withdrawal, is a pathophysiological determinant of chronic heart failure (CHF) [1]. Although direct evidence linking increased sympathetic nerve activity, assessed through microneurography, to morbidity and mortality in CHF patients is limited [2], substantial indirect evidence supports the critical role of sympathovagal imbalance [3–6], sustained by the resetting of visceral feedbacks (namely baroreflex, chemoreflex, and ergoreflex) [7–11], in these patients.

While the vascular–sympathetic limb of the barore-flex is the main determinant of blood pressure regulation, reduced cardio-vagal baroreflex gain has demonstrated strong prognostic significance in patients with cardiovascular disease. Most notably, in the ATRAMI study, which enrolled 1284 patients with recent myocardial infarction, a lower cardio-vagal baroreflex gain was associated with a threefold higher risk of cardiac death during follow-up, independent of confounders [7]. These findings have been confirmed in patients with CHF and were not affected by the use of beta-blockers [8] or other anti-neurohormonal drugs [9]. Although the precise mechanisms remain unknown, the evaluation of cardio-vagal baroreflex gain has been proposed as a surrogate of the parasympathetic cardiovascular control and, therefore, of the overall autonomic balance. Indeed, although reduced cardio-vagal baroreflex gain was associated with other markers of disease severity [8, 9], clinical variables explained only 43% of its variance in patients with CHF [8]. Accordingly, the use of baroreceptor activation therapy (BAT) has been tested in CHF patients, showing benefits on quality of life, exercise capacity, and levels of neurohormonal activation [12]. Nevertheless, data on hard outcomes are awaited, while the invasive nature of BAT remains a major drawback. [13]

The low-level electrical stimulation of the afferent fibers of the auricular branch of the vagus nerve [i.e., trans-cutaneous vagus nerve stimulation (tVNS)] is emerging as a noninvasive alternative to improve sympathovagal balance [14]. On the basis of encouraging preclinical studies [15–17], tVNS has been shown to reduce sympathetic activity and increase heart rate variability (HRV) in healthy individuals [18, 19], and to improve quality of life, and inflammatory markers in patients with CHF with preserved left ventricular ejection fraction [HFpEF; left ventricular ejection fraction (LVEF) $\geq$ 50%] [20]. Though the precise mechanisms are still unclear, some evidence from healthy individuals suggests that the beneficial effects of tVNS may be associated with an upward resetting of baroreflex operating point [19, 21]. Nevertheless, the autonomic effects of tVNS have never been tested in patients with CHF with mildly reduced LVEF (HFmrEF; LVEF 41–49%) and patients with CHF with reduced LVEF (HFrEF; LVEF $\leq$ 40%), who could benefit from this neuromodulation strategy. [22]

The optimal stimulation side (i.e., right versus left ear) is however unclear [23]. On the basis of the preclinical evidence that the stimulation of the right vagus may result in a stronger effect on heart rate [24, 25], the left side has been arbitrarily chosen in most clinical studies testing tVNS to minimize bradycardia, even if in the absence of well-designed comparative studies. [23]

Therefore, this study aimed to assess the effects of right-sided versus left-sided tVNS on cardio-vagal baroreflex gain in patients with CHF and LVEF < 50% on guideline-recommended therapies.

Methods

Subjects

The research protocol has been approved by the locally appointed ethics committee, according to the Declaration of Helsinki and privacy rules. The presented findings are derived from the pilot phase of the Transcutaneous Vagus Nerve Stimulation in Patients with Chronic Heart Failure (TRAGUS-HF; NCT06355388) study, which will explore the autonomic, biohumoral, and functional consequences of acute and chronic tVNS in patients with HFmrEF and HFrEF.

Consecutive stable adults with a diagnosis of CHF and LVEF < 50% according to the latest guidelines were screened to be enrolled in the study [26]. Only patients in sinus rhythm were selected, and patients with implanted cardiac devices [namely, pacemakers, implantable cardioverter-defibrillators, cardiac resynchronization therapy, BAT, invasive vagus nerve stimulation (VNS), and cardiac contractility modulation] were excluded. Other exclusion criteria were any condition of clinical instability including acute coronary syndrome, acute decompensated heart failure, or therapy changes within 3 months; severe renal, hepatological, and pulmonary diseases; and neurological conditions associated with dysautonomia (e.g., diabetic neuropathy and Parkinson's disease).

All patients underwent a comprehensive cardiological evaluation, including clinical assessment [i.e., anthropometric measures, comorbidities, New York Heart Association (NYHA) functional class, and therapies] and resting transthoracic echocardiography (Philips iE33 or EPIQ7, Andover, Massachusetts, USA) to assess LVEF. [27]

Study procedures

Following the clinical assessment and a first familiarization with the laboratory environment and instrumentation, each patient underwent the study session on a different day, in the same quiet environmental conditions and time window (between 11 a.m. and 4 p.m.). All patients were asked not to consume coffee, tea, or alcohol in the 12 h before the study, to avoid large meals and physical exercise in the 6 h before the study, and to empty their bladder before the protocol. The intake of prescribed drugs was not changed.

All recordings were performed with the experimental subject lying semi-recumbent on a chair with the back at

45° and were asked to not speak, to breathe normally, and to stay awake. The following signals were then acquired: surface echocardiogram (ECG) through three chest electrodes (BioAmp, PowerLab, ADInstruments, Sydney, Australia), sampled at 2 kHz (bandpass 0.3 Hz–1 kHz); beat-to-beat finger blood pressure (BP) by pulse plethysmography (Finapres Medical Systems BV, Enschede, Netherlands), sampled at 400 Hz and calibrated with an arm sphygmomanometer; and respiratory rate through a belt transducer (ADInstruments), sampled at 100 Hz. All signals were stored on a computer via a data acquisition and analysis system (PowerLab 16SP™ and LabChart™, v7.1.2.5 software; ADInstruments).

Study protocol and tVNS

For each patient, the protocol lasted consecutive 40 min. The first 10 min consisted of signals recording without tVNS, i.e., "baseline." Left-sided or right-sided tVNS (as detailed below) was then applied. The order allocation (left-sided tVNS versus right-sided tVNS first) was randomly selected (1:1) for each patient, and the two tVNS phases were separated by a 10-min recovery. The Parasym Device (Parasym Ltd, London, UK) was used to deliver tVNS at the tragus level, as previously reported [20]. The electrical current was continuously applied for each phase with the following parameters: pulse amplitude 200 μs, frequency 30 Hz, and intensity 1 mA below the discomfort threshold (as assessed for each patient and stimulation side 30 min before starting the protocol).

Data analysis

All the recorded signals were analyzed by one of the investigators (E.D.E.), blinded to the allocation of experimental phases (i.e., baseline, left/right-sided tVNS, and recovery).

For each phase, the signals recorded in the last 5 min were averaged and analyzed. Respiratory and BP signals were analyzed through LabChart™. Heartbeat series were extracted by using the HRV tool of LabChart™ and analyzed through Kubios (Kubios HRV 2.2, Kuopio, Finland). The raw normal-to-normal (NN) interval tachograms were visually inspected to assess the quality of signal acquisition, and artifacts were automatically corrected using a piecewise cubic spline interpolation method. A first-order detrending method was applied to remove low-frequency aperiodic trends. From each series, heart rate variability (HRV) was assessed according to the Task Force rules [28]. For time-domain HRV, the standard deviation of the NN intervals (SDNN) and the root mean square of the successive differences between NN intervals (rMSSD) were calculated [28]. For frequency-domain HRV, a power spectrum analysis of NN intervals using a fast Fourier transform was performed, applying a Welch's periodogram (256 s window with 50%

overlap) to reduce spectral leakage. The areas under the low frequency (LF; 0.04–0.15 Hz) and high-frequency (HF; 0.15–0.40 Hz) band were calculated and expressed as power (HF_{power} and LF_{power}, ms^2), Ln-transformed power, normalized units [$HF_{nu} = HF/(HF + LF)$ and $LF_{nu} = LF/(HF + LF)$, n.u.], and LF/HF ratio [28].

Cardio-vagal baroreflex gain was calculated through the standard deviation (SD) method, as the ratio between SDNN and the corresponding SD in systolic BP (SDsBP) [29].

$$Cardio-vagal\ baroreflex\ gain\ (ms/mmHg)$$
$$= SDNN\ (ms)/SDsBP\ (mmHg)$$

Briefly, this method is based on the ratio between the global rather than specific variabilities of the successive NN intervals and sBP [29]. Validated against other six established methods for spontaneous cardio-vagal baroreflex gain assessment [29], the standard deviation method showed prognostic significance in a large cohort of CHF patients [9]. As originally proposed, cardio-vagal baroreflex gain was calculated for 5-min series, after linear detrending and interpolation of ectopic beats. [29]

Statistical analysis

Statistical analysis was performed by using SPSS (version 25.0, 2017, IBM Statistics, Armonk, New York, USA), and R software (version 3.4.0), and a two-tailed p-value ≤ 0.05 was considered significant. Quantitative values were tested for normal or skewed distribution (Kolmogorov–Smirnov test) and reported as mean $\pm$ standard deviation (SD), or median (interquartile range), as appropriate, and qualitative values as numbers or percentages.

Baseline data were reported and compared between HFmrEF versus HFrEF patients.

As anticipated, the values of vital parameters, cardio-vagal baroreflex gain, and time- and frequency-domain HRV averaged in the last 5 min of baseline recording, left-sided, and right-sided tVNS were averaged and compared. The baseline was considered the reference category. A one-way analysis of variance (ANOVA) test for repeated measures was used to compare variables across the protocol phases. In the case of statistical significance, pairwise comparison were performed using a Bonferroni post hoc test. Subgroup analyses were performed to assess the possible influence of simulation-side order allocation (left-first versus right-first), patient sex (men versus women), LVEF class (HFmrEF versus HFrEF), and CHF etiology (ischemic versus nonischemic) on the effects of tVNS on cardio-vagal baroreflex gain.

Results

Patient population

Out of 18 patients selected, 2 were excluded since they were unable to maintain the semi-recumbent position for the whole duration of the study protocol, due to orthopedic reasons. Finally, 16 patients with either HFmrEF or HFrEF (8 per group) were included in the study (Table 1). The mean age was 65 ± 8 years, most patients (63%) were men, and half had an ischemic etiology of CHF, with a mean LVEF of 40 ± 5%. Patients were mildly symptomatic (NYHA class II in 63% of the cases), and received an optimal medical therapy as recommended by the latest guidelines. Of note, 88% of the patients were on beta-blockers, titrated up to 38% (25–94%) of the recommended target dose.

At the baseline multichannel recording, in line with the use of beta-blockers, patients had mild bradycardia (mean heart rate 59 ± 11 bpm), while beat-to-beat BP values were in the normal ranges (Table 2). The mean cardio-vagal baroreflex gain was 5.6 ± 3.1 ms/mmHg, and time-domain and frequency-domain HRV parameters were significantly lower compared with reference values in the general population (Table 2).

Except for body mass index and LVEF, no significant differences were observed comparing patients with either HFmrEF or HFrEF (Tables 1, 2).

Acute effects of tVNS

The maximum tolerated current intensity was similar for left-sided and right-sided tVNS, with a median value of 23 (range 10–44) mA at the left tragus and 23 (range 12–33) mA at the right tragus, respectively. No patients complained of discomfort or any other adverse effects during the stimulation phases.

As detailed in Table 3, tVNS did not affect vital parameters, observing only mild and nonsignificant reductions in heart rate and increase in BP values over the protocol.

Right-sided—but not left-sided—tVNS was associated with a significant increase in cardio-vagal baroreflex gain with a Δ of 1.9 ± 1.6 ms/mmHg from baseline (Bonferroni-corrected p-value < 0.001; Fig. 1). The association between right-sided tVNS and cardio-vagal baroreflex gain increase was confirmed at subgroup analysis, comparing stimulation-side order (p-value for interaction 0.59), patient sex (p-value

Table 1 Characteristics of the study population distinguished into HFmrEF and HFrEF categories

Variables	All patients ($n=16$)	HFmrEF ($n=8$)	HFrEF ($n=8$)	p-Value
Clinical features				
Age, years	65 ± 8	66 ± 8	64 ± 9	0.620
Men, n (%)	10 (63)	6 (75)	4 (50)	0.608
BMI, kg/m^2	27 ± 6	30 ± 5	23 ± 5	0.013
Ischemic etiology, n (%)	8 (50)	4 (50)	4 (50)	1.000
Hypertension, n (%)	8 (50)	6 (75)	2 (25)	0.132
Diabetes, n (%)	4 (25)	2 (5)	2 (25)	1.000
COPD, n (%)	2 (13)	0 (0)	2 (25)	0.467
NYHA class I, n (%)	6 (37)	3 (37)	3 (37)	1.000
NYHA class II, n (%)	10 (63)	5 (63)	5 (63)	1.000
LVEF, percentage (%)	40 ± 5	44 ± 3	36 ± 3	< 0.001
Treatments				
Beta-blockers, n (%)	14 (88)	8 (100)	6 (75)	1.000
Beta-blockers, dose percentage (%)	38 (25–95)	38 (24–94)	44 (26–91)	0.970
ACEi/ARB, n (%)	4 (25)	2 (25)	2 (25)	1.000
ARNI, n (%)	11 (69)	5 (63)	6 (75)	1.000
MRA, n (%)	15 (94)	7 (88)	8 (100)	1.000
SGLT2i, n (%)	8 (50)	4 (50)	4 (50)	1.000
Furosemide, n (%)	3 (19)	2 (25)	1 (13)	1.000

Values are mean $\pm$ SD, median (interquartile interval), or n (%). *ACEi* angiotensin-converting-enzyme inhibitors, *ARB* angiotensin receptor blockers, *ARNI* angiotensin receptor–neprilysin inhibitors, *BMI* body mass index, *COPD* chronic obstructive pulmonary disease, *HFmrEF* heart failure with mildly reduced ejection fraction, *HFrEF* heart failure with reduced ejection fraction, *LVEF* left ventricular ejection fraction, *MRA* mineralocorticoid receptor antagonists, *NYHA* New York Heart Association, *SGLT2i* sodium–glucose cotransporter-2 inhibitors

Table 2 Baseline vital parameters and autonomic assessment in the study population distinguished into HFmrEF and HFrEF categories

Variables	All patients ($n=16$)	HFmrEF ($n=8$)	HFrEF ($n=8$)	p-Value
Time domain				
Respiratory rate, breaths per minute	18 ± 4	19 ± 4	16 ± 4	0.164
Heart rate, bpm	59 ± 11	60 ± 13	59 ± 8	0.842
NN, ms	1042 ± 169	1040 ± 193	1043 ± 155	0.968
SDNN, ms	25 ± 7	25 ± 9	25 ± 6	0.947
rMSSD, ms	25 ± 20	26 ± 22	25 ± 20	0.913
Systolic BP, mmHg	106 ± 13	111 ± 11	100 ± 12	0.072
Diastolic BP, mmHg	60 ± 11	64 ± 13	57 ± 9	0.229
Mean BP, mmHg	76 ± 11	80 ± 11	73 ± 10	0.201
Systolic BP-SD, mmHg	4.9 (3.5–6.4)	5.2 (3.5–11.3)	4.7 (3.4–5.7)	0.584
Baroreflex gain, ms/mmHg	5.6 ± 3.1	5.1 ± 2.9	6.2 ± 3.4	0.497
Frequency domain				
Total power, ms^2	763 (236–929)	817 (169–1260)	574 (254–874)	0.833
LF power, ms^2	139 (57–232)	136 (79–193)	139 (56–241)	0.916
Ln(LF)	4.9 ± 0.8	4.8 ± 0.9	5.1 ± 0.7	0.232
LF, n.u.	58 ± 21	61 ± 22	56 ± 21	0.649
HF power, ms^2	94 (39–182)	113 (36–182)	88 (41–221)	0.916
Ln(HF)	4.7 ± 1.3	4.6 ± 1.3	4.9 ± 1.4	0.306
HF, n.u.	42 ± 21	39 ± 21	45 ± 21	0.615
LF/HF	1.9 ± 1.3	2.0 ± 1.1	1.8 ± 1.6	0.783

Values are mean $\pm$ SD and median (interquartile interval). *BP* blood pressure, *HF* high frequency, *HFmrEF* heart failure with mildly reduced ejection fraction, *HFrEF* heart failure with reduced ejection fraction, *LF* low frequency, *NN* normal-to-normal heartbeat interval, *rMSSD* root mean square of the successive differences between normal-to-normal intervals, *SDNN* standard deviation of the normal-to-normal intervals

for interaction 0.17), LVEF category (p-value for interaction 0.85), and CHF etiology (p-value for interaction 0.38; Fig. 2).

As for HRV, compared with the baseline, right-sided tVNS was associated with nonsignificant changes toward vagal predominance (Fig. 3; all $p > 0.05$).

Discussion

This is the first study evaluating the acute effects of tVNS, obtained through the Parasym Device, in patients with CHF and LVEF $<50\%$. In this cohort, tVNS was well-tolerated, and no adverse effects were reported. Right-sided tVNS was associated with a statistically significant increase in cardio-vagal baroreflex gain, accompanied by nonsignificant changes in HRV parameters toward vagal predominance, while left-sided tVNS was not. These findings were consistent independently of stimulation side order, patient sex, CHF etiology, and LVEF category.

Despite the recent advances in drug and device treatment, patients with CHF still are at high risk of recurrent hospitalizations, malignant arrhythmias, and early mortality [26]. Since residual sympathovagal imbalance contributes to disease progression and adverse events even in patients on

optimal therapy [1, 9], novel approaches for neuromodulation have been proposed not only to decrease sympathetic activity but also to increase vagal signaling [22, 30]. Despite encouraging preliminary findings, no strategy has shown satisfactory results in clinical studies such that it has been translated into routine practice so far [30]. Most notably, while invasive VNS failed to show clinical benefits in the context of randomized trials [31], optimal criteria for patient selection and the invasive nature have limited the use of BAT despite its potential efficacy. [12, 13]

To overcome these limits, tVNS has been proposed as a noninvasive alternative to improve sympathovagal balance [30]. After various preclinical studies demonstrating that tVNS may improve sympathovagal balance and prevent adverse cardiac remodeling [15, 17, 32], preliminary clinical studies have shown some benefits in the short term for patients with myocardial infarction [33], and in the medium term for those with atrial fibrillation [34], or HFpEF. [20]

To date, the autonomic effects of tVNS have been tested almost exclusively in healthy subjects, mainly evaluating HRV as the endpoint and reporting mixed findings [35]. Considering that multiple endogenous and/or exogenous factors may influence HRV (e.g., respiration, body temperature, comorbidities, etc.) [28, 36], the use of alternative markers of autonomic function has been advocated to

Table 3 Comparisons of vital parameters and autonomic measures in the study population between baseline recording and tVNS phases

Variables	Baseline	Left tVNS 23 (10–44) mA	Right tVNS 23 (12–33) mA	ANOVA main effect *p*-Value
Time domain				
Respiratory rate, breaths per minute	18 ± 4	17 ± 3	18 ± 3	0.110
Heart rate, bpm	59 ± 11	59 ± 11	58 ± 10	0.189
NN, ms	1042 ± 169	1051 ± 174	1057 ± 170	0.274
SDNN, ms	25 ± 7	31 ± 12	33 ± 17	0.127
rMSSD, ms	25 ± 20	26 ± 18	28 ± 21	0.378
Systolic BP, mmHg	106 ± 13	107 ± 11	108 ± 13	0.508
Diastolic BP, mmHg	60 ± 11	62 ± 10	61 ± 10	0.508
Mean BP, mmHg	76 ± 11	78 ± 10	77 ± 10	0.568
Systolic BP-SD, mmHg	4.9 (3.5–6.4)	4.6 (3.9–7.3)	4.7 (3.8–6.1)	0.507
Baroreflex gain, ms/mmHg	5.6 ± 3.1	6.1 ± 2.4	7.5 ± 3.8	0.003[*]
Frequency domain				
Total power, ms^2	763 (236–929)	765 (482–1125)	717 (483–1282)	0.589
LF power, ms^2	139 (57–232)	122 (76–178)	130 (75–229)	0.848
Ln(LF)	4.9 ± 0.8	4.9 ± 0.8	4.9 ± 0.9	0.804
LF, n.u	58 ± 21	53 ± 24	50 ± 24	0.138
HF power, ms^2	94 (39–182)	104 (41–164)	138 (54–311)	0.571
Ln(HF)	4.5 ± 1.5	4.6 ± 1.4	4.9 ± 1.3	0.143
HF, n.u.	42 ± 21	47 ± 24	50 ± 24	0.129
LF/HF	1.9 ± 1.3	1.9 ± 1.7	1.7 ± 1.6	0.559

Values are mean ± SD and median (interquartile interval). *BP* blood pressure, *HF* high frequency, *HFmrEF* heart failure with mildly reduced ejection fraction, *HFrEF* heart failure with reduced ejection fraction, *LF* low frequency, *NN* normal-to-normal heartbeat interval, *rMSSD* root mean square of the successive differences between normal-to-normal intervals, *SDNN* standard deviation of the normal-to-normal intervals, *tVNS* transcutaneous vagus nerve stimulation. [*]Bonferroni post hoc test pairwise comparisons *p*-values: left tVNS versus baseline *p* = 0.914; right tVNS versus baseline *p* < 0.001; and left tVNS versus right tVNS *p* = 0.163

assess tVNS efficacy. In this respect, while direct neural recording represents the gold standard method, the assessment of cardio-vagal baroreflex gain has been proposed as well [19, 21]. Indeed, among patients with CHF, reduced cardio-vagal baroreflex gain was associated with a worse clinical profile and identified as an independent predictor of mortality in multivariable regression analyses. [8, 9]

For the first time, in the present study, the tolerability and potential efficacy of acute tVNS were tested in patients with CHF and systolic dysfunction, which are characterized by a more severe sympathovagal imbalance [1, 37]. Accordingly, in our cohort, both baroreflex [38] and HRV measures [39] were significantly lower compared with those reported in the general population, despite the fact that most of the patients were receiving optimized therapies according to the latest guidelines (88% on beta-blockers, 69% on sacubitril-valsartan, 94% on mineralo-corticoid-receptor antagonists, and 50% on sodium–glucose cotransporter-2 inhibitors). Of note, right-sided tVNS was associated with a mean 34% increase in cardio-vagal baroreflex gain (*p* < 0.001), while no significant changes were observed for HRV parameters. Furthermore, tVNS

was not associated with any adverse effects or discomfort for the patients.

Though the precise link between tVNS and baroreflex is unclear, similar findings had been previously reported in healthy subjects [19, 21]. In the study by Antonino et al., 15-min active tVNS, but not sham stimulation, was associated with a mean 24% increase in cardio-vagal baroreflex gain compared with baseline values in 13 volunteers [21]. Similar findings were reported by Bretherton et al. in 69 individuals aged ≥ 55 years free of cardiovascular disease, in which tVNS was associated with a mean 22% increase in cardio-vagal baroreflex gain. [19]

The mechanisms behind this relation, as well as the potential clinical implications, are not completely understood [40]. By using functional magnetic resonance during tVNS, a consistent activation of the nucleus tractus solitarius has been documented [41]. Since the nucleus tractus solitarius represents the main relay station for arterial baroreflex afferents [42], a central interaction between tVNS and baroreflex gain has been hypothesized. In this respect, also the direct stimulation of afferent vagal fibers has been associated with improved baroreflex function in an experimental rat model
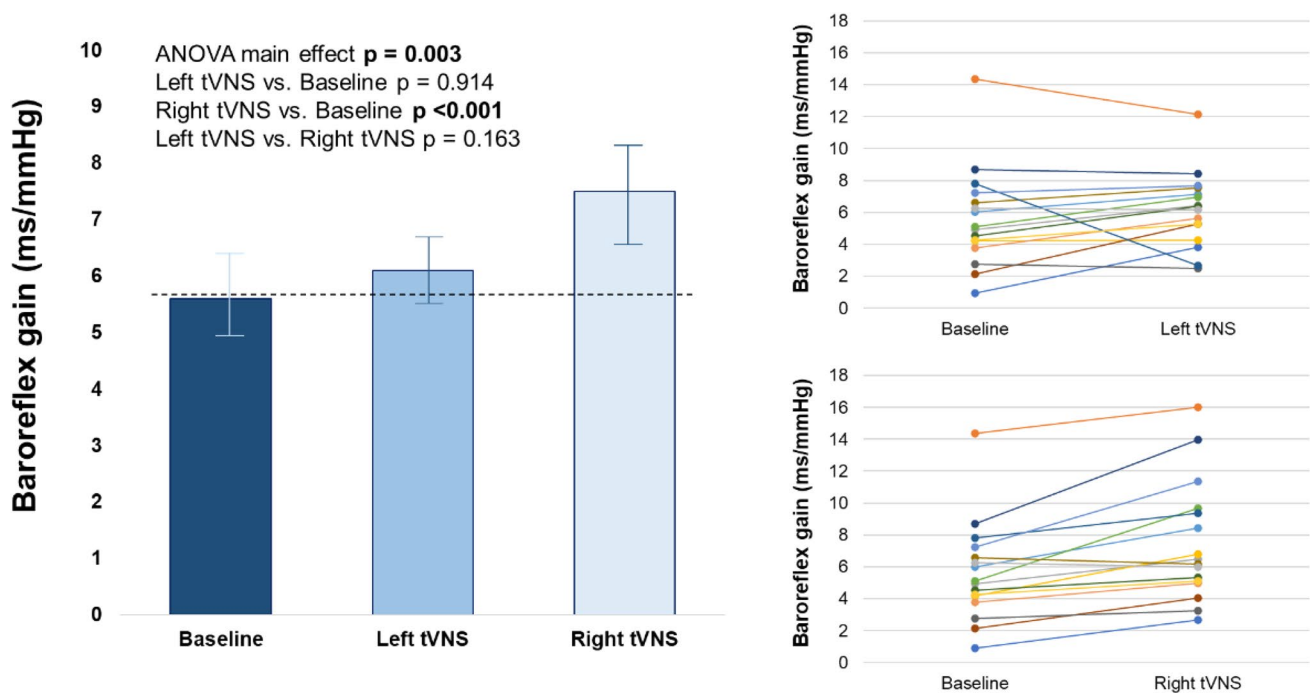
Fig. 1 Effects of tVNS on cardio-vagal baroreflex gain in patients with chronic heart failure. Acute right-sided—but not left-sided—tVNS increased cardio-vagal baroreflex gain in the study population. Each patient underwent left-sided and right-sided tVNS during the same experimental session, in a randomized order, and separated by a 10-min recovery. One-way ANOVA for repeated measure was used, with Bonferroni correction for post hoc pairwise companions. *tVNS* transcutaneous vagus nerve stimulation

of myocardial infarction [43]. However, since baroreceptors also modulate sympathetic efferences, cardio-vagal baroreflex gain cannot be considered independent by other hemodynamic and autonomic influences. Therefore, to gain a deeper understanding of the autonomic effects of tVNS, future studies evaluating both the cardio-vagal and vascular-sympathetic limbs of baroreflex are warranted.

While the used stimulation side had not been reported in the previous studies [19, 21], only right-sided tVNS was associated with improved cardio-vagal baroreflex gain in this work. While concerns about the safety of right-sided stimulation had been hypothesized for invasive VNS [24, 25], due to the potential risk of a stronger sinus node inhibition [24, 25], right and left vagus stimulation were associated with similar changes in heart rate in the CHF patients enrolled in the ANTHEM-HF trial [44]. Furthermore, while left-sided stimulation has been arbitrarily chosen in most of the clinical studies conducted so far, no adverse effects have been reported with either right-sided or left-sided tVNS [23]. Interestingly, some functional asymmetry has been reported for baroreflex function. Indeed, right carotid baroreflex activation was more effective than left stimulation in modulating HRV [45, 46], and a role of the ipsilateral central projections to the nucleus tractus solitarius was hypothesized. While the clinical efficacy of right versus left BAT has not been compared in CHF so far, right-sided BAT ($n=127$) was more

effective than left-sided BAT ($n=88$) in lowering BP among 215 patients with resistant hypertension. [47]

Whether the stimulation side may affect the efficacy of tVNS on other endpoints beyond cardio-vagal baroreflex gain remains to be investigated. Nevertheless, taken together, these findings underscore the importance of comparing and reporting the stimulation side when testing novel neuromodulation strategies.

Study limitations

The small sample size may not allow for the immediate translation of the findings to other cohorts, considering the heterogeneity characterizing the CHF population. Nonetheless, the inclusion of patients on stable optimized medical therapy followed-up at the outpatient clinic of our tertiary center, the study cohort may constitute a snapshot of modern well-treated real-life CHF patients. In the absence of definitive evidence about the interactions with the Parasym Device, only patients with no implantable cardiac devices were enrolled. Though no safety issues were reported in patients with pacemakers [20], dedicated studies should assess the safety of tVNS in this subset. In the present study, no "sham stimulation" protocol was performed: while the optimal option for a reliable "sham stimulation" is an object of debate due to the observation that lobe stimulation may
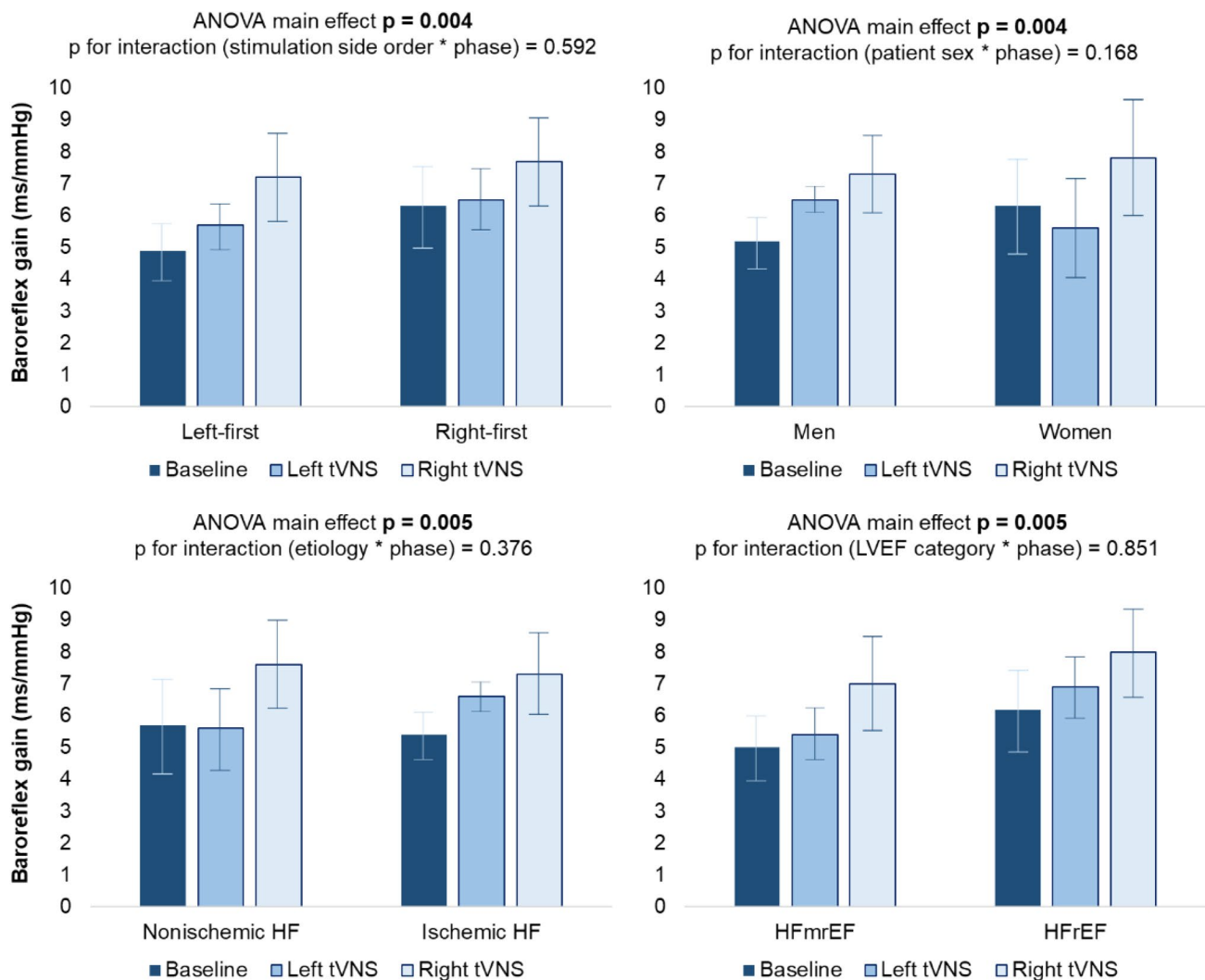
Fig. 2 Effects of tVNS on cardio-vagal baroreflex gain across patient subgroups. The efficacy of acute right-sided tVNS in increasing cardio-vagal baroreflex gain was consistent across different subgroups in the study population. One-way ANOVA for repeated measure was used. *HFmrEF* heart failure with mildly reduced ejection fraction, *HFrEF* heart failure with reduced ejection fraction, *tVNS* transcutaneous vagus nerve stimulation

itself activate central autonomic areas [23, 48], performing both left-sided and right-sided tVNS in a randomized order constituted a fair "control condition," beyond providing an answer to an open research question [23]. In this respect, confirming that only right-sided tVNS was effective in increasing cardio-vagal baroreflex gain independently of stimulation side order ruled out concerns about the carryover effect related to the initial stimulation side over the protocol.

The optimal parameters for tVNS are unclear and may vary according to the device used and the study endpoint [23]. In patients with postural tachycardia syndrome, a stimulation frequency of 25 Hz was identified as the optimal one to increase the HF component of HRV [49]. Therefore, we could not exclude that having used a stimulation frequency

of 30 Hz may have affected the findings of the current study. Considering the differences in the study populations (e.g., age, sex, severity of autonomic imbalance, and medications), further studies should investigate the optimal stimulation parameters in patients with CHF. [23]

While spontaneous cardio-vagal baroreflex gain is an accurate, reproducible, and widely available noninvasive parameter [29], which has been shown to retain a strong prognostic value in CHF [9], the clinical significance of its modulation remains to be confirmed. Indeed, the reduction in cardio-vagal baroreflex gain observed in CHF patients may not exclusively indicate a pathophysiological process. As evidenced in healthy sedentary adults [50], lower cardio-vagal baroreflex gain can result from arterial stiffening, a natural aspect of aging, particularly in sedentary individuals,
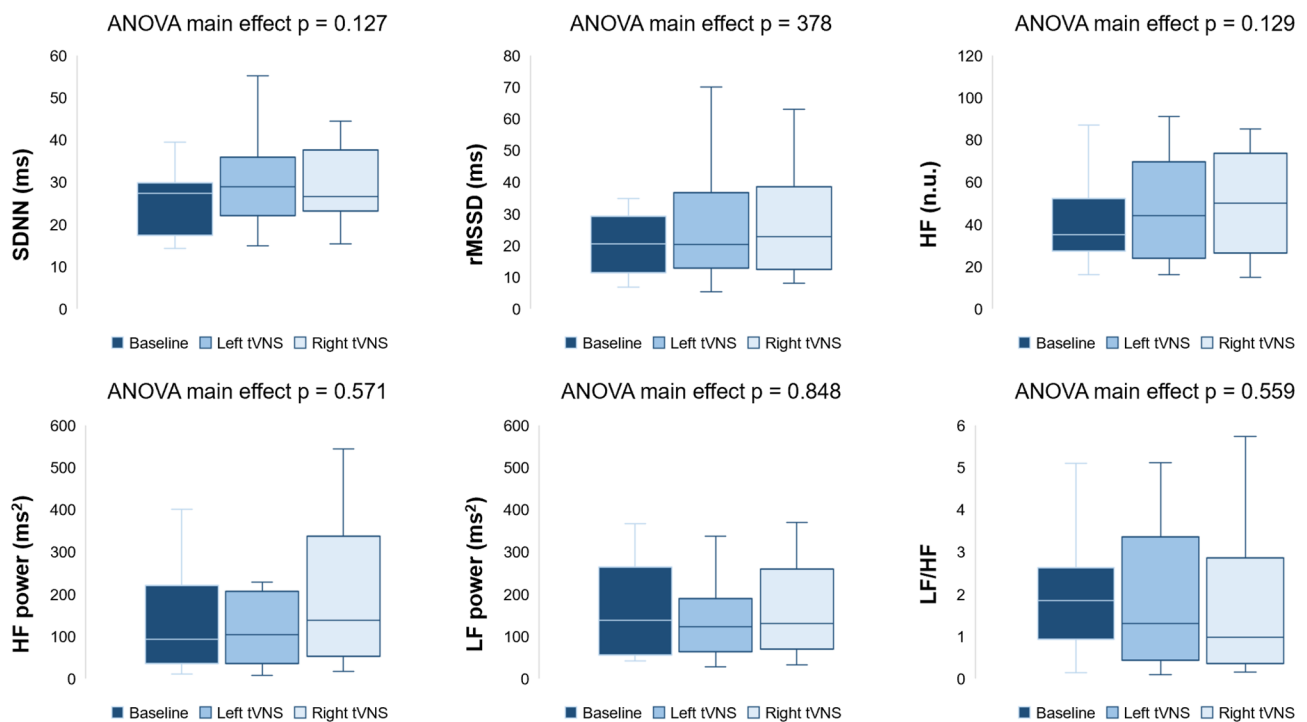
Fig. 3 Effects of tVNS on HRV parameters in patients with chronic heart failure. Acute right-sided tVNS was associated with nonsignificant changes in time-domain and frequency-domain HRV parameters toward vagal predominance (all $p > 0.05$). One-way ANOVA for repeated measure was used. Individual data points for each parameters are reported in Supplemental Figures 1, 2, and 3. *HF* high frequency, *LF* low frequency, *rMSSD* root mean square of the successive differences between normal-to-normal intervals, *SDNN* standard deviation of the normal-to-normal intervals, *tVNS* transcutaneous vagus nerve stimulation

rather than serving as a direct marker of disease [51]. However, a significant improvement in cardio-vagal baroreflex gain through physical training in patients with CHF has been associated with reduced cardiac mortality during follow-up [52]. While the contribution of other beneficial mechanisms secondary to physical training cannot be excluded, these findings underscore the potential importance of enhancing cardio-vagal baroreflex gain in CHF management. Notwithstanding, other studies are expected to assess the autonomic effects of tVNS in CHF patients by using direct measures of sympathetic and vagus nerve activity, assessed through microneurography.

The use of anti-neurohormonal drugs can impact both cardiovascular parameters (such as BP and heart rate) and autonomic functions (including cardio-vagal baroreflex gain). However, due to safety concerns, these therapies were not discontinued in study patients. While this may have influenced the effects of tVNS on these parameters, it is valuable to provide data on CHF patients treated in accordance with the latest guidelines. This approach reflects the potential clinical application of tVNS as an adjunct therapeutic strategy.

Finally, in this study, only the acute effects of tVNS were evaluated, which are unlikely to provide clinical benefits. However, together with the promising findings derived from preclinical models [15, 32], these results encourage designing further studies to evaluate the efficacy of chronic tVNS.

Conclusions

Acute right-sided tVNS is safe and well tolerated and improves cardio-vagal baroreflex gain in adults with systolic CHF. Considering the pathophysiological and prognostic significance of reduced baroreflex function in this population, right-sided tVNS may prove valuable as a novel noninvasive and cost-effective strategy for neuromodulation.

Future studies should now test the safety and effectiveness of chronic tVNS in patients with systolic CHF, evaluating the potential benefits on autonomic balance, neurohormonal activation, cardiac function, and other clinically relevant endpoints.

Data availability Upon reasonable request.

Declarations

Conflict of interest None.

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