Study of Autonomic and Gastrointestinal Function in Patients Suffering From Diabetes with Gastrointestinal Symptoms Treated With Vagus Nerve Stimulation

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STUDENT REPORT

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Abstract

Background

Diabetic autonomic neuropathy (DAN) may cause gastroenteropathy which leads to gastrointestinal (GI) symptoms in a high proportion of the diabetic population. Existing treatment is ineffective and associated with severe side-effects. A new therapeutic option, transcutaneous vagal nerve stimulation (tVNS), is hypothesized to improve GI motility and reduce inflammatory responses through modulation of the vagally innervated coordination of the GI tract.

Methods

52 participants with type 1 or type 2 diabetes who experienced autonomic dysfunction and gastrointestinal symptoms was randomized and assigned 1:1 to active or sham tVNS, both applied bilaterally four times daily. Primary outcome was gastrointestinal symptom relief measured by the self-reported questionnaires Gastroparesis Cardinal Symptom Index (GCSI) and Gastrointestinal Symptoms Rating Scale (GSRS). Secondary autonomic outcomes included blood pressure, heart rate, cardiac vagal tone, electrochemical sudomotor function and Cardiac Autonomic Neuropathy-score. All outcomes were performed at baseline and following seven days treatment. The study is ongoing and only unblinded into treatment A and B.

Results

Primary outcome: in comparison to treatment A, treatment B showed a significant symptom decline from baseline GCSI (-0.3 vs. -0.9, p=0.01). Analysis of subscale domain showed similarly a significant decline from baseline in nausea (p=0.05) and early satiety (p=0.02). No other outcomes showed any statistically significant difference between the two treatments.

Conclusion

If treatment B is active and A is sham, it can be concluded that tVNS has an alleviating effect on gastrointestinal symptoms.

On the contrary, if treatment A is active and B is sham it can be concluded that tVNS is inferior to a robust placebo response in reducing gastroparesis symptoms.

Table of Contents

Abbreviations

CAN - Cardiac autonomic neuropathy

CVT - Cardiac vagal tone

DAN - Diabetic autonomic neuropathy

GCSI - Gastroparesis Cardinal Symptom Index

GSRS - Gastrointestinal Symptom Rating Score

tVNS - transcutaneous vagus nerve stimulation

Introduction

Diabetes mellitus is a global challenge that affects 463 million people (2019) and the incidence is predicted to increase to 700 million in 2045[1]. One of the most burdensome complications to diabetes is diabetic neuropathy, which can be diagnosed in up to half of the diabetic population [2], typical after several years of disease duration of type 1 diabetes. Diabetic neuropathy may already be present in at least 10-15% of newly diagnosed individuals with type 2 diabetes[3]. Essentially, diabetes leads to hyperglycemia which activates different biochemical and metabolic pathways, creating oxidative stress, and resulting in microvascular damage, growth factor deficiency, and neuroinflammation[4].

The clinical expression of neuropathy typically reflects the damaged nerves, including the autonomic nervous system. Consequently, diabetic autonomic neuropathy (DAN) leads to diminished parasympathetic tone which impairs the metabolism and function of the cardiovascular, genitourinary, sudomotor, and gastrointestinal systems. Gastroenteropathy, causes a wide range of symptoms from the esophagus, intestines and rectum including gastroparesis, which is one of the most bothersome outcomes of DAN[2]. Gastroparesis is defined as a delayed gastric emptying without any mechanical obstruction concomitant with the presence of six cardinal symptoms: Nausea, early satiety, vomiting, abdominal pain, postprandial fullness, and abdominal distension[5]. The pathophysiology is multifactorial, including hypomotility, lack of fundic accommodation, diminished numbers of intestinal cells of Cajal and pyloric dysfunction[5] but macrophage-driven inflammation has also been shown[6].

Up to 40% of patients with gastrointestinal complications report a poor quality of life, due to persistent symptoms, depression, anxiety, smoking, consumption of alcohol, and weight gain[7]. The existing data on associations between symptoms and gastroenteropathy are ambiguous, as several studies report association between cardinal symptoms and gastric emptying time, whilst others do not [8–11]. Similarly, symptoms have been associated with other motility disorders which give rise to abdominal pain, diarrhea, constipation, and fecal incontinence[12].

Symptom relief and treatment of gastroparesis can be pharmacological and non-pharmacological. These treatment options are however often insufficient and can cause serious adverse effects [5,13]. In treatment refractory cases, surgical treatment options can be utilized, including feeding jejunostomy or gastric electrical stimulation[14,15]. The underlying mechanism is unclear, mainly because inconsistent associations between improvement of gastric emptying time and

symptom relief [16]. Thus it has been speculated that gastric electrical stimulation may modulate the sympathetic withdrawal, affecting the sympatico-vagal balance[17].

Preclinical trials have shown the importance of vagal input in regulating basal gastric tone, gastric contractions and fundic relaxation[18,19]. Theoretically, such shift could be activated through vagal nerve stimulation. Several approaches have been used to activate the parasympathetic tone[20,21] including physiologically via deep breathing[22], cervical implanted electrodes[23], and transcutaneous vagal nerve stimulation (tVNS) in healthy individuals (auricular and cervical)[24,25]. Recently two studies showed 35-40% clinical symptomatic improvements in patients suffering from drug-refractory idiopathic/diabetic gastroparesis following treatment with tVNS [26,27].

However, a knowledge gap on these topics still exists, and a viable treatment without serious adverse effects is still missing.

Consequently, we hypothesized that tVNS could alleviate gastrointestinal symptoms in individuals suffering from diabetes and therefore the aim of this study is to assess the response to tVNS in terms of:

1) GI symptoms relief.
2) Objective measurements to evaluate the autonomic function.
3) Differentiating between responder versus non-responder of treatment.

Method

Study Design

This study (n=50) is part of a multicenter randomized, parallel-group, placebo-controlled collaborative study, between the Steno Diabetes Centers in Northern Jutland, Aarhus, and Copenhagen (NCT04143269). The study is still recruiting and will enroll in total 120 participants. The participants were randomized 1:1 in blocks of 8. The randomization list was generated centrally, and the GammaCore tVNS stimulators were numbered consecutively to mask whether the participant received tVNS active or sham treatment.

Study Population

The study included participants with diabetes mellitus type 1 or 2, who suffer from GI symptoms and DAN. Inclusion and exclusion criteria is specified in the protocol[28]. Invitation was done via "e-Boks", where patients with a confirmed diagnosis of diabetes were contacted digitally. All subjects were screened with the Gastroparesis Cardinal Symptom Index (GCSI) and Gastrointestinal Symptom Rating Scale (GSRS) from which a combined weighted score of 2.3 was needed to participate in the study. As different numbers of questions exist in the two questionnaires, a weighted score is needed to combine a total score where each questionnaire score weighs the same. Additionally, to secure the diagnoses of DAN, participants should have one of either: At least one abnormal cardiovascular reflex test (Cardiac autonomic neuropathy (CAN) score of ≥1) assessed with the VAGUS device [29,30], or impaired electrochemical conductance of hands (<50 µS) and/or feet (<70µS) assessed with SUDOSCAN[31], or a Composite Autonomic Symptom Score >16[32].

Ethics

Prior to inclusion all participants provided informed signed consent and the study was conducted in accordance with the Helsinki Declaration, Clinical investigation of medical devices for human subjects – Good Clinical Practice, and local regulations and laws. It has prospectively been approved by the North Denmark Region Committee on Health Research Ethics (N-20190020) and the Danish Health and Medicine Authority (CIV-19-07-029105).

Study Setting

The participants met on the study location at Aalborg University Hospital between 8 and 9 a.m., refrained from coffee, tobacco, food, liquids, and strenuous exercise.

Stimulations

Following randomization all participants received thoroughly instruction in how to use the GammaCore (ElectroCore LLC, Basking Ridge, New Jersey, USA) by an otherwise non-contributing un-blinded person. Hereafter, participants were instructed to use the device four times daily. One stimulation accounted for two consecutive bilateral doses lasting 2x120 sec. The peak Intensity of the stimulation was 24 peak voltage and 60 mA peak output current. Intensity is adjustable, and participants were imposed to use the highest tolerable intensity. Pulse duration was 1 ms, with a frequency of 5 Hz.

The sham GammaCore device is a copy of the active device. The only difference being instead of electrical stimulation, the sham device provides a sound to mimic the active treatment. The participants in the sham group received the same instructions as the active.

Outcome and Measures

Gastrointestinal Symptoms

The primary outcome was measured on baseline and day 7 as a symptom-score of both gastroparesis symptoms and overall GI-symptoms with the validated self-reporting questionnaires GCSI and GSRS, respectively. Additionally, the weighted score was calculated from GCSI and GSRS. The endpoint was the change in symptom score from baseline, after one week of stimulation.

Autonomic Function

Secondary outcomes were measured on baseline and day seven in terms of: 1) Blood pressure, 2) Heart rate, 3) Cardiac vagal tone (CVT)[33], 4) Sudomotor function, and 5) CAN-score. CVT was retrieved from a 5-minute recording by use of the eMotion FAROS device (Mega Electronics, Kuopio, Finland). Additionally, CVT was measured immediately after first stimulation at baseline. Moreover, sudomotor function was tested with the SUDOSCAN device (Impeto Medical, California, San Diego, USA)[31]. Lastly, CAN-score were determined through the results of three standardized test a) posture change b) deep breathing c) Valsalva maneuver using

the VAGUS device (Medicus Engineering, Aarhus, Denmark). CAN-score is calculated on age-related cut-off value and ranges from 0 (absent) 1 (borderline) 2 & 3 (definitive)[34].

Definition of Responders Versus Non-responders

A reduction of ≥30% in questionnaires score was defined as a response to the tVNS treatment.

Statistical Analysis

Statistical analyses were done with a significance level of 0.05 by use of the IMB SPSS Statistics 27 (IBM Corp., Armonk, New York, USA)

Data were analyzed using the per protocol approach. Variables were inspected for normality with histograms and using Shapiro-Wilk test. Parametric variables were presented as mean +/- standard deviation and nonparametric variables were presented as median with interquartile range. Continuous variables were compared using an unpaired t-test, and categorical variables were compared using Chi-square test or Fisher's exact test.

Change from baseline was calculated by subtracting values at day 7 from the values at baseline, thus negative values represent symptom alleviation. If a value from day 7 was missing, the participant was excluded from this calculation. For parametric variables the changes from baseline were tested in an ANCOVA model with baseline values as a covariate, to calculate adjusted mean change with standard error and a between-group difference with a 95% confidence interval. For nonparametric variables the changes from baseline were used in a Mann-Whitney Wilcoxon test to observe any between-group difference. To show any difference in CAN score between treatments at baseline and day 7 a Kruskal-Wallis test was performed. A Chi-square test was used to determine any statistical difference between responder and non-responders for each questionnaire. Logistic regression was used to analyze the relationship of CVT change on the probability of being a responder of treatment. This was preformed both with the immediate CVT change and the change after 7 days of treatment as the predicting value, on both the results of the weighted score and the GSCI score.

Justification of Sample Size

It was calculated that at least 42 participants were needed to show a statistically significant difference with a power of 90% and a two-sided significance level of 0.05. This was based on the estimation that a 30% difference between the groups[27] with a previously reported baseline GCSI score of 2.0 +/- 1 (SD) [35]. Accounting for a dropout rate of up to 30%, 58 participants were enrolled.

Results

Participant Flow

There were 1478 invited participants, 178 of which wished to participate. Following a telephone interview, 46 withdrew or were not eligible, which left 132 to fill out questionnaires of GI symptoms. 13 did not respond and 44 did not have a high enough weighted score. 75 were screened for the remaining inclusion criteria where 17 did not fulfil this. After the screening process, 58 patients were eligible in this study (see figure 1).

Table 1 shows the baseline characteristics of the groups. A statistical difference was found between the two randomization groups. There was a significant difference at baseline in GCSI-score, satiety-score, BMI, total cholesterol, and heart rate between treatment A and B.

Primary Outcomes

Table 2 presents the change in symptom score and the between-group differences in questionnaires. The weighted score showed a reduction in treatment A at -0.3 and treatment B at -0.9 with a significant between-group difference at -0.6 (p=0.01). GCSI showed a reduction in mean at -0.1 in treatment A and -0.4 in treatment B with a statistically significant between-group difference at -0.3 (p=0.04). GSRS between-group differences were not significant (see figure 2).

The between-group differences in the subscale symptom score of GCSI shows significant decline in nausea (p=0.05) and satiety (p=0.02). There was no significant difference in any of the subscale scores in GSRS between the groups (detail are presented in Table 2 and Figure 3).

Secondary Outcomes

The objective measures of autonomic nerve tonus: Blood pressure, heart rate, CVT (both immediate response and after 7 days), SUDOSCAN, and CAN-score shown no significant changes (see Table 2).

Responders Versus Non-responders

In GCSI we observed that six participants out of 24 (25%) in treatment A and 15 participants out of 26 (57.7%) in treatment B responded to the treatment, with a significantly higher proportion of responders in treatment B (p=0.02). In GSRS four participants (16.7%) in treatment A and five in treatment B (19%) responded to treatment. In weighted score five participants (20.8%) in treatment A and 11 in participants B (42.3%) responded to treatment. The proportion of responders did not differ between the groups in GSRS and weighted score. (See Table 3).

For both treatments, no significant relationship between the probability of being a responder of treatment (measured by GCSI or total weighted score) and the change in CVT both immediate response and after 7 days were shown (see Table 4).

Side Effects

Among the participants, 1/24 (4.2%) in treatment A and 6/26 (23.1%) in treatment B reported at least one side effect. All were minor and no serious adverse events were reported. Treatment-related side effects were non-serious and infrequent, with three cases of headache, all in treatment B. No cases required further intervention.

Discussion

Our results show a significant decline in GCSI and weighted score from baseline to day 7 in treatment B compared to treatment A. The weighted score is obtained through both GCSI and GSRS scores, the reduction in GCSI score contributed to the majority of the weighted score decline, driven by a reduction of upper GI symptoms.
None of the autonomic variables showed any statistically significant change.
The proportions of responders were significantly larger in treatment B compered to treatment A in regard to weighted score and GCSI but not GSRS.

The vagus nerve is a crucial bidirectional, parasympathetic modulator throughout organs innervated by the autonomic nervous system among these the gastrointestinal system. It maintains the homeostasis of the gut, among others motility, sensitivity, and immunity[36]. The vagus nerve is one of the longest nerves of the body and consists of 80% afferent and 20% efferent fibers. The afferent fibers are activated through pancreatic and gastrointestinal hormones, ingested micronutrients and luminal osmolarity, and are involved in the complex regulation of pancreatic excretion, and rhythm generation of the heart and lungs[37].

If Treatment A is Sham and B is Active:

Transcutaneous VNS has previously been used as a treatment for diabetic/idiopathic gastroparesis symptoms, however it was conducted in a non-RCT setting[26,27]. Paulon et al. and Blackmore et al. showed a rate of response at 35% and 40% following 3- and 4-weeks stimulation period, respectively, assessed with GSCI score. In our data we observed nearly 60% symptom alleviation in response to treatment B. The relatively higher symptom-relief may be interpreted in the context, that the referenced studies included patients with confirmed gastroparesis, who were therapy refractory[26]. Thus, our cohort may have been included at a time where it was possible to affect the parasympathetic tone, and thereby alleviating symptoms.

It is noteworthy, that there has not been established any consensus of what a clinical meaningful change in symptoms is. Revicki et al.[38] estimated that a minimal important difference for GCSI total scores are 0.4-0.5 points and that a change of 0.5-0.75 points are clinically significant in populations suffering from gastroparesis. Our study has chosen the definition of a minimum of 30% reductions based on the most up-to-date FDA recommendation on GCSI[39]. In support of

our findings, Paulon et al.[26] showed that the most significant symptom benefit was found in the nausea GCSI subscale, while Blackmore et al.[27] showed the largest improvement in the early satiety subscale. This study showed that treatment B significantly improved both nausea and early satiety subscale scores, which agitate for a strong vagal component in the generation and maintenance of especially these symptoms.

It is generally accepted that the anti-inflammatory effect of the vagus nerve conveyed through dual pathways. Firstly, the neuroendocrine hypothalamic-pituitary-adrenal axis is activated via the vago-nucleus tractus solitarius-paraventricular nucleus of the hypothalamus pathway resulting in the release of the anti-inflammatory hormone cortisol. Secondly, the cholinergic anti-inflammatory pathway stimulates the alpha-7nAChR receptor expressed on proinflammatory macrophages, leading to reduced release of proinflammatory cytokines, including TNF-alpha [36]. This study did not measure any anti-inflammatory markers. However, an open-label study by Brock et al. showed that the level of serum TNF-alpha was reduced in response to short-term tVNS stimulation in 20 healthy subjects [24] indicating that tVNS has an anti-inflammatory effect in healthy participants with intact autonomic nervous system. As diabetic autonomic neuropathy also may yield a neuroinflammatory component, it is plausible that tVNS may modulate the macrophages-driven inflammation as part of the pathomechanisms of diabetic gastroparesis. Therefore, one could speculate that the same inflammatory pathogenesis is present in the entire gut.

Several studies have shown that it is possible to increase the cardiac vagal tone, a measurement of efferent vagal activity, by inducing short-term tVNS in a healthy population [33,40]. In support of these, Stocker et al. showed increased cholinergic function in response to 1 year of gastric electrical stimulation in patients suffering from gastroparesis. However, direct comparison is obscured because only approximately 25% of the population had diabetic gastroparesis, whereas the others suffered from idiopathic gastroparesis[41]. Nevertheless, the above-mentioned studies indicate that it is also possible to increase parasympathetic tone in both healthy and participants with gastroparesis.

In our study we did not observe between-group difference in autonomic variables, including blood pressure, heart rate, CVT, SUDOSCAN, and CAN-score. This was unexpected because in theory, stimulation of the vagus nerve should increase the parasympathetic tonus resulting in decreased blood pressure, lower heart rate, and increase CVT. It is more complex with the measures of sudomotor function as this is sympathetically innervated. One explanation could

be that short-term stimulation does not alter autonomic variables. In contrast to the study of Stocker et al. who stimulated continuously over one year, participants only underwent one week of stimulation in our study. Long-term stimulation may affect the vagal tone in a different way with a more profound impact on the parasympathetic tonus resulting in altered autonomic variables [41].

Another possible explanation is that the nerves of the patients included in our study may be too damaged to observe any direct effect of tVNS on the heart rate variability, CAN-score, and SUDOSCAN, as we only included patients with verified diabetic autonomic neuropathy. In contrast to Brock et al.[24], which only included healthy volunteers, and Stocker et al.[41] primarily investigated a combined cohort consisting of idiopathic and diabetic gastroparesis patients, our study included patients with GI symptoms and verified diabetic autonomic neuropathy. Our findings could be due to both study length and participant selection.

Even though the between-group difference of GCSI score is significant, it does not necessarily mean that it is clinically relevant. However, one study found that an improvement of 0.75 points was clinically relevant and that 0.40-0.50 points is the minimally important difference in GCSI[38]. We showed a GCSI difference at 0.3, and thus it cannot be categorized as clinically relevant albeit significantly different. The difference in GSRS was not statistically significant at 0.2, which is insufficient in comparison to a clinically relevant difference of 0.5 suggested by Talley et al.[42]. It is interesting that the weighted score is decreased 0.7, however this composite score has not undergone validation and accordingly, we do not know whether it is clinical meaningful.

If Treatment A is Active and B is Sham:

The results of this study were unexpected. The expectation was that tVNS would alleviate experienced GI symptoms in accordance with Paulon et al. and G. Blackmore et al. [26,27]. Both studies showed a response to treatment in GCSI measures of 35% and 40% following 3- or 4-weeks stimulation period, respectively. In contrast, this study showed 25% responders in the active group and even more intriguing, a 60% of the participants showed a response in the sham group. These results suggest that the sham-device has greater effect than the active tVNS treatment combined with an unexpectedly high placebo response.

It is widely known that placebo can have a great effect on, for instance trials investigating analgesic effect where the opioid-agonist worked as expected, however the placebo drug was also effective, even though the underlying mechanisms were different[43]. Petrovic et al. suggests that expectations have a lot of weight in how placebo works and that placebo is not a passive control but an active state in itself [43]. Most studies describe expectation of a positive treatment experience as a main contributor to the explanation of the phenomenon, but also classical conditioning has a significant influence[44]. A systematic review by Quinn et al. found that nausea is easily affected by placebo intervention[45].

However, the placebo effect is also affected by study-design. A systematic review by Enck et al. explains that placebo has the least amount of impact in 1:1 trials (50% placebo: 50% active treatment), since our study design is a 1:1 RCT it cannot explain why we see a greater response in the placebo group compared to the active group[46].

It is important to emphasize that the primary outcome was subjective in form of perceived GI symptoms, and as pain perception can be altered by placebo, it is possible that the same mechanism is responsible for GI symptoms. However, we could not show any objective measures of parasympathetic modulation even though a study by Wilhelm et al. found that placebo has a significant effect on the blood pressure, but no effect on heart rate[47]. A possible explanation could be that 1 week of stimulation is not sufficient to alter any objective measurements.

Limitations

Limitations of this study include recall bias of the self-reported questionnaire. The significant difference between GCSI score between the groups at baseline is unfortunate but must be a coincidence due to the study design. However, this should not affect the results due to the statistical analysis chosen.

Furthermore, it should be noted that even though the GSCI and GSRS questionnaires are validated in the United States, it has not been validated in Danish. However, it is likely generalizable to other populations than the United States[38].
Another bias includes the “self”- stimulation at home. It has not been possible to control if the simulations have been applied correctly. Errors could have occurred in the application at home, though clear instruction has been given each patient.

Conclusion

The results are unblinded into treatment A and treatment B because the study is still ongoing. The results have been evaluated on the following presumption

If Treatment A is Sham and B is Active

This study shows that short-term tVNS has an effect on gastroparesis symptoms and it affects the same subscale symptoms as previous studies [26,27]. It has significant effect on GSRS or any of the measured autonomic variables. Though it has a statistically significant effect on GCSI, it cannot be concluded that this is clinically relevant.

If Treatment A is Active and B is Sham

This paper shows that the placebo effect can be of great influence and tVNS does not reduce gastroparesis symptoms nor improve any autonomic variables. Placebo has a significant decrease in symptom severity compared to tVNS. The reason for this cannot be fully explained compared to previous studies.

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Tables

Table 1. Baseline characteristics of treatment A and B as mean (standard deviation) or otherwise specified and p-value (<0.05=significant) calculated with [A]= unpaired t-test, [B]= Mann-Whitney U, [C]= Chi-square test and [D]= Fisher's exact test. IR= Interquartile range.

Day 0	**Treatment A (n=24)**	**Treatment B (n=28)**	**p-value**
Gender, n (%)	18 men (75) 6 women (25)	16 men (57.1) 12 women (42.9)	0.18[C]
Age, years	64 (10)	59 (12)	0.14[A]
BMI, kg/m^2	29.3 (4.8)	32.6 (6.2)	0.04[A]
Systolic blood pressure, mmHg	133 (13)	136 (15)	0.48[A]
Diastolic blood pressure, mmHg	78 (10)	80 (8)	0.60[A]
Heart rate, bpm	69 (10)	77 (12)	0.02[A]
Median [IR] CVT, before stimulation	3.2 [3.6]	2.3 [1.9]	0.12[B]
Median [IR] disease duration, years	17 [14]	15 [9]	0.76[A]
Type 1 diabetes, n (%)	2 (8.3)	5 (17.9)	0.43[D]
Type 2 diabetes, n (%)	22 (91.7)	23 (82.1)	
Median [IR] HbA1c, mmol/ml	60 [15]	63 [17]	0.21[B]
Total cholesterol, mmol/l	3.6 (0.7)	4.2 (0.8)	0.02[A]
HDL Cholesterol, mmol/l	1.2 (0.4)	1.2 (0.4)	0.82[A]
Median [IR] eGFR, ml/min/1.73m^2	90 [19]	90 [17]	0.98[B]
Oral antidiabetic drugs, n (%)	21 (87.5)	21 (80.8)	0.25[C]
Insulin, n (%)	16 (66.7)	20 (71.4)	0.71[C]
Statin therapy, n (%)	22 (91.7)	22 (78.6)	0.19[C]
Anti-hypertensive therapy, n (%)	19 (79.2)	18 (64.3)	0.24[C]
Median [IR] Suduscan feet, µS	81 [17]	79 [15]	0.51[B]
Median [IR] Sudoscan hands, µS	61 [20]	60 [13]	1.0[B]

Absent CAN n/N (%)	12/23 (52.2)	14/28 (50.0)	0.82[C]
borderline CAN n/N (%)	6/23 (26.1)	5/28 (17.9)	
Definitive CAN n/N (%)	5/23 (21.7)	9/28 (32.1)	
Weighted total-score	3.85 (1.84)	4.37 (1.33)	0.24[A]
GCSI-score	1.18 (0.95)	1.67 (0.73)	0.04[A]
Median nausea-score [IR]	0.33 [1.00]	0.84 [0.67]	0.18[B]
Median satiety-score [IR]	1.13 [1.5]	1.63 [1.69]	0.04[B]
Median bloating-score [IR]	1.5 [3]	2.25 [2.50]	0.07[B]
GSRS-score	2.86 (0.92)	2.69 (0.71)	0.46[A]
Median reflux-score [IR]	2.00 [1.88]	2.00 [1.88]	0.78[B]
Median abdominal pain-score [IR]	2.67 [2.25]	2.5 [1.83]	0.60[B]
Median indigestion- score [IR]	3.13 [2.38]	2.38 [1.75]	0.27[B]
Median diarrhea-score [IR]	3.33 [2.33]	2.67 [2.34]	0.24[B]
Median constipation-score [IR]	3.33 [2.00]	3.17 [2.08]	0.69[B]

Table 2. The change from baseline with standard error, unless otherwise is displayed, in treatment A and B. The difference between groups with 95%-confidence interval and p-value (<0.05=significant) calculated with [A]=ANCOVA, [B]=Mann-Whitney U, and [C]=Chi-square [D] =Kruskal Wallis. Both questionnaires, sub-scales, and autonomic measurements are displayed. CAN= Cardiac autonomic neuropathy. GSRS= Gastro-intestinal Symptom Rating Score. GCSI= Gastrointestinal Cardinal Symptom Index. IR= Interquartile range.

Variables	**N**	**Gruppe A**	**N**	**Gruppe B**	**Between-group difference**	**P-value**
Weighted score	24	-0.3 (0.2)	26	-0.9 (0.2)	-0.6 (-1.2; -0.2)	0.01[A]
GCSI	24	-0.1 (0.1)	26	-0.4 (0.1)	-0.3 (-0.6; 0.0)	0.04[A]
Median [IR] Nausea		0 [0.33]		-0.33 [0.67]		0.05[B]
Median [IR] Satiety		0 [0.30]		-0.25 [0.75]		0.02[B]
Median [IR] Bloating		0 [1]		-0.75 [1]		0.06[B]
GSRS	24	-0.3 (0.1)	26	-0.5 (0.1)	-0.2 (-0.5; 0.1)	0.13[A]
Median [IR] Reflux		0 [0.30]		0 [1]		0.29[B]
Median [IR] Abdominal pain		0 [0.33]		-0.34 [1]		0.06[B]
Median [IR] Indigestion		0 [0.75]		-0.38 [1]		0.27[B]
Median [IR] Diarrhea		-0.5 [1.33]		-0.33 [1.33]		0.77[B]
Median [IR] Constipation		-0.66 [0.84]		-0.67 [1.33]		0.86[B]
Blood Pressure systolic	23	-1.0 (2.6)	25	-3.6 (2.5)	-2.4 (-9.9;4.8)	0.48[A]
Blood Pressure diastolic	23	-0.5 (1.7)	25	-1.2 (1.6)	-0.7 (-5.3;3.9)	0.76[A]
Heart rate	23	-1.2 (1.6)	25	0.4 (1.5)	-1.6 (-3.0;6.2)	0.48[A]

Median [IR] Cardiac Vagal Tone (Day 0, immediate response)	20	0.6 [4.6]	26	0.4 [2.2]	0.93[B]
Median [IR] Cardiac Vagal Tone (day 7 response)	18	-0.2 [2.4]	21	0.3 [1.9]	0.71[B]
Median [IR] Sudoscan, feet	23	-2.5 [6.0]	26	0.3 [7.1]	0.30[B]
Median [IR] Sudoscan, hands	23	-3.0 [11]	26	-1.0 [10.4]	0.34[B]
Absent CAN n/N (%)		15/23 (65.2)		9/24 (37.5)	0.17[D]
Borderline CAN n/N (%)		4/23 (8.7)		9/24 (37.5)	
Definitive n/N (%)		4/23 (17.4)		6/24 (25)	

Table 3. The number of responders (%) measured in questionnaires (Gastrointestinal Cardinal Symptom Index, Gastrointestinal Symptom Rating Score and weighted score) divided in treatment A and B. Responders are defined as a minimum 30% decline in questionnaire score from baseline to day 7.

Scores	**Gruppe A (n=24) Number of responders (%)**	**Gruppe B (n=26) Number of responders (%)**	**p-value**
Weighted score	5 (20.8)	11 (42.3)	0.10
GCSI	6 (25)	15 (57.7)	0.02
GSRS	4 (16.7)	5 (19.2)	0.81

Table 4: Logistic regression between cardiac vagal tone (CVT) both immediate and day 7 response and the probability of being a responder in gastro cardinal symptom index (GCSI) and total weighted score. ΔCVT = CVT change from baseline. CI=confidence interval.

	Odds	**95% CI**
Treatment A		
ΔCVT immediate response as predictor for responder measured by GCSI	1.021	0.819; 1.272
ΔCVT day 7 response as predictor for responder measured by GCSI	1.435	0.818; 2.516
ΔCVT immediate response as predictor for responder measured by weighted score	0.873	0.638; 1.196
ΔCVT day 7 response as predictor for responder measured by weighted score	1.311	0.853; 2.014
Treatment B		
ΔCVT immediate response as predictor for responder measured by GCSI	0.925	0.792; 1.080
ΔCVT day 7 response as predictor for responder measured by GCSI	1.039	0.918; 1.177
ΔCVT immediate response as predictor for responder measured by weighted score	0.931	0.806; 1.074
ΔCVT day 7 response as predictor for responder measured by weighted score	1.047	0.935; 1.173

Figures

Figure 1: CONSORT flow diagram

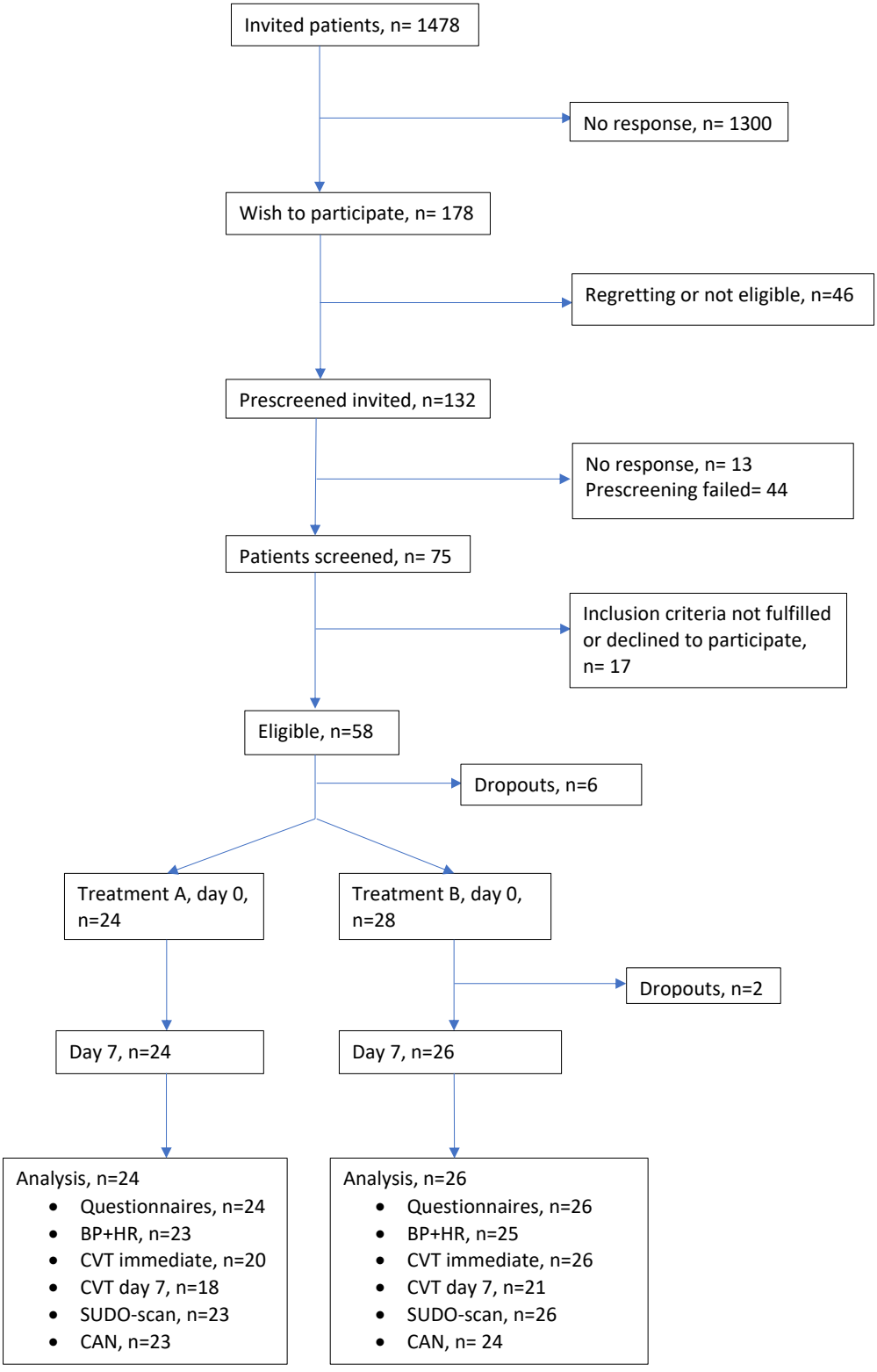

Figure 2: Change in the underlying questionnaires and the weighted score from baseline to day 7 in treatment A (grey) and B (white). x-axis shows the questionnaires and y-axis shows the change from baseline. The delta values between A and B are depicted as Δ. p-values are depicted (p-value <0.05 is significant).

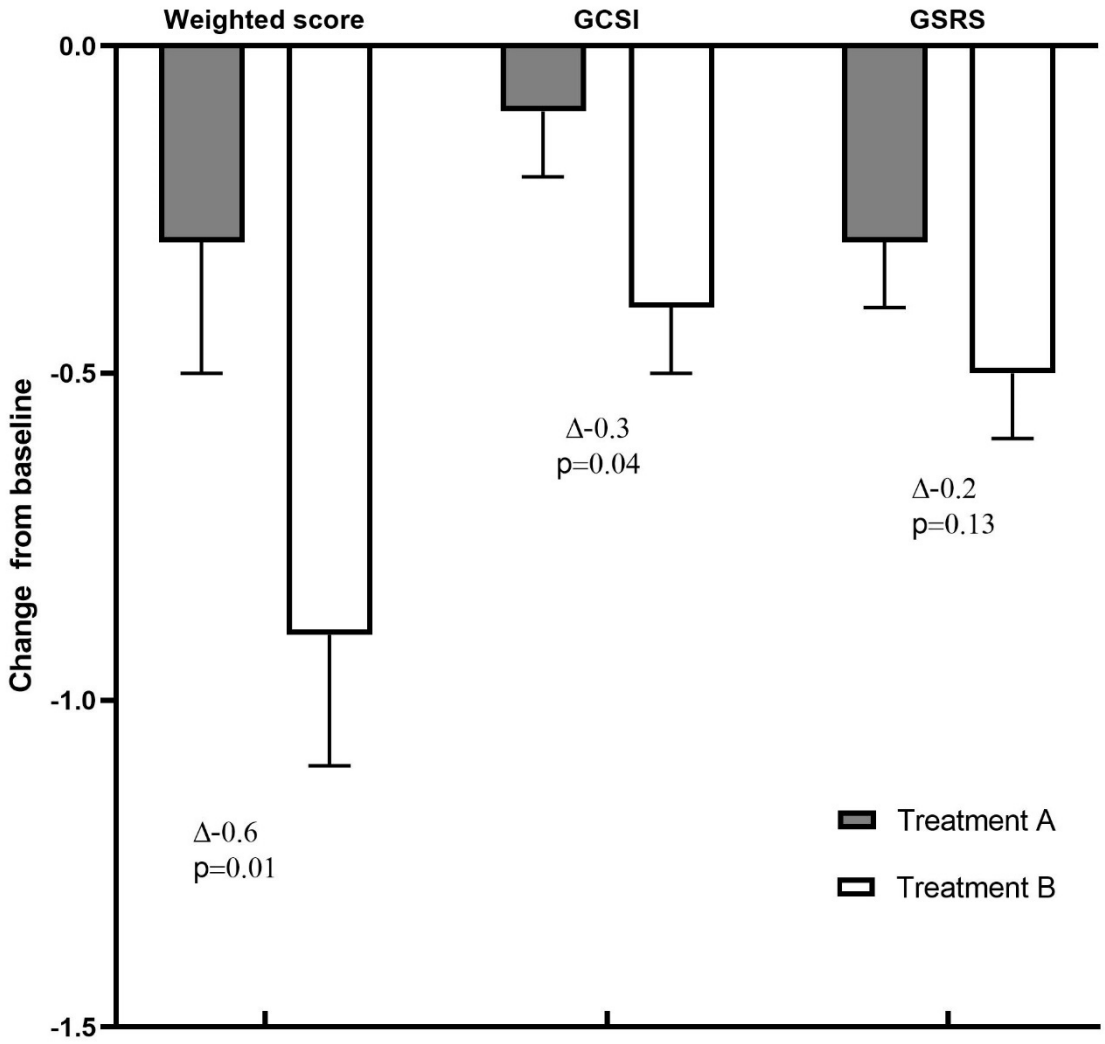

Figure 3: Boxplots and whiskers show the change in subscale scores in GCSI and GSRS from baseline to day 7 in treatment A (grey) and B (white). x-axis shows which subscale symptoms and y-axis shows the change from baseline. p-values are depicted (p-value <0.05 is significant).