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Effects of Chronic Non-Invasive Vagus Nerve Stimulation in an Asplenic Individual with Rheumatoid Arthritis: A Single Subject Trial

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Abstract

Background: Rheumatoid arthritis (RA) is an autoimmune disease for which the goal is disease remission or near-remission on a treat-to-target basis. Noninvasive vagus nerve stimulation (nVNS) represents a new era as an alternative to drug-resistant therapy, providing evidence of the anti-inflammatory neuroimmunological (NIM) effect of vagal stimulation along with the restoration of autonomic nervous system balance.

Objective: We aimed to assess recovery outcomes and neuroimmunological effects of 24-month nVNS on an asplenic participant suffering from RA.

Methods: This study was single subject trial. A man on his 60's suffering from RA was enrolled and at baseline he was shown how to stimulate remotely at home the auricular branch of the vagus nerve. The primary outcome measure was the change in the simple disease activity index (SDAI), and the secondary outcome measure was the change in the sympathovagal balance (SVB); additionally, we tracked the change in the NIM index across three periods (blocks) with different stimulation settings (20 Hz–300 μs, 12 Hz–300 μs and 10 Hz–300 μs) as another endpoint. Data collection was performed at baseline and every two months during the 24-month run-in period.

Results: Compared with the baseline, the SBV was significantly lower after the first block of treatment (period 1) for a stimulation parameter of 20 Hz–300 μs ($p<0.01$; $p<0.001$); the SDAI significantly increased from moderate to high during the last period ($p<0.05$); and the NIM index only moderately improved during period 1. No other significant improvement was found in the outcomes for the other stimulation parameters during the treatment periods.

Conclusion: Chronic nVNS was safe and well tolerated, but the overall effect of nVNS on an asplenic patient with RA was unremarkable. Our results suggested poor benefits of nVNS on disease activity, the sympathovagal balance and the NIM index after splenectomy. Future controlled trials of nVNS on asplenic subjects are suggested to corroborate our findings.

Clinical Trial Registration: ClinicalTrials.gov NCT07646470.

Keywords: Rheumatoid Arthritis; Splenectomy; Disease Activity; Noninvasive Vagus Nerve Stimulation; Heart Rate Variability; Vagus Nerve

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Introduction

Rheumatoid arthritis (RA) is a heterogeneous, complex, progressive, and debilitating immune-mediated inflammatory disorder of the musculoskeletal system that can affect any joint, although it affects mainly the synovial tissue of small diarthrodial joints [1]. RA is among the most prevalent chronic inflammatory diseases, and 18%-40% of patients experience variable extra-articular manifestations, making it more characteristic than an articulatory disorder and more reflective of a systemic disease [2, 3]. This autoimmune disease affects 0.5% to 1.0% of the adult population of industrial countries worldwide [4]. Because of the common high variability and severity of disorders accompanying RA, it is difficult to reach a consensus on the definition of disease activity (DA), along with activity-predicting biomarkers and treatment-predicting responses, in patients with RA, where ultimately, the treatment goal is to achieve disease remission or near remission on a "treat-to-target" basis [1]. Unlike rheumatoid factor, whose specificity and sensitivity are limited [5], C-reactive protein (CRP) is correlated with DA and plays a crucial role in modulating the inflammatory

immune reaction associated with RA [6].

The vagus nerve (VN), also known as the Great Nerve, is the main component of the parasympathetic nervous system (PNS) and plays dual anti-inflammatory roles through complex afferent and efferent reflexes, e.g., central modulation of the central autonomic network (CAN), which represents interconnected brain subcortical and cortical neuraxial structures that regulate body homeostasis [7]. Typically, through these short and long reflexes, vagally mediated processes can initiate central-afferent immune-suppressive actions *via* hypothalamic–pituitary–adrenal release of glucocorticoids [8] and efferent vago-vagal stimulation of the cholinergic anti-inflammatory pathway (CAP) (e.g., targeting the spleen macrophage α7 acetylcholine receptors to damp the release of specific proinflammatory cytokines) [9]. Moreover, the tonic activity of the VN is a crucial physiological mechanism for maintaining immune homeostasis [10]. A point of significance is the suggestion that an intact subdiaphragmatic vagus nerve is necessary to promote immune-suppressor action on spleen macrophages [11], which, when stimulated, attenuates TNF-α and the production of other proinflammatory cytokines, as interestingly observed by Koopman et al. in RA patients under vagus nerve stimulation (VNS) [12]. Therefore, the spleen is considered a primary target of the inflammatory reflex. Furthermore, patients with RA exhibit an altered sympathetic–parasympathetic balance in favor of the former compared with the normal population, as demonstrated by heart rate variability (HRV) analysis [13].

Vagus nerve stimulation, which represents a new era as a possible alternative to drug-resistant therapy for the management of RA because of its good tolerance, safety, and lower cost than pharmacological approaches, has been under investigation for more than a decade. Preclinical and clinical studies have shown that invasive and noninvasive vagus nerve stimulation (nVNS), also called transcutaneous vagus nerve stimulation, is a promising immunomodulatory therapy because it provides evidence of the anti-inflammatory and neuroimmunomodulation (NIM) effects of vagal stimulation in patients with RA, as well as the restoration of the balance of the autonomic nervous system [14-16].

This 24-month, open-label n-of-1 interventional study in three treatment blocks with different stimulation settings aimed to assess (a) the effects of chronic nVNS on the DA, sympathovagal balance (SVB) and NIM over time and (b) whether a specific treatment protocol alters the outcomes. In addition, we aimed to show that long-term nVNS is safe, well tolerated, and feasible remotely at home.

Materials and Methods

Selection and Description of the Participants

Prior to this study, the following research hypothesis was formulated: chronic nVNS improves DA, the SVB, and the NIM index in an asplenic male suffering from RA.

The study was conducted in accordance with the WMA Declaration of Helsinki and was approved by our investigator Affiliation Ethics Committee. The patient was a man in his 60s who was diagnosed with RA in 2004. Written informed consent was provided.

At baseline, a relevant medical intake revealed a family history of RA (sister and niece) and posttraumatic splenectomy in 1976. In 2013, hydroxychloroquine was prescribed after a flare-up in 2012. The prescription of methotrexate in 2015 was stopped in 2016 after the patient developed a shingle rash. From 2017 to 2019, he was on a daily low dose of CBD oil and, inconsistently, ibuprofen for arthritis. In 2019, the patient chose to be drug free. On physical examination, the patient was normocardic and normotensive, had a 20.3 kg body mass index, and presented moderate swelling in multiple joints, including both the wrists, metacarpophalangeal joints, and knees. The simplified disease activity index (SDAI) was 10.9, and the serum CRP concentration was 16.52 mg/L.

Data collection and measurements

The study used a repeated measures design. After being instructed on the use of a standard nVNS device, the patient was given an "at-home" nVNS treatment protocol for a 24-month run-in period, which was divided into three 8-month treatment blocks where each block period consisted of active nVNS with specific settings and a daily stimulation time. The blocks were divided into 4 groups, AAAA-BBBB-CCCC, once every 2 months (Figure 1). No washout period was included. Disease activity was defined as the primary outcome, the SVB was defined as the secondary outcome, and the NIM indices were defined as other prespecified outcomes; both the SVB and NIM indices were derived from the HRV analysis. Disease activity was monitored via the SDAI for RA, where <3.3 indicates remission, ≤11 indicates low activity, ≤26 indicates moderate activity, and >26 indicates high activity. The root mean square of successive differences between normal heartbeats (RMSSD) was used to estimate the vagally mediated change in the HRV. The SD2/SD1 ratio, where SD1 (Poincare plot width) reflects short-term HRV and SD2 (Poincare plot length) reflects short-term and long-term HRV, respectively, provides the SVB [17]. The CRP level yields the NIM index to express the effect of vagal modulation on inflammation, where the NIM index = RMSSD/CRP [18]. Importantly, to avoid significant short-term variability in C-reactive protein (CRP) levels, both HRV and CRP levels were obtained near measurements [19]. Patient outcome measures (POMs) were reported at the baseline and follow-up visits.

HRV measurement

Heart rate variability analysis was based on short-term ECGs, which were sampled at 250 Hz in agreement with the Task Force of the European Society Guidelines [20]. Ten minutes of resting ECG data were collected via an ambulatory device (Bittium Emotion Faros 90, 1 channel, 3-lead wearable ECG with disposable electrodes), and 5 min epochs were analyzed. Heart rate variability analysis was performed via Kubios HRV Premium software (ver. 3.1.0) [21], and the data were processed according to the recommendations and methodological considerations in the HRV analysis [22]. Importantly, the patient was instructed to control his or her breathing to stay in the ECG-derived respiration high-frequency band from 0.15 Hz to 0.40 Hz (between 9 and 24 breath cycles per minute) to ensure that the RMSSD reflected vagal tone HRV-mediated [23], as autonomic rhythms are influenced by controlled breathing [24].

nVNS treatment protocol

Noninvasive vagus nerve stimulation was performed by means of a standard lightweight and portable TENS stimulator (TU7000 Digital TENS device, RMP Ltd., NS, Canada) and two ear electroclips to stimulate the afferent fibers of the left auricular branch of the VN in the outer ear, as described in a previous study [25]. The device was preset for home treatment, and the patient was instructed on how to use it properly. He was told that he should feel only a comfortable 'vibrotactile' or 'fibrillation'-like sensation and maintain this sensation throughout the treatment session by adjusting the intensity

Table 1: Descriptive of measurements and changes between periods.

POMs	BL	Period 1 AAAA	Change (BL *vs* AAAA)		Period 2 BBBB	Change (BL *vs* BBBB)		Period 3 CCCC	Change (BL *vs* CCCC)	
		mean±sd	Δx	*p*-value	mean±sd	Δx	*p*-value	mean±sd	Δx	*p*-value
SDAI	10.90	17.907 ± 5.865	7.007	0.097	24.975 ± 12.110	14.075	0.103	21.505 ± 12.110	10.605	*
CRP (mg/L)	16.52	16.647 ± 5.394	0.127	0.965	22.265 ± 10.848	5.745	0.367	20.042 ± 4.800	3.542	0.253
RMSSD	19.7	18.900 ± 3.964	-0.800	0.730	17.550 ± 5.028	-2.150	0.455	19.375 ± 5.190	-0.325	0.908
SD2/SD1	3.52	2.55 ± 0.11	-0.97	***	3.46 ± 106	-0.06	0.914	3.05 ± 1.30	-0.47	0.525
NIM I	1.19	1.285 ± 0.696	0.095	0.803	1.035 ± 0.734	-0.185	0.701	0.992 ± 0.247	-0.228	0.208

The SDAI score tended to increase from low activity (>3.4–11.0<) to moderate activity (>11.1–26.0<. The CRP level increased, with only mild changes (period 1 *vs* baseline). The RMSSD trend decreased. SD2/SD1 improved significantly only in period 1. The NIM I only moderately improved in period 1.

Abbreviations used: Δx, change in the means; POMs, patient outcome measures; BL, baseline; SDAI, simplified disease activity index; CRP, c-reactive protein; RMSSD, root mean square of the successive differences; SD2, Poincare plot width; SD1, Poincare plot length; NIM I, neuroimmunological index. (*$p < 0.05$; ***$p < 0.001$: paired *t* test).

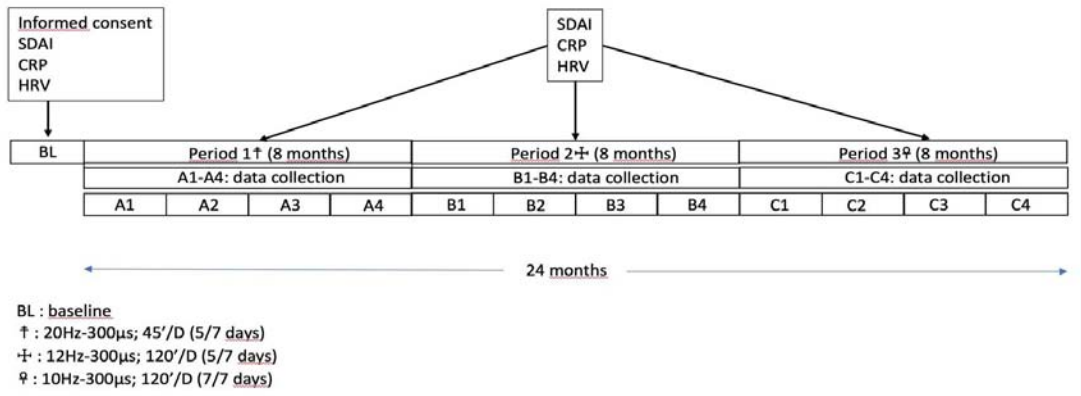

Figure 1: Study overview. A 24-month study was divided into 3 different periods or blocks of home-based treatment, with 4 assessments per block.

under the pain threshold. Additionally, he was instructed to perform the stimulation when he could find proper moments to rest and relax. The first 8-month block A (AAAA) consisted of 20 Hz–300 μs of stimulation for 45 min and 5/7 days; the second block B (BBBB) consisted of 12 Hz–300 μs and 120 min and 5/7 days; and the third block C (CCCC) consisted of 10 Hz–300 μs and 120 min and 7/7 days.

Statistical analyses

Prism 9® software was used for the data analysis (GraphPad Software, LCC. Released 2021, Prism for Mac, version 9.3.1 (350). Quantitative variables are expressed as the means and standard deviations with 95% confidence intervals (95% CIs). All study variables were normally distributed according to the Shapiro–Wilk test. Paired sample t tests were computed to compare the scores of the outcomes between baseline and after each treatment. Repeated measures analysis of variance was also conducted to evaluate the differences in disease activity and HRV between the stimulation parameters. If statistically significant results were found, a Bonferroni adjustment was applied to limit the risk of type I error, with 3 contrasts (considered baseline AAAA, baseline BBBB, and baseline CCCC). $p < 0.05$ indicated statistical significance.

Sample size = 12 (total number of measurements over three 8-month treatment periods). The 24-month intervention was carried out for 3 periods (8 months) of different nVNS protocols, and differences between the baseline and experimental periods were detected (Table 1). Paired t tests were performed to compare the SDAI scores, NIM indices and SD2/SD1 ratios before and after each treatment block.

Results

The changes between blocks are shown in Table 1. Statistically significant differences were found only for the SDAI score; baseline (M=10, SD=0.00); block C (M=21.51, SD=6.27) conditions; t(3)=3.39, p=0.043; and for the SD2/SD1, baseline (M=3.52, SD=0.00) and SD2/SD1 block A (M=2,56, SD=0.11) conditions; t(3)=17, p=0.0004.

Analysis of variance revealed that there was no significant difference among the three treatment periods for the SDAI (F(3, 9) = 1.694, $p > .05$), for the NIM (F(3, 9) = 0.6264, $p > .05$), or for the SD2/SD1 index (F(3, 9) = 0.4295, $p > .05$). Post hoc testing revealed statistically significant differences in only the means for SD2/SD1 between pairs at baseline (M=3.52, SD=0.0) and treatment period 1 (block A) (M=2.56, SD=0.11).

These results suggest that, compared with baseline, 10 Hz–300 μs (block C) worsens the SDAI score from moderate activity to high activity. Compared with those at baseline, the SVB at a stimulation frequency of 20 Hz-300 μs (block A) was significantly greater (Figure 2). Overall, the results did not indicate a conclusive improvement in disease activity or HRV, as shown in Figure 2. No adverse events were reported.

Discussion

To the best of our knowledge, this study provides the first in-human demonstration of the effects of chronic nVNS in an asplenic patient with RA. In this n-of-1 interventional study, the aim was to assess the clinical and physiological responses to three different nVNS settings, one every three months, for a 24-month period. This

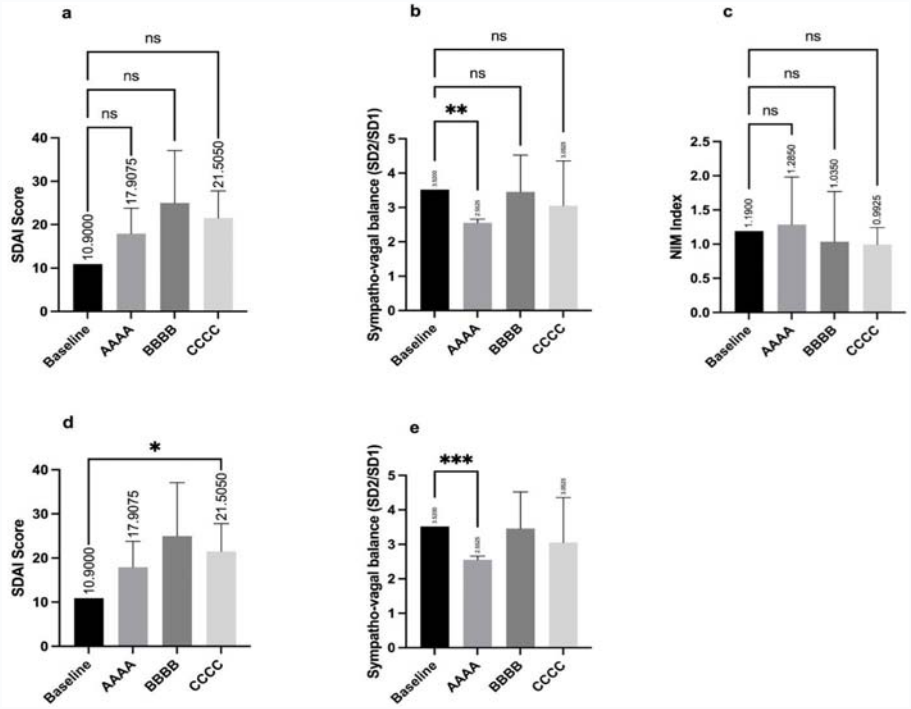

Figure 2: Repeated-measures ANOVA and paired t tests (baseline and treatment blocks). POMs were recorded at baseline and at follow-up visits. The column bar graphs show the means with sd. Repeated-measures ANOVA across treatment blocks (a–c) revealed improvements in only the SVB between baseline and AAAA (20 Hz-300 μs) (** p<0.01), (b), and paired t tests (d–e) revealed significant changes in the SDAI score between baseline and CCCC (10 Hz-300 μs) (*p<0.05) (d) and in the SVB between baseline and AAAA (***p<0.001) (e). SDAI, simplified disease activity index; SVB, sympathovagal balance.

study is the first to demonstrate that long-term nVNS is safe and well tolerated, as no adverse effects were reported. Second, in an n-of-1 long-term study, the home-treatment nVNS protocol was feasible and allowed valuable patient–practitioner collaboration, with a focus on individual (person-centered) care. However, RA is a very challenging autoimmune disorder, especially when, as in this case, the patient decided to be drug free. Moreover, although several invasive and noninvasive studies have demonstrated encouraging clinical and biological effects of VN in multidrug-resistant rheumatoid arthritis patients with high, moderate, and low disease activity [12, 15, 26], none of them included aslpenic participants. Unfortunately, the results of the present study were unable to confirm these findings, as they failed to yield a globally significant clinical and physiological effect after the 24-month duration of treatment; therefore, the null hypothesis that chronic nVNS does not improve DA, the SVB, or the NIM index in an asplenic male suffering from RA was accepted.

The three different nVNS settings did not improve the DA (exacerbating it in the case of block C) or the NIM index. Only a significant difference was observed in the SVB in the 20 Hz–300 μs stimulation setting. A possible explanation for these findings is that, first, the spleen is required to modulate the autoimmune response via the CAP [27]. Second, only high frequencies that are known to stimulate the afferent fibers of the vagus nerve might explain the temporary effects on the SVB through modulation of the CAN [28]. Third, the activation of the HPA axis alone might not be strong enough to trigger a significant immune response. Finally, the overall poor response of the HRV to stimulation might be explained by the use of only left nVNS compared with the observations of De Couck et al. [29], who reported that right nVNS might have greater effects on the HRV than left nVNS does. The ability to assess the effect of chronic nVNS (which has three different stimulation parameters) on an asplenic patient with RA is strong, as has never been reported, and indicates that nVNS might not be an alternative to classical pharmacologic treatment in RA or other autoimmune diseases in aslpenic patients. The major limitation of this study is that the findings are case study-based and therefore cannot be generalized. The other limitation is the self-administered treatment, as it represents an important bias, as there was no means to control that the patient would 100% respect the treatment as per the study protocol (stimulation time/day). Consequently, compliance is low.

Conclusion

Chronic nVNS for an asplenic patient suffering from RA was not conclusive. Long-term self-administered noninvasive stimulation was possible and was well tolerated without any side effects. Limitations of this study should be addressed in future randomized controlled studies wishing to look deeper into the impact of nVNS in asplenic populations suffering from RA. Further controlled studies with larger sample sizes are encouraged to corroborate our findings.

Compliance with Ethical Standards

The study was compliant with the WMA Declaration of Helsinki and approved by the Ethical Committee, UFV Madrid, Spain.

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