

ORIGINAL ARTICLE

Effect of acupuncture on heart rate variability in individuals with multiple sclerosis: a protocol for a double-blinded randomized controlled trial

Luciana Nagato^{a,b*}, Andrea Fernanda Leal^{a,b*}, Íbis Ariana Peña de Moraes^{b,c}, Amanda Farage Frade Barros^d, Alice Estevo Dias^e, Anna Maria Canzonieri^e, Juan G. Quispe-Cabanillas^{f,g}, Marcelo Ferreira^a, Carlos Bandeira de Mello Monteiro^{b,c}, Talita Dias da Silva^{a,b,c,h}, Celso Ferreira^a.

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^aDepartamento de Medicina (Cardiologia), Universidade Federal de São Paulo (UNIFESP), São Paulo, Brazil

^bGrupo de Pesquisa e Aplicações Tecnológicas em Reabilitação (PATER), Escola de Artes, Ciências e Humanidades, Universidade de São Paulo (EACH-USP), São Paulo, Brazil

^cPrograma de Pós-Graduação em Ciências da Reabilitação, Faculdade de Medicina da Universidade de São Paulo (FMUSP), São Paulo, Brazil

^dUniversidade Brasil, São Paulo, Brazil.

^eAssociação Brasileira de Esclerose Múltipla (ABEM), São Paulo, Brazil

^fNeuroimmunology Unit, Department of Genetics, Evolution and Bioagents, University of Campinas, Campinas, SP, Brazil.

^gAcupuncture Course Hermínio Ometto University Center, Araras, Brazil

^hFaculdade de Medicina, Universidade Cidade de São Paulo (UNICID), São Paulo, Brazil

*Co-first author: Luciana Nagato and Andrea Fernanda Leal are joint first authors.

Corresponding author
luciana.nagato@unifesp.com

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Abstract

Background: the growing of patients with multiple sclerosis seeking acupuncture treatment is based on clinical reports of improvements in symptoms. Considering that autonomic impairment, including cardiovascular autonomic dysfunction, is not uncommon in patients with MS, neuromodulation with acupuncture could be an interesting tool to change heart rate variability in this population.

Objective: to evaluate heart rate variability in patients with multiple sclerosis, during the application of acupuncture, in order to analyze the behavior of the autonomic nervous system before, during and after therapy and changes in condition after a longitudinal intervention.

Methods: a double-blinded randomized sham-controlled crossover trial with a 1:1 allocation ratio will be conducted, with 40 individuals without a previous illness, who will constitute the control group, and 40 individuals with Multiple Sclerosis, who will constitute the experimental group, paired by age and sex. All participants will undertake active or sham acupuncture sessions.

Discussion: according to the studies found, cardiovascular autonomic dysfunction is expected, with alterations in heart rate variability. Although neuromodulation with acupuncture can control pain and inflammation, there are still difficulties in affirming whether the balance between the sympathetic and parasympathetic systems can be changed by acupuncture.

Trial registration: We registered this trial on ClinicalTrials.gov, ID: NCT05523466

Keywords: multiple sclerosis, heart rate, acupuncture.

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Authors summary

Why was this study done?

The growing search for acupuncture treatment by patients with multiple sclerosis is based on clinical reports of improvements in symptoms, however further studies are necessary to provide a greater scientific basis for these reports. Autonomic impairment, including cardiovascular autonomic dysfunction, is not uncommon in patients with MS. Cardiovascular involvement may be expressed as poor physical fitness, fatigue, orthostatic hypotonia, or cardiac arrhythmias. In this way neuromodulation with acupuncture could provide vital information about the functional organization of the nervous system.

What did the researchers do and find?

This study is a protocol for a randomized clinical trial that aims to assess autonomic function at the time of acupuncture in patients with multiple sclerosis.

What do these findings mean?

If the hypothesis for this protocol is confirmed, the findings will suggest that acupuncture can contribute to autonomic system balance and thus collaborate in the evolution of the disease.

INTRODUCTION

Multiple sclerosis (MS) is a severe autoimmune demyelinating disease that affects the nervous system, has high morbidity and mortality, and for which no effective targeted therapies are available. There are currently approximately 3 million people worldwide who are affected by MS. The disease typically begins between the ages of 20 and 50 and is twice as common in women as in men¹. This equates to 1 in 3,000 people in the world living with MS, and in countries with the highest prevalence, as many as 1 in every 300 people have MS².

The correlations between clinical presentations and pathological alterations are extremely complex³. The clinical subtypes of Multiple Sclerosis (MS) are determined as Relapsing-Remitting, Secondarily Progressive, and Primarily Progressive. These names are widely incorporated in clinical practice, being used for better communication with the patient in relation to the course of the disease and to define specific populations in research, however, to date, no biomarkers or MRI signals have been identified which can distinguish between them^{4,5}.

The Relapsing-Remitting type is defined by clinical outbreaks or increased lesions in the central nervous system (CNS), which generate major phenotypic alterations. In turn, the Primarily Progressive type also shows alterations, generating lesions in the CNS, but there is no inflammatory activity, that is, no outbreaks^{4,5}. Progression in this subtype occurs within months or years, with a deterioration process that occurs in a slow and irreversible way⁶. The Secondarily Progressive type is an evolution of the RR phase, promoting a progressive and continuous neurological disability, and this transition mechanism is still poorly described^{7,8}.

Although, to date, there is no cure for MS, there are several therapies aimed at improving function after an outbreak and preventing disability. Therapies, including medications and neuro rehabilitation, can improve some symptoms, but they cannot change the course of the disease¹.

Autonomic impairment, including cardiovascular autonomic dysfunction, is not uncommon in patients with MS. Cardiovascular involvement may be expressed as poor physical fitness, fatigue, orthostatic hypotonia, or cardiac arrhythmias. The autonomic dysfunction observed in MS is believed to be associated with plaques located

in the brainstem and spinal cord, which affect autonomic areas and their connections⁹.

The Autonomic Nervous System (*i.e.* sympathetic and parasympathetic systems) is responsible for involuntary control of vital physiological functions, such as heart rate at rest and in response to stress. Therefore, adequate responses depend on the balance between the sympathetic and parasympathetic systems¹⁰⁻¹³.

To evaluate the activity of the ANS (Autonomic Nervous System) under physiological and pathological conditions, HRV measurement is a non-invasive technique able to provide important information about the individual's physical and mental health^{12,14}. The presence of high HRV represents efficient autonomic mechanisms, while low HRV may indicate abnormal adaptation of these mechanisms and insufficient ANS response^{12,15}. HRV is a widely accepted means of assessing cardiac autonomic regulation¹⁶.

Some studies have documented abnormal HRV in patients with MS, showing significant loss of vagal tone and increased sympathetic tone^{17,18}. Dysautonomia in MS patients was more likely to occur in patients with a greater extent of physical disability¹⁹ and most pronounced in the progressive variant of MS disease²⁰. In turn, Studer *et al.*²¹ and Shirbani *et al.*²² showed that autonomic balance appears to be intimately linked to both the inflammatory activity of multiple sclerosis, which is featured by an overall hypoactivity of the sympathetic nervous system, and its compensatory plastic processes, which appear to be inefficient in the case of worsening and progressive multiple sclerosis.

Neuronal stimulation is a promising emerging field in modern medicine for the control of organ function and reestablishing physiological homeostasis during illness^{23,24}. Multiple recent studies show the potential of nerve stimulation to control inflammation and improve organ function in MS disorders. Acupuncture is a practice of Traditional Chinese Medicine that aims to both treat and prevent diseases through various methods, including the insertion of needles at specific points of the body²⁴, and which should be analyzed as a complementary therapeutic measure in the treatment of MS. The locations of the points used in Acupuncture are found in channels or meridians through which the qi (energy) circulates, aiming to regulate the functions of the organism and

allow the free flow of qi. The 1997 National Institutes of Health consensus considers the use of acupuncture as a complementary therapy in the management of painful conditions²⁵.

Furthermore, neuromodulation with acupuncture can provide vital information about the functional organization of the nervous system²⁴. In this way Quispe-Cabanillas *et al.*²⁶ evaluated thirty-one patients with Relapsing-Remitting Multiple Sclerosis undergoing treatment with immunomodulators, and concluded that Electroacupuncture improved various aspects of quality of life, including a reduction in pain and depression. In addition, according to Anderson *et al.*²⁷ the ability of acupuncture to modulate HRV could imply that acupuncture may function by regulating and integrating the various physiological systems in the body. In their review all the included randomized placebo-controlled trials (a total of 16) suggest that acupuncture improves HRV, but 12 of these studied healthy subjects, four studied subjects with medically diagnosed conditions, and none studied these effects in people with MS.

Given the above, the objective of this study is to evaluate heart rate variability in individuals with MS during the application of Acupuncture, in order to analyze the behavior of the autonomic nervous system before, during, and after therapy and the changes in the condition. It is necessary to emphasize the need for studies that provide evidence of the benefits of the adopted procedures, for better understanding and consequent better application, to assist rehabilitation professionals in choosing the therapeutic program based on scientific evidence. Considering the deliberations presented, as a hypothesis, we believe that individuals with MS will present greater sympathetic modulation that will tend to balance during and after the applicability of Acupuncture.

METHODS

Trial design

A double-blinded randomized sham-controlled crossover trial with a 1:1 allocation ratio will be conducted. In total, 40 individuals without a previous illness will be evaluated, who will constitute the control group and 40

individuals with MS, who will constitute the experimental group, paired by age and sex. All participants will undertake active or sham acupuncture sessions. Group A-S will start with 5 sessions (1 per week) of active acupuncture for 20 min combined with HRV evaluation. After a 2-week washout, this group will be reallocated to another 5 sessions (1 per week) of sham acupuncture for 20 min combined with HRV evaluation. Meanwhile, Group S-A will carry out the opposite protocol, participants will start with the allocated 5 sessions (1 per week) of sham acupuncture combined with HRV evaluation, and after a 2-week washout period will be reallocated to 5 sessions (1 per week) of active acupuncture combined with HRV evaluation.

As previously described by Shen *et al.*²⁸, Tseng, Tseng and Chang²⁹, Li *et al.*³⁰ and Shin *et al.*³¹, a 2-week washout period was shown to be sufficient to reset the effects of the first 5 sessions. Figure 1 summarizes the planned experimental design. This research protocol follows the SPIRIT recommendations. A total of 40 participants with MS will be recruited through referral by the coordinators of the Brazilian multiple sclerosis association (ABEM), located in São Paulo state.

Those interested in participating will undergo detailed screening according to the study eligibility criteria. The sample size was calculated using statistical software (GPower 3.1.5) on the main outcome measure (Heart Rate Variability indices). This calculation was based on data from a systematic review which indicated that Brazil has a prevalence of 1.36 to 27.2 cases per 100,000³². The study has a power of 0.80, alpha of 0.05, and effect size of 0.65 (Cohen's d). The sample estimate indicated that 40 participants would be needed (i.e., 20 per group), with an adjustment to allow for withdrawal (20%). This article is reported in accordance with the Consolidated Standards of Reporting Trials (CONSORT)^{33,34}. The study was approved by the Research Ethics Committee of UNIFESP, CAAE: 01515118.3.0000.5505. All participants will sign the Free and Informed Consent Form.

This article is reported in accordance (tables 1, 2) with the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT)^{33,34}.

Table 1: SPIRIT - description of the study protocol, schedule of enrolment, interventions, and assessments.

TIMEPOINT	STUDY PERIOD												
	Enrolment		Allocation		Post-allocation								close-out
	t ¹	0	t ¹	t ²	t ³	t ⁴	t ⁵	t ⁶	t ⁷	t ⁸	t ⁹	t ¹⁰	t ¹¹
		1 day	7 day	14day	21 day	28 day	42 day	49 day	56 day	63 day	77 day	84 day	
ENROLMENT													
Eligibility screen	x												
Informed consent	x												
Assessment scales and tools	x												
Allocations		x											
INTERVENTIONS													
Group S-A			←	←	←	←	←	←	←	←	←	←	←
Group A-S			→	→	→	→	→	→	→	→	→	→	→
ASSESSMENTS													
EDSS	x						x	x				x	x
MoCA	x												x
BRUMS			x									x	
BDI-II	x												x
EVA (pain)		x	x	x	x	x	x	x	x	x	x	x	x
PHYSIOLOGICAL ASSESSMENT													
Ryodoraku			xx				xx	xx				xx	
Thermography			xx				xx	xx				xx	
Heart Rate Variability			x	x	x	x	x	x	x	x	x	x	x

S-A: Sham-Acupuncture; A-S: Acupuncture – Sham; EDSS: Expanded Disability Status Scale; MoCA: Montreal Cognitive Assessment; BRUMS: Brunel Mood Scale; BDI-II: Beck Depression Inventory Second Edition; VAS: Visual Analogue Scale.

Table 2: Trial characteristics based on WHO Trial Registration Data Set.

Data category	trial information
Primary registry and trial identifying number	clinicaltrials.Gov, id: nct05523466
Date of registration in primary registry	august 2022
Secondary identifying numbers	ethical committee of university
Caae 01515118.3.0000.5505 – January 18th, 2019.	
Source(s) of monetary or material support	coordenação de aperfeiçoamento de pessoal de nível superior – brasil (capes)
Primary sponsor	federal university of são paulo– unifesp
Secondary sponsor(s)	na
Contact for public queries	tds, cf
Contact for scientific queries	tds, cf
Public title	effect of acupuncture on heart rate variability of people with multiple sclerosis: a study protocol for a double-blinded randomized controlled trial
Scientific title	analysis of autonomic cardiac modulation during acupuncture in individuals with multiple sclerosis.
Country of recruitment	Brazil
Health condition(s) or problem(s) studied	multiple sclerosis
Interventions	<p>for data collection, the following instrument will be used: visual analogue scale of pain. This scale will be applied at the beginning of each intervention day. In sequence, the participants will perform the thermography protocol, which comprises capturing images in various postures in the orthostatic position (in the initial and final assessments).</p> <p>Afterwards, the participants will remain in the supine dorsal position on a stretcher at rest for 10 minutes to start the ans analysis with the ryodoraku (device that measures the electrical conductivity of certain points on the skin) through the digital reading of the 24 points called ryodos³⁵.</p> <p>Immediately after the placement of the instrument for analyzing hrv, the polar v800, , the needles will be placed at the points carefully selected for this research. The needling will proceed for the experimental group using specific traditional chinese acupuncture points: st36, sp6, lr3, li4, for 20 minutes while the control group will receive needling at sham points (points not recognized as acupuncture points), for the duration of 20 minutes.</p> <p>Final phase, new reading with ryodoraku and thermography.</p>
Key inclusion and exclusion criteria	<p>Inclusion criteria</p> <p>This study will include individuals diagnosed with ms, aged over 18 years, with motor and intellectual capacity to understand the evaluations, with light and moderate functional classification levels (edss scale) and who agree to participate in the study through signing the informed consent form.</p> <p>Exclusion criteria</p> <p>Participants will be excluded if they (1) do not understand the evaluations; (2) have a cardiac disease that impedes the assessment of hrv; (3) have a cardiac pacemaker.</p>
Study type interventional allocation:	randomized

Continuation - Table 2: Trial characteristics based on WHO Trial Registration Data Set.

Data category	trial information
Masking:	double-blind
Assignment:	crossover
Primary purpose:	treatment
Date of first enrolment	december 2022
Target sample size	80 participants
Recruitment status	recruitment not started
Primary outcome(s)	heart rate variability improvement
Key secondary outcome(s)	ryodoraku and thermography improvement

Study Location and Period

Randomization

To perform the allocation procedure, the encoded groups will be placed inside a closed opaque envelope, which will be labeled with the code for each participant. Envelopes will be opened only during the first acupuncture or sham intervention.

To blind the evaluators, they will receive the results without any group description.

Study Population and Eligibility Criteria

Inclusion Criteria

This study will include individuals diagnosed with MS, aged over 18 years, with motor and intellectual capacity to understand the evaluations, with light and moderate functional classification levels of MS (EDSS scale), and who agree to participate in the study by signing the informed consent form.

Exclusion Criteria

Participants will be excluded if they (1) do not understand the evaluations; (2) have a cardiac disease that impedes the assessment of HRV; (3) have a cardiac pacemaker.

Withdrawal Criteria

Participants will be withdrawn from the study if they are not willing to continue, cannot be present on the day of the experiment, or miss any treatment session. Screening tools.

Functional assessments

Expanded Disability Status Scale (EDSS)

The Expanded Disability Status Scale was developed by Kurtzke³⁶. This scale evaluates the functional systems of the central nervous system and is used to describe disease progression in patients with MS and to assess the effectiveness of therapeutic interventions in clinical trials. It is subdivided into eight functional systems: pyramidal, cerebellar, brainstem, sensitive, bladder, intestinal, visual, and mental functions³⁷. The scale consists of an ordinal rating system ranging from 0 (normal neurological status) to 10 (death due to MS) in increments of 0.5. Lower values on the scale of the EDSS measure impairments based on the neurological examination, while the upper range of the scale (> EDSS 6) measures disabilities in patients with MS. The determination of EDSS 4 – 6 is heavily dependent on aspects of walking ability³⁸.

Cognitive assessments

Montreal Cognitive Assessment, MoCA

The MoCA screening test is a useful tool for the detection of mild dementia or mild cognitive impairment. It covers 10 cognitive domains using rapid, sensitive, and easy-to-administer cognitive tasks³⁹. It is a 10-minute test, during which patients answer items including the following: the short-term memory recall task, visuospatial abilities, a three-dimensional cube, executive functions, a phonemic fluency task, a verbal abstraction task, a sustained attention task, a serial subtraction task, digits forward and backward, language, and orientation to time and place 40. Each domain generates a different score, totaling a 30-point cognitive screening test. The suggested cut-off point on the MoCA is ^{26 39}.

Emotional and behavioral assessments

Brunel Mood Scale (BRUMS)

The Brunel Mood Scale was developed to provide a quick measurement of the mood state of populations composed of adults and teenagers. The scale consists of 24 items to assess mood change in the six mood dimensions - tension, depression, anger, vigor, fatigue, and confusion. The question asked is “How do you feel now?”. The items are assessed on a 5-point scale anchored from “not at all” (0) to “extremely” (4). Each subscale contains four items. From the sum of the responses of each subscale, a score is obtained that can vary from 0 to 16. The scale takes around one to two minutes to complete ⁴¹. The BRUMS has been presented as a tool for detection of over-training syndrome, and has been used in different populations ⁴² and contexts in Brazil⁴³ and other countries, ⁴⁴ including for evaluations in populations with cardiac disorders⁴⁵.

Beck Depression Inventory Second Edition (BDI-II)

The BDI-II is a relevant psychometric instrument, presenting high reliability and the capacity to discriminate between depressed and non-depressed subjects⁴⁶. The main factors of the inventory are a cognitive and a somatic factor, and some items can shift between factors depending on which population the sample represents⁴⁷. Examples of these items include questions regarding changes in sleep patterns, difficulty concentrating, sadness, self-dislike, crying, loss of energy, and suicidal thoughts⁴⁸. The BDI-II is a self-report measure of depression. Twenty-one symptoms of depression are rated on a 4-point (0 to 3)

scale, considering the time frame of the previous 2 weeks. The total scores are obtained by summing the ratings for all items. Scores ranging between 0 and 13 are indicative of minimal depression; scores between 14 and 19 are considered to reflect a mild level of depression; scores of 20 to 28 are considered moderate; and scores ranging from 29 to 63 are labeled severe⁴⁹.

Evaluation tools

Visual Analogue Scale (VAS)

The VAS is by far the most frequently used assessment instrument⁵⁰. Visual analogue scales are psychometric measuring instruments designed to document the characteristics of disease-related symptom severity in individual patients, and to achieve a rapid (statistically measurable and reproducible) classification of symptom severity and disease control⁵¹. In the current study, we will use this scale to measure pain. The VAS consists of a 100 mm line giving a score ranging from 0 to 10, whose endpoints are designated as 'no pain' and 'the worst pain imaginable'. Although some pain sensations may be equally intense and unpleasant, the patient needs to judge the two aspects independently, to indicate the relative intensity of their pain sensation: the higher the number, the greater the intensity⁵².

Blinding

Both the participants and assessors will be blind to group allocation during the study. To ensure proper blinding, participants will receive the same care, but with points outside the area of the correct acupuncture points in the sham treatment. Considering that the needle application must be performed by a qualified professional, it is not possible for this individual to be blinded.

Intervention

The VAS Scale will be applied at the beginning of the data collection.

In sequence, the participants will perform the Thermography protocol, which comprises capturing images in an orthostatic position.

Subsequently, the participants will remain in the supine dorsal position on a stretcher at rest for 10 minutes to start the ANS analysis with the Ryodoraku (device that measures the electrical conductivity of certain points on the skin) through the digital reading of 24 points called ryodos³⁵.

Immediately after the placement of the instrument for analyzing heart rate variability, the Polar V800, the needles will be placed at the points carefully selected for this research. The needling will proceed for the experimental group using specific Traditional Chinese Acupuncture points: Zusanli (ST36), Sanyinjiao (SP6), Taichong (LR3), Hegu (LI4), for 20 minutes, while the control group will receive needling at sham points (points not recognized as Acupuncture points), for a duration of 20 minutes. Finally, new Ryodoraku and Thermography readings will be performed.

Ryodoraku

Based on the Ryodoraku theory, developed by Dr. Yoshio Nakatani⁵³ there are 12 meridians on each of the right and left sides of the human body. The property of Ryodoraku can reflect the condition of certain organ(s) by analyzing and comparing their mutual relations and changes using a micro-electrical current. The electrical state of the acupuncture points of the human subject is measured by a computerized testing instrument using a very low electrical current⁵⁴. From the traditional theory, the mechanism can be explained by the viscera-skin sympathetic nerve reflex. The impulses from the viscera radiate to the spinal cord and the reflex zones are then reflected onto the skin surface via the efferent sympathetic nerves and appear as a longitudinal connecting system, just like meridian lines⁵⁵.

Electrodermal screening measures either skin impedance or conductance at acupuncture points, as diagnostic aids for planning treatment strategies. Skin impedance is the skin's opposition to the flow of current. The practice of measuring skin impedance is based on the widely held assumption that acupuncture points are loci of decreased impedance compared to skin sites where there are no known acupuncture points⁵⁶.

Recently, researchers have begun to explore electrical conductance at acupoints as a potential physiological outcome measure for use in clinical trials of acupuncture. The technique consists of measuring electrical conductance at representative points for each meridian, generally the Yuan points on the wrists and ankles. Because of the high variability in skin conductance measurements, readings are never taken as absolute but rather as relative to all other measurements on the same patient and reported on a normalized scale. Increased conductance (decreased resistance) is believed to represent an excess in the meridian being measured, while decreased conductance (increased resistance) represents a deficiency⁵⁷.

Thermography

Infrared cameras measure the wavelength of the infrared radiation an object emits and converts it to electrical energy, which is then used to measure surface temperature. As an imaging technology, it has numerous advantages resulting from its non-invasive and non-contact approach. In this way, thermal information can be assessed from a distance, a valuable feature for field biology, without the need for surgical interventions or handling that have prolonged effects on body temperature⁵⁸. This is one of the most promising methods of probing the psychological status of human beings⁵⁹.

The human skin acts as a black-body radiator, operating at the body temperature to generate an infrared radiation spectrum with a broad peak at around $\lambda = 9.5\mu\text{m}$. The body's core temperature is developed in the central part of the body where the major organs are located (heart, lungs, liver, etc.) and heat is transported by the blood through the arteries to the rest of the body. Because the environment temperature is usually lower than the core temperature (37 °C), there is a cooling effect from the skin to the environment and heat transfer takes place from the blood into the surrounding tissue and to the environment⁶⁰.

With the aid of thermometry, infrared electromagnetic waves of 0.7–1000 M (which the human eye is unable to identify) can be visualized; healthy human bodies emit waves with a length of 3–10 M. Temperature differences as low as 0.1°C can be perceived and may help track the response to acupuncture as opposed to needling of non-acupoints in order to objectively estimate effects of needling⁶¹.

Lastly, when the external environment is stable (no changes in irradiative, conductive, or convective heat transfer), changes in temperature detected will be related to changes in blood flow and thus sensitive to acute changes in sympathetic activity which would induce vasoconstriction as the vasculature is not parasympathetically innervated. This temperature rise under extreme stimuli may be due to increases in cardiac output, which could increase perfusion pressure or the release of local factors, such as nitric oxide from nearby endothelial cells, and may be sympathetically mediated but could also be related to parasympathetic withdrawal, that induces cardiac acceleration, suggesting a complex interplay of autonomic control⁶².

Analysis of Heart Rate Variability

After the initial evaluation, the capture strap will be placed on the volunteer's chest, and the heart rate receiver (V800, Polar) on their wrist. This equipment was previously validated for capturing the heart rate, beat by beat, and the use of the data for HRV analysis^{14,63}. Then individuals will be positioned in a supine position and will remain at rest for 10 minutes, breathing spontaneously. The analysis will then be carried out during the performance of Acupuncture. For analysis of HRV data, 256 consecutive intervals will be used. Subsequently, digital filtering will be performed to eliminate premature ectopic beats and artifacts; there will be no substitution, only the elimination of artifacts¹². HRV analysis will be performed using linear methods (time and frequency domains) and non-linear methods, assessed by Kubios HRV software.

In the time domain, the RMSSD, pNNS50, and SDNN indices will be used. The RMSSD is the square root of the mean of squared differences between successive beat intervals^{64,65}; the pNNS50 index, in turn, is a sensitive and easily interpretable marker of the parasympathetic ANS modulation, defined as the percentage of successive differences in the R-R intervals whose absolute value exceeds 50ms; and the SDNN reflects the participation of both branches of the ANS and corresponds to the standard-deviation of the mean of all normal RR intervals, expressed in ms⁶⁶⁻⁶⁹.

For the analysis of HRV in the frequency domain, low frequency spectral components (LF – range between 0.04 to 0.15 Hertz) will be used in absolute units and high frequency components (HF – range from 0.15 to 0.4Hertz), in normalized units, as well as the ratio between these components (LF/HF), which represents the relative value of each spectral component in relation to the total power, minus the exceptionally low frequency components (VLF). The algorithm used for the spectral analysis will be the fast Fourier transform - FFT (256 s window with 50% overlap)^{12,70,71}.

For HRV analysis using nonlinear methods, the DFA

quantifies the presence or absence of fractal correlation properties of RR intervals and has been validated using time series data. This method calculates the fluctuation in the mean square root of the integral and deparates the time series, allowing the detection of intrinsic self-similarity embedded in the non-stationary time series. The DFA graph is not strictly linear, but consists of two distinct regions of distinct curves, separated at one point, suggesting that there is a short-term fractal scale exponent (a1) during periods of 4-11 beats (or 4 to 13), and a long-term exponent (a2), for longer periods (greater than 11 beats)⁷¹⁻⁷³.

Primary Outcome

We will evaluate changes in the ANS after the intervention with active acupuncture and sham acupuncture in patients with MS, during a period of 28 days, after the 2-week follow-up, and during the further 28 days of intervention.

Secondary Outcome

We will analyze changes in Ryodoraku and Thermography regarding both interventions (active and sham acupuncture).

Data analysis

Statistical analysis will be performed using IBM-SPSS (version 26.0, IBM Corp., Armonk, NY, USA). As dependent variables, all HRV indices will be considered, as well as test scores of all assessment scales, Ryodoraku and Thermography. If the data meet the assumptions of normality, multiple analysis of variance will be used to compare the study groups (Control and Multiple sclerosis) and intragroup comparison (A-S and S-A, when comparing the same subject), with least significant difference post-hoc test. If the data do not meet the assumptions of normality, the differences between the groups will be analyzed using the Kruskal–Wallis test with Dunn–Bonferroni post-test on each pair of groups. The same tests will be applied for $p < 0.05$, which will be considered significant.

Risks and benefits

The research is based on analyzing the variability of the ANS – Autonomous Nervous System during the therapeutic use of an Acupuncture protocol and a complementary protocol of Thermography in individuals with Multiple Sclerosis. Therefore, it is considered research with human beings and as such has a duty to safeguard the integrity of those involved. This safeguard will be in relation to the questions of privacy, secrecy, and the minimization of any discomfort.

Risks

The risks of participation in the research are minimal and related to the use of acupuncture needles, which are sterile, of single and individual use, and discarded at the end of the procedure in a suitable receptacle for sharp performing material.

Regarding the use of Ryodoraku – Model NKL, the risks are also minimal, which may include slight discomfort in relation to the pressure of the wet cotton tip

on the skin applied on the points to be evaluated during the reading. Therefore, there will always be a responsible person in attendance to provide any necessary guidance to the participant.

The procedures adopted in this research comply with the Criteria of Ethics in Research with Human Beings according to resolution 44/12 of the National Health Council. None of the procedures used puts the participant's dignity at risk.

Confidentiality: all information collected will be kept strictly confidential, and restricted only to the researcher (a) and the supervisor (a). Only these individuals will have knowledge of the data collected for the development of the research.

Benefits

Research participants will receive only indirect benefits, from future information that may improve the quality of life of the participants. Therefore, there is no immediate benefit foreseen to the participants of this research. After the conclusion of this work, the findings could contribute in a significant way to the adequate, organized, and effective elaboration of the diagnostic methods and treatment with Acupuncture.

Corroborating, we hope that this study will contribute significantly to rehabilitation programs, so that any knowledge observed and acquired from this research can contribute to the advancement of science and to the health of these patients. The researcher commits to disseminating the obtained results.

Trial status

Participant recruitment will start in December 2022. It was paused due to quarantine in March 2020 and has not yet restarted. We expect to finish recruitment in May 2023. Study completion is estimated by November 2023.

DISCUSSION

As mentioned at the beginning of this study, autonomic impairment, including cardiovascular autonomic dysfunction, is not uncommon in patients with MS. Cardiovascular involvement can be expressed as low physical fitness⁷⁴, even in daily activities of daily living, as observed in the study by Coote S, O'Dwyer C.⁷⁵, in which people with MS took significantly fewer steps in activities of daily living, used bilateral support, and had higher energy expenditure per step for common activities such as walking and negotiating stairs, which causes fatigue, and greater autonomy of the autonomic nervous system⁷⁶⁻⁷⁸. Furthermore, Brevezinova *et al.*¹⁷ and Tombul¹⁸ defend that HRV is lower in patients with MS, presenting a significant loss of vagal and sympathetic tone, with an increase in LF/HF index alterations.

In addition, neuromodulation with acupuncture

can control pain and inflammation, and provide vital information about the functional organization of the nervous system²⁴, as well as improving various aspects of quality of life, including a reduction in depression²⁶. Research recognizes the difficulty of finding safe parameters to define dysautonomia in Multiple Sclerosis due to its complexity⁷⁹, but more studies are needed, as the balance between the sympathetic and parasympathetic systems is a sensitive predictor of the capacity for emotional regulation of intrinsic and extrinsic stress responses¹¹⁻¹³.

The choices of points used in this protocol proposal consider that the proper combination of the chosen points is important in traditional Chinese methods⁸⁰. Among several interconnections between acupuncture points, LI 4, LI 11, ST 36, and SP 6 indicate important combinations but also have individual functions, as in the study by Zheng Y, *et al.*⁸¹ who observed that acupuncture at LR3 specifically activated the functional brain network that participates in visual function, associative function, and emotional cognition, which are similar to LR3 characteristics in traditional Chinese medicine. Previous evidence also clarified that the ST 36, LI11, and LI4 points exert a beneficial effect on promoting neurogenesis and cell proliferation in the central nervous system⁸². Furthermore, early electroacupuncture at ST 36 in mice with ALS (Amyotrophic Lateral Sclerosis) was related to effects in suppressing nerve inflammation, and reducing activation of small and medium glial cells in the brainstem and spinal cord⁸³.

Author Contributions

Conceptualization, L.N., A.F.L.; methodology, T.D.S., C.B.M.M., C.F., M.F.; formal analysis, Í.A.P.M.; writing - original draft preparation, L.N., A.F.L., A.F.F.B.; writing - review and editing, A.E.D., A.M.C., Í.A.P.M., J.G.Q.C.; supervision, T.D.S., C.B.M.M., C.F., M.F.; project administration, Í.A.P.M.; funding acquisition, T.D.S. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Resumo

Introdução: o crescimento de pacientes com esclerose múltipla que procuram tratamento com acupuntura é baseado em relatos clínicos de melhora dos sintomas. Considerando que o comprometimento autonômico, incluindo a disfunção autonômica cardiovascular, não é incomum em pacientes com EM a neuromodulação com acupuntura pode ser uma ferramenta interessante para alterar a variabilidade da frequência cardíaca nessa população.

Objetivo: avaliar a variabilidade da frequência cardíaca em pacientes com esclerose múltipla, durante a aplicação da Acupuntura, a fim de analisar o comportamento do sistema nervoso autônomo antes, durante e após a terapia e as mudanças na condição após uma intervenção longitudinal.

Métodos: será realizado um ensaio clínico cruzado, randomizado, placebo-controlado, duplo-cego, com proporção de alocação de 1:1, com 40 indivíduos sem doença prévia, que constituirão o grupo controle, e 40 indivíduos com Esclerose Múltipla, que constituirão o grupo experimental. grupo, pareado por idade e sexo. Todos os participantes realizarão sessões de acupuntura ativas ou simuladas.

Discussão: de acordo com os estudos encontrados, é esperada disfunção autonômica cardiovascular, com alterações na variabilidade da frequência cardíaca. Embora a neuromodulação com acupuntura possa controlar a dor e a inflamação, ainda há dificuldades em afirmar se o equilíbrio entre os sistemas simpático e parassimpático pode ser alterado pela acupuntura.

Registro do estudo: registramos este estudo em ClinicalTrials.gov, ID: NCT05523466

Palavras-chave: esclerose múltipla, frequência cardíaca, acupuntura.

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