Biomarkers of Auricular Stimulation - Protocol of Systematic Review and Meta-analysis of Randomized Controlled Clinical Trials

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Protocol

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Abstract

Background: The number of randomized controlled trials using auricular stimulation (AS) such as transauricular vagus nerve stimulation, or auricular electrostimulation or auricular acupuncture or acupressure, in experimental and clinical settings has increased markedly over the last three decades. Various clinical scores and self-reported outcomes are used as primary outcome measures to evaluate the effects of AS; they have been already analysed in an array of systematic reviews. But regarding the effect of AS on objective, physiological measures (biomarkers), for example blood values, heart rate, electrophysiological measurements and brain imaging, a systematic analysis is missing. This systematic review protocol was developed following the PRISMA guidelines to explore and evaluate for the first time the existing literature examining the impact of AS on biomarkers in randomized controlled trials as reported in their primary or secondary outcomes.

Methods and analysis: The following databases will be searched: MEDLINE (PubMed), EMBASE, Cochrane Central Register of Controlled Trials (CENTRAL), ISI Web of Science, and Scopus Database. RCTs will be included if an abstract is available in English. Data collection and analysis will be conducted by two reviewers independently. Quality and risk assessment of included studies will be done using the Cochrane 6.1.0 handbook criteria and RoB 2 tool and meta-analysis of the effect of the most frequently assessed biomarkers will be conducted using the statistical software RevMan V.5.3.

Ethics and dissemination: This systematic review will evaluate the effect of AS and related techniques on various blood values and physiological measures. Since all data used in this systematic review and meta-analysis will have been published, this review does not require an ethical approval. The results will be published in a peer-reviewed journal as well as presented in relevant conferences.

Registration number: PROSPERO CRD42021231885

Introduction

1.1 Background

Research evaluating the effectiveness of auricular stimulation (AS) has markedly increased in the last thirty years. Smoking and drug cessation, postoperative pain relief, chronic pain, heart rhythm disorders, epilepsy, insomnia and depression, and obesity treatments are among the most frequently evaluated conditions [1–7]. AS trials apply a variety of techniques such as acupuncture or acupressure, or electrostimulation or transauricular vagus nerve stimulation (taVNS). Evaluation of biomarkers, such as blood samples measuring metabolic profiles, inflammatory or immunological markers, as well as electrophysiological measurements and functional neuroimaging are used as outcomes. The potential mechanism of AS effects is attributed to the neuroanatomical conditions of the external auricle. It is presumed, that auricular stimulation exerts its effects via the involvement of cranial nerves V, VII and X [8], which lead to the modulation of the brain areas involved in the stress response, including the limbic system, locus coeruleus and hypothalamus [9, 10].
An overview of these postulated effects of AS on biomarkers, which may further clarify the mechanisms of this treatment modality, is missing [11]. This protocol was developed following the PRISMA guidelines to explore and evaluate - for the first time - the existing literature regarding the effect of biomarkers in randomized controlled trials comparing AS with sham AS or AS with no intervention. The checklist is provided as additional file. This review also aims to investigate whether or not systemic effects from ear stimulation are clinically significant.

The review will also try to identify the potential for future clinical research for AS.

**Methods**

This systematic review protocol has been registered on PROSPERO CRD42021231885. No ethical approval is needed, since included data in this systematic review and meta-analysis have been published. Systematic review and meta-analysis is conducted according the Cochrane 6.1.0 Handbook for Systematic Reviews of Interventions.

**2.1. Eligibility Criteria for including studies in the review**

**2.1.1. Types of studies**

Only randomized controlled trials (RCTs) in European languages will be included. The funding source will be registered.

**2.1.2. Types of participants**

No restriction on the study population will be made in order to broaden the scope of the review. Thus there will be no restrictions regarding the age, gender or ethnicity of participants. However, subgroup analysis will divide study population into different sections if this is practical.

**2.1.3. Types of interventions/comparators**

Following interventions will be included: auricular stimulation alone or in addition to routine care. All interventions from traditional auricular stimulation (i.e. auricular acupuncture, auricular acupressure) to related techniques such as transcutaneous auricular vagus nerve stimulation taVNS or cranial electrotherapy stimulation (CES) etc. will be included. Any comparison with control conditions (sham acupuncture, sham acupressure, placebo stimulation, routine care etc.) will be included.

**2.1.4. Types of outcome measures**

Owing to the exploratory approach of this systematic review there will be no ranking of outcomes. All biomarkers that are reported with results will be registered and evaluated. Among the blood values there will be lipids, cortisol, and inflammatory markers. Other evaluated outcomes will include urine samples, electroencephalograms (EEG), electrocardiograms (ECG), fMRI, and Doppler measures.
2.1.4.1. Safety of intervention.

Assessing safety of auricular stimulation reported adverse events and serious adverse events will be analysed.

2.2. Search methods for identification of studies

2.2.1. Electronic searches.

The following databases will be screened from inception until 12.02.2021: MEDLINE (PubMed), EMBASE, Cochrane Central Register of Controlled Trials (CENTRAL), ISI Web of Science, Scopus Database. The complete search strategy is listed in Table 1.
### Table 1
Complete search strategy adapted for MEDLINE database

| # number | Search term (title/abstract) (combined with OR) |
|----------|-----------------------------------------------|
| 1        | randomized controlled trial                   |
| 2        | controlled clinical trial                     |
| 3        | randomized                                     |
| 4        | trial                                          |
| 5        | RCT                                            |
|          | **AND**                                        |
| 6        | auricular acupuncture                         |
| 7        | auricular acupressure                         |
| 8        | auricular electro-acupuncture                 |
| 9        | auricular stimulation                         |
| 10       | auriculotherapy                               |
| 11       | ear acupuncture                               |
| 12       | taVNS                                         |
| 13       | auricular vagus nerve stimulation             |
| 14       | tVNS                                          |
| 15       | transcutaneous vagus nerve stimulation         |
| 16       | transauricular vagus nerve stimulation         |
| 17       | percutaneous auricular vagus nerve stimulation|
| 18       | auricular laser stimulation                   |
| 19       | CES                                           |
| 20       | cranial electrotherapy stimulation            |

### 2.3. Data extraction and management

#### 2.3.1 Study identification

Two researchers (JD, KH) will independently: screen titles, abstracts and full texts for eligibility. Disagreements will be resolved by discussion with a third author (TU). If an article does not provide
enough information to decide about eligibility, one researcher will attempt to contact the trial authors via e-mail.

### 2.3.2. Data extraction.

Two researchers will independently extract data according to the standardized form designed by the review group (Table 2). Accuracy of data entering into Review Manager software (RevMan 5.3. 2011) will be checked by a third researcher.

#### Table 2

**Content of data extraction**

| N  | Categories               | Items extracted                                                                 |
|----|--------------------------|-------------------------------------------------------------------------------|
| 1  | General information      | Author, year of publication, title, journal (title, volume, pages), country, language of publication |
| 2  | Research method          | Random allocation, allocation concealment, blinding, baseline level            |
| 3  | Participants             | Total sample size, number in experimental group, number in control group, gender, age, ethnicity, underlying condition, setting |
| 4  | Intervention             | Type of study (experimental, clinical), type of intervention (auricular acupuncture, auricular acupressure, auricular electro-acupuncture, etc.), selection of auricular sites/auricular acupuncture points, selected for stimulation, type of device/needles, used for auricular stimulation, length of auricular stimulation, additional stimulation if applied, control condition |
| 4.1| Stimulation, if applicable| Current intensity (mA), pulse width (µs), frequency (Hz), duty cycle (s), Voltage (mV), device description (anode/cathode, pulse/burst stimulation) |
| 5  | Outcome parameter        | Ranking: primary or secondary outcome. Changes in biomarker, such as, but not limited to: blood sampling, urine samples, ECG, blood pressure, respiratory rate, sweating reaction, EEG, fMRI, doppler etc. |
| 6  | Safety of Intervention   | Reporting of adverse events                                                   |

### 2.3.3. Assessment of risk of bias in included studies

Risk of bias will be assessed according to the Cochrane Collaboration assessment tool (RoB 2). Two researchers will assess all included trials independently. The following values will be checked: random sequence generation, allocation concealment, blinding of participants and personnel, incomplete outcome data, selective reporting and other potential sources of bias. Risk of bias will be stated as low, high or unclear. Any disagreements will be resolved by discussion or by involving a third researcher to adjudicate.

### 2.3.4. Measures of treatment effects
The general approach of this review is exploratory. If biomarkers are presented as continuous data an analysis of treatment effect will be feasible. Therefore, they will be presented as mean differences with 95% confidence intervals (CI), or as standardized mean differences (SMD).

2.3.5. Dealing with missing data.

Trials with more than 20% missing data will be excluded from analysis of treatment effect. All outcomes will be analyzed where possible on an intention-to-treat basis.

2.3.6. Assessment of heterogeneity

RevMan 5.3. 2011 software will calculate in each analysis using the $T^2$, $I^2$ and Chi² statistics for statistical heterogeneity. If $T^2$ is greater than zero or P value is low in the Chi² test (less than 0.10), heterogeneity will be regarded as relevant.

2.3.7. Assessment of reporting biases

A funnel plot with asymmetry test will be provided, if meta-analysis includes more than 10 investigations.

2.4. Data synthesis

RevMan 5.3. 2011 software will be used for statistical calculation. In the first steps fixed-effect meta-analysis will be performed to estimate heterogeneity. In case of relevant heterogeneity random effects (RE) meta-analysis will be performed. If RE analyses will be necessary, their results will be presented as the average treatment effect with its 95% confidence interval, and the estimates of $T^2$ and $I^2$.

2.4.1. Subgroup analysis and investigation of heterogeneity

Subgroup analysis will be performed where data is available, and outliers removed from meta-analyses where there is a high level of heterogeneity.

2.4.2. Sensitivity analysis

Sensitivity analysis will be performed to assess the contribution of different factors where data is available. For example meta-analysis will be repeated only including trials with a low risk of bias if possible.

2.4.3. Quality of outcome evidence

Assessing clinical relevance for our outcome the Grading of Recommendations Assessment, Development and Evaluation (GRADE) will be performed to summarize our findings. Each comparison from meta-analysis will be rated as: high, moderate, low or very low certainty.

2.4.4 Summary of findings

In case of inappropriate quantitative analysis due to insufficient data, trials will be narratively summarized.
2.5. Discussion

Auricular stimulation is an easy to apply treatment option for selected indications and has shown to have a good safety profile [12, 13]. Application of non-invasive acupressure devices or clip-on electrical stimulators can be delegated to non-physicians. This review will contribute to clarify how much the stimulation of the auricle can alter biomarkers and organ function. This non-pharmacological and safe access to organ function is of high interest in modern medicine [14].

A systematic evaluation of evidence for the impact of auricular stimulation on biomarkers is not available so far. This review and meta-analysis will fill this gap, analysing the RCTs based on this protocol, which was designed according to the PRISMA statement. The review will calculate and summarize the data on efficiency of auricular stimulation where possible.

The results of this systematic review may be biased, since only the trials described in European languages will be considered which published an abstract in English, excluding the full format papers in native languages from the countries of the Far East, where auriculotherapy is widely used in traditional medicine [15]. Furthermore, the trials using transauricular vagal nerve stimulation (taVNS) and cranial electrotherapy stimulation (CES) will be included in this review, since increasing numbers of trials apply this variant of electrical auriculotherapy [16, 17].

The results of this review will provide the basis for better understanding of the effect of AS on internal organs and will help to apply this method in basic and clinical research trials as well as in clinical practice in defined indications.

Registry

This protocol has been registered in the PROSPERO registry (International prospective register of systematic reviews) under the record CRD42021231885.

Abbreviations

RCT: randomized controlled trial, AA: Auricular acupuncture, AS: auricular stimulation; CI: confidence intervals, SMD: standardized mean differences, RE: random-effects, RoB: Risk of bias, taVNS: transcutaneous auricular vagus nerve stimulation; tVNS: transcutaneous vagus nerve stimulation.

Declarations

Ethics approval:

not applicable

Consent for publication:
Availability of data and materials:
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests:
The authors declare that they have no competing interests.

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Data collection: JD, KH, TU
Formal analysis: KH, MC
Supervision: MC
Writing original draft: JD, KH, TU, BB
Writing review & editing: JD, KH, TU, MC, BB

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