Efficacy and safety of acupuncture adjunct to donepezil in patients with mild Alzheimer's disease: a randomized, sham-controlled trial (NCT05078944)

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Statistical Analysis Plan (SAP)
Version 4.0-30/9/2022

1 Administrative Information

SAP version

4.0-30/9/2022

Protocol version

4.0

SAP revisions

SAP revised history-V2.0

6.1 The score of Clinician's Interview-Based Impression of Change with caregiver input (CIBIC plus) was removed from the secondary outcome, and the score of Neuropsychiatric Inventory (NPI) was added.

SAP revised history-V3.0

3.3 The original sample size of 143 cases considering 10% dropout rate was revised to 160 cases considering 20% dropout rate

SAP revised history-V4.0

6.2 Time since diagnosis and AD drug use history were added as fixed effects in the model (reason: It was originally thought that more than 95% of AD patients used drugs, but it was found that the rate of drug use in mild AD was not so high, so this confounding factor was taken into account in the statistics). Additionally, the original method for handling missing data in sensitivity analysis, last observation carried forward (LOCF) method, was revised to multiple imputation (MI) method (reason: reduce the risk of bias).

Roles and responsibility

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2 Introduction

2.1 Background and rationale

Preclinical studies suggest acupuncture may offer symptomatic relief or modify disease progression in Alzheimer's disease (AD), yet clinical evidence remains limited.

2.2 Objectives

This study evaluated whether adding acupuncture to donepezil improves outcomes in patients with mild AD and whether any benefits persist after treatment ends.

3 Study Methods

3.1 Trial design

This is a prospective, randomized, parallel (1:1), sham-controlled trial, with each eligible participant randomly assigned to either active or sham acupuncture added to donepezil treatment.

3.2 Randomization

The randomization sequence is generated by a third party in permuted blocks of randomly varying sizes (4 and 6) using the 'proc plan' procedure of the SAS 9.4. Consecutively numbered opaque sealed envelopes for each group are stored in a secure location and opened sequentially upon enrollment of a study participant by the research assistant.

3.3 Sample size

Sample size was estimated using SAS version 9.4, with ADAS-cog12 at week 14 as the designated primary outcome measure. Based on the results of previous studies, we assume a treatment difference of approximately 3.0 with a standard deviation of 6.0. Considering a 10% non-adherence to treatment and a 10% loss to follow-up, 80 randomized patients per arm or 160 randomized patients in total (128 completers), will have more than 80% power and a type I error rate of 5% for the ADAS-cog12 comparison to detect a significant treatment difference.

3.4 Framework

Difference between active acupuncture and sham acupuncture

3.5 Statistical interim analyses and stopping guidance

One interim analysis was pre-specified and scheduled to be conducted after approximately 50% of participants had completed the week 14 assessments. The interim analysis was intended to evaluate efficacy and safety outcomes, including the primary endpoint (change in ADAS-cog12 score from baseline to week 14) and adverse events.

To control the overall type I error rate at 0.05 (two-sided), the Haybittle–Peto principle was applied. Under this approach, an extremely stringent boundary ($P < 0.001$) was used at the interim analysis for declaring early statistical significance, which consumes a

negligible portion of the overall alpha. Therefore, the final analysis was performed using the conventional significance level (two-sided P < 0.05) without further adjustment.

According to the Haybittle–Peto stopping guideline, the trial would be considered for early termination only if the interim analysis demonstrated overwhelming evidence of efficacy (P < 0.001) or raised serious safety concerns based on recommendations from the independent Data and Safety Monitoring Board (DSMB). Otherwise, the study would continue as planned until the final analysis.

3.6 Timing of final analysis

All outcomes were analyzed collectively after trial ended

3.7 Timing of outcome assessments

From baseline to week 28, once every 7 weeks (see Protocol P9 in details).

Visit No.	***V1***	***V2***	***V3***	***V4***	***V5***	***V6***
End of Week Relative to Study Treatment Start	-4	0	7	14	21	28
Tolerance Interval for Visit (d)	0	0	±3	±3	±7	±7
Entry and Administrative						
Eligibility	X	X				
Informed consent	X					
Physical/neurological examination	X					X
Demographics	X					
Medical history	X					
Randomization		X				
Interventions						
Active/Sham acupuncture		◆—	—	—◆		
Donepezil	◆—	—	—	—	—	—◆
Outcomes Measures						
ADAS-cog12		X	X	X	X	X
MMSE		X	X	X	X	X
ADCS-ADL		X	X	X	X	X
NPI		X	X	X	X	X
Fecal samples collection		X		X		X
Additional Assessment						
Adverse events		◆—	—	—	—	—◆
Treatment compliance		◆—	—	—	—	—◆

4 Statistical Principles

4.1 Confidence intervals and P values

All statistical tests were two-sided with a significance threshold of 0.05. Adjustments for multiple comparisons were not applied for secondary endpoints. Continuous outcomes were summarized as means with 95% confidence intervals (CIs).

4.2 Adherence and protocol deviations

In both groups, we consider participants who attend 85% or more (≥ 36 of 42) of acupuncture sessions and take at least 12 times of donepezil hydrochloride in every 14 consecutive days to have completed a full course of treatment. Concomitant donepezil treatment will be continued unchanged during the study. Participants will return the unused tablets at each follow-up visit. Number of acupuncture sessions and unused tablets will be counted and recorded. Any other concomitant treatments for AD are considered to violate the study protocol, for which participants will be withdrawn.

4.3 Analysis populations

Modified intention-to-treat (mITT) principle, including all randomized participants with at least one post-baseline efficacy assessment, and a per-protocol (PP) analysis was performed as a sensitivity analysis

5 Trial Population

5.1 Screening data

The following information will be summarized:

- Total number of participants screened;
- Number and percentage of participants who were ineligible (did not meet inclusion/exclusion criteria);
- Number and percentage who declined participation;
- Number and percentage who were randomized;
- Reasons for screen failure (e.g., inclusion/exclusion criteria not met, consent not provided).

Screening data will be presented descriptively as part of the CONSORT flow diagram.

5.2 Eligibility

Eligible participants were between 50 and 80 years of age, met the diagnostic criteria for AD, and had a Clinical Dementia Rating (CDR) global score of 1.0. Key exclusion criteria included: presence of a clinically relevant or unstable psychiatric disorder; unstable or severe systemic disease; ongoing use of AD therapies other than donepezil that could not be discontinued; presence of a cardiac pacemaker or metal allergy; any prior exposure to electroacupuncture (manual acupuncture permitted); and premenopausal women.

5.3 Recruitment

Recruitment data will be summarized to describe the accrual of participants into the trial.

The following information will be presented:

- Total number of participants randomized;

- Recruitment over time (e.g., by month or quarter);
- Recruitment by trial site/center (for multi-center studies);
- Comparison with planned recruitment targets.

Recruitment information will be summarized descriptively and presented in the CONSORT flow diagram.

5.4 Withdrawal/follow-up

All participant withdrawals and losses to follow-up will be summarized by treatment group.

The following will be reported:

- Number and percentage of participants who withdraw from intervention but continue follow-up;
- Number and percentage of participants who withdraw completely from the study (no further follow-up);
- Timing of withdrawal/loss to follow-up (e.g., before randomization, during intervention, post-intervention);
- Primary reasons for withdrawal or loss to follow-up (e.g., adverse event, withdrawal of consent, protocol deviation, lost contact).

These data will be presented in tabular form in the CONSORT flow diagram and in the result section.

5.5 Baseline patient characteristics

Age (years), sex (male, female), education (years), time since diagnosis (years), AD drug use history (yes or no), TCM pattern (4 patterns), and baseline ADAS-cog12 (points), MMSE (points), ADCS-ADL23 (points), NPI scores (points). Categorical variables were described as n (%) and continuous variables as means (SD).

6 Analysis

6.1 Outcome definitions

The primary outcome was the change from baseline to week 14 in the Alzheimer's Disease Assessment Scale–Cognitive Subscale, 12-item version (ADAS-cog12; score range 0–75, higher scores indicating greater cognitive impairment).

Secondary outcomes included the change from baseline in ADAS-cog12 score at week 28; the change from baseline in the Mini-Mental State Examination (MMSE; range 0–30, lower scores indicating more severe impairment); the Alzheimer's Disease Cooperative Study–Activities of Daily Living, 23-item version (ADCS-ADL23; range 0–78, lower scores indicating greater functional decline); and the Neuropsychiatric Inventory (NPI; range 0–144, lower scores indicating fewer behavioural disturbances).

6.2 Analysis methods

Changes from baseline in ADAS-cog12 scores were analysed using linear mixed-effects models (LMMs) with repeated measures. The models included fixed effects for treatment group (active vs. sham), time, and their interaction, as well as sex, AD drug

use history and TCM pattern; age, years of education, time since diagnosis, and baseline ADAS-cog12 score were included as covariates. A random intercept for each participant was used, with an unstructured covariance matrix. MMSE, ADCS-ADL23, and NPI outcomes were analysed using the same model structure. Pre-specified subgroup analyses were conducted by TCM pattern, age, sex, and education levels. Continuous outcomes were summarized as means with 95% confidence intervals (CIs). The LMM framework accommodated missing data under the missing-at-random assumption. In addition, multiple imputation was conducted for primary outcome as sensitivity analyses.

6.3 Missing data

The linear mixed-effects models (LMM) framework accommodated missing data under the missing-at-random assumption. In addition, multiple imputation was conducted for primary outcome as sensitivity analyses.

6.4 Additional analyses

Treatment credibility was evaluated after the first and final acupuncture sessions using a four-item, 5-point scale questionnaire completed by participants.

6.5 Harms

An adverse medical event that occurs in a patient or clinical trial subject after receiving the intervention, which does not necessarily have a causal relationship with the treatment. From the time the patient signs the informed consent form and is enrolled in the trial until the end of the trial, any adverse medical event occurring during this period, regardless of its causal relationship with the study intervention, shall be determined as an adverse event.

Assessment and grading shall be conducted according to the definitions of the NCI-CTCAE version 4.0, divided into Grades 1–5 (please refer to NCI-CTCAE v4.0 for specific grading criteria). The possible association between the AE and the investigational drug or medical device shall be assessed using a five-level classification. The expectedness is assessed based on previously observed adverse events. If the nature, severity, or frequency of an AE does not match the risk information described in previous studies of the intervention, it shall be considered unexpected.

Once an AE occurs, details including the time of occurrence, management process, duration, and outcome shall be recorded in the case report form (CRF). For adverse events of moderate severity or above, the principal investigator, department head, and ethics committee shall be notified within 24 hours. For SAE, regardless of the cause, the investigator shall provide appropriate and timely medical treatment for the subject. This responsibility shall be explicitly stated in the study protocol and reaffirmed during investigator training. A SAE Report Form shall be completed and promptly submitted to the regulatory authorities, while simultaneously informing all participating sites and notifying the study sponsor.

6.6 Statistical software

SAS software, version 9.4 (SAS Institute, Cary, NC, USA)

6.7 References

No nonstandard statistical methods are used in this study. All statistical analyses will be performed using standard, widely accepted methods as described in the SAP.