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Shanghai Research Institute of Acupuncture and Meridian
Shanghai University of Traditional Chinese Medicine

Protocol

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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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1 Background

In 2019, Alzheimer's Disease International estimated that there were over 50 million people living with dementia globally, a figure set to increase to 152 million by 2050 [1]. Alzheimer's disease (AD) is the most common type of dementia in older people, characterized by early accumulation of amyloid beta (Aβ) in the brain, which triggers tau pathology, synaptic loss, and neuronal death. These processes ultimately lead to cognitive decline and dementia. Recent advances have produced disease-modifying therapies such as aducanumab [2], lecanemab[3], and donanemab [4], which promote amyloid clearance and may provide clinical benefit. However, their long-term safety remains uncertain, and their high cost limits accessibility, particularly in countries such as China. For most patients, treatment remains focused on symptomatic therapy with two classes of FDA-approved drugs: cholinesterase inhibitors (donepezil, rivastigmine, galantamine) for mild to moderate AD, and the N-methyl-D-aspartate–receptor antagonist memantine for moderate to severe disease. Higher doses of cholinesterase inhibitors may improve symptoms in advanced stages but are often poorly tolerated due to cholinergic side effects, including nausea, vomiting, and diarrhea [5,6]. There remains a pressing need for both safer symptomatic options and affordable disease-modifying strategies.

Acupuncture, a widely practiced complementary therapy, involves stimulating specific acupoints to influence physiological processes. Over the past decade, influential clinical and preclinical research on acupuncture has expanded [7–9]. Experimental studies suggest that acupuncture may modulate multiple neurotransmitter systems—including acetylcholine, serotonin, and glutamate—thereby supporting cognitive function [10]. An adjunctive therapy that acts via a different neurotransmitter system to cholinesterase inhibitors may offer enhanced cognitive benefits. Moreover, acupoints commonly used for dementia, such as PC6 and ST36, are associated with antinausea and antidiarrheal effects [11–14], which may counteract side effects of cholinesterase inhibitors. Meta-analyses indicate that combining acupuncture with donepezil, the most widely used AD drug in China, may improve outcomes compared with donepezil alone[15]. Yet, existing trials are limited by short observation periods and small sample sizes, but more critically by inadequate blinding, which leaves major uncertainty about whether the observed benefits of acupuncture are genuine or primarily placebo-driven. Preclinical studies further suggest that acupuncture may influence AD pathology directly by modulating Aβ metabolism and tau phosphorylation [10], raising the possibility of disease-modifying effects. To date, however, little is known about whether any benefits persist after treatment cessation, a critical marker of disease modification [16].

In recent years, a plethora of studies have indicated the development of AD can be linked to gut microbiota that naturally reside in the body based on the following results: (1) Microbiome profiling of both AD patients [17,18] and mouse models [19,20] for AD has suggested alterations in the gut microbiota; (2) Modulations of the gut microbiota through germ-free rearing, dietary alterations, antibiotic treatment or fecal microbiota transplantation alter learning and memory-related behaviors [21–23]. Further, acupuncture was reported to treat diseases such as abdominal obesity,

irritable bowel syndrome by gut-brain axis. A recent study demonstrated that the acupuncture at Baihui (GV20), Yintang (GV29), and Zusanli (ST36) benignly modulated gut microbiota dysbiosis and improved cognitive function in AD mouse model [24]. Collectively, it is suggestive that gut microbiome-brain axis may be a potential mechanism of acupuncture for AD.

2 Objectives

1) To evaluate if adding acupuncture to donepezil improves outcomes in patients with mild AD;
2) If there is an effect of acupuncture, whether the previous effect of acupuncture treatment is present after washout;
3) If acupuncture has a long-term effect on AD, is it related to changes in gut microbiota?

3 Study design

This is a prospective, randomized, participant-masked, sham-controlled trial, with each eligible participant randomly assigned to either active or sham acupuncture added to donepezil treatment. All participants or their legal representatives will provide written informed consent and be treated in accordance with the tenets of the Declaration of Helsinki (version 2013).

Table 1 Trial summary

Title	An efficacy and safety trial of acupuncture as adjunct to donepezil in patients with mild AD
Clinical indication	Treatment of mild AD
Trial type	Interventional
Type of control	Sham/Placebo
Route of intervention	needle stimulation + oral
Trial blinding	Single-blind
Treatment groups	Active acupuncture for 14 weeks
	Sham acupuncture for 14 weeks
Concomitant therapy	Donepezil 5 mg QD for 28 weeks
Number of trial subjects	160 in total
Estimated duration of trial	The sponsor estimates that the trial will require approximately 2.5 years from the time the first participant signs the informed consent until the last participant's last visit.
Duration of participation	Each participant will participate in the trial for approximately 30 weeks from the time the participant signs the Informed Consent Form through the final contact. After a screening phase, each subject will receive assigned treatment for 28 weeks. After the end of treatment each participant will be followed for at least 14 days.
Randomization ratio	1:1

4 Methodology

4.1 Participant recruitment

The study will take place in the Outpatient Department, Shanghai Research Institute of Acupuncture and Meridian, Shanghai, China. The recruitment will be announced via our official account on social media and forwarded once a month. Placement of brochures and study posters in Yueyang Hospital of Integrated Traditional Chinese and Western Medicine, Shanghai University of Traditional Chinese Medicine and Ruijin Hospital, Shanghai Jiaotong University School of Medicine (collaborating hospitals) and community centers will be used to assist recruitment. In addition, we conducted disease-related science lectures in the elderly community and put up posters in the community to further promote the recruitment. Participants or their representatives will be instructed to read the informed consent and those who showed interest in the study will be scheduled for the screening visit. After presenting written informed consent, participants will be examined, and if all entry criteria are met, will commence a 4-week run-in period of treatment with donepezil hydrochloride (5 mg/capsule, China) 5 mg daily (prior AD treatments will be terminated by then). To further avoid potential confounding effects on the clinical outcomes and gut microbiota, participants will be instructed to maintain their regular diet and activity level, and refrain from probiotics, prebiotics, or synbiotics use. Upon successful completion of the 4-week run-in, participants will be scheduled to complete baseline assessments and undergo randomization. The study flowchart and proposed trial schedule are shown in Figure 1 and Table 1, respectively.

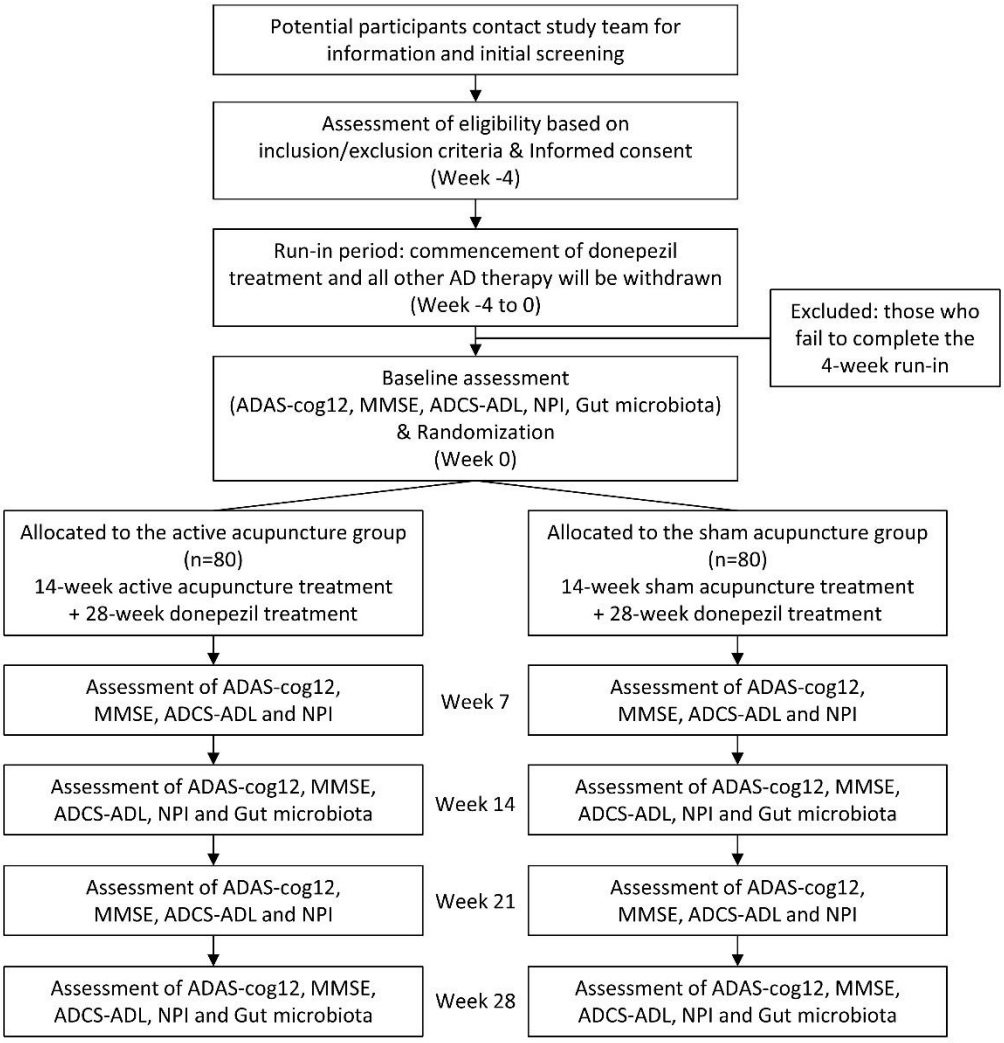

Figure 1 Flow diagram of the study protocol

Table 2 Study schedule

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		♦	———	———	———	♦

ADAS-cog12, Alzheimer's Disease Assessment Scale; MMSE, Mini-Mental State Examination; ADCS-ADL, the Alzheimer's Disease Cooperative Study Activities of Daily Living; NPI, Neuropsychiatric Inventory.

4.2 Entry criteria

4.2.1 Inclusion criteria

- Aged 50-80 years (inclusive), no gender limitation
- Meets the diagnostic criteria for AD

diagnostic criteria include:

a. dementia established by clinical examination and documented by the Mini-Mental State Examination, Blessed Dementia Scale (MMSE), or some similar examination, and confirmed by neuropsychological tests

b. deficits in 2 or more areas of cognition

c. progressive worsening of memory and other cognitive functions

d. no disturbance of consciousness

e. onset between ages 40 and 90, most often after age 65

f. absence of systemic disorders or other brain diseases that, in and of themselves, could account for the progressive deficits in memory and cognition.

supported by:

g. progressive deterioration of specific cognitive functions such as language (aphasia), motor skills (apraxia), and perception (agnosia)

h. impaired activities of daily living and altered patterns of behavior

i. family history of similar disorders, particularly if confirmed neuropathologically

j. laboratory results of normal lumbar puncture as evaluated by standard techniques, normal pattern or nonspecific changes in electroencephalogram, evidence of cerebral atrophy on CT with progression documented by serial observation.

•Scored 1.0 by the Clinical Dementia Rating Scale (CDR) Global Score

4.2.2 Exclusion criteria

•Evidence of a clinically relevant or unstable psychiatric disorder

•Has unstable or severe cardiovascular, hepatic, renal, respiratory, endocrinologic, neurologic diseases and other conditions that, in the investigator's opinion, could interfere with the analyses of safety and efficacy in this study

•Use of AD therapy (except for donepezil hydrochloride) which cannot be stopped

•Has visual or hearing disorder, defeating completion of evaluation

•Without a reliable caregiver who will accompany the participant during treatment and assessment, and monitor administration of the prescribed medications

•Has irritable bowel syndrome or inflammatory bowel disease

•Use of antibiotics within 1 month prior to enrollment

•Has a history of gastrointestinal surgery (except for appendicitis and hernia surgery)

•With cardiac pacemaker or metal allergy

•Once experienced electroacupuncture treatment before at any time (manual acupuncture is allowed)

•Premenopausal woman

4.3 Randomization and blinding

Eligible participants are randomly allocated to active or sham acupuncture group at a ratio of 1:1 via a computer-generated randomization. The randomization sequence is generated by a third party in permuted blocks of randomly varying sizes using the 'proc plan' procedure of the SAS 9.4 (SAS Institute Inc). 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. The participants, assessors and the statisticians, except the licensed acupuncturist, are masked to the treatment allocation. Participants will be informed about the active and sham acupuncture in the study as follows [25]: "In the present study, different types of acupuncture will be compared. One acupuncture protocol is based on previous studies. The other acupuncture protocol is chosen as a contrast and has also been associated with positive outcomes in clinical studies. There is no evidence that either acupuncture protocol being utilized is more effective than the other, which is why we are conducting this research". To avoid communication, the participants will be treated and assessed separately at different times during the trial. Moreover, the credibility questionnaire will be used after the 1st and the 36th (final) acupuncture sessions as a check that the active and sham treatments are equivalent in their psychological impact. The treatment allocation will only be unblinded if there is a serious medical or safety reason.

4.4 Interventions

4.4.1 Treatment regimens

Acupuncture will be performed by an experienced, licensed acupuncturist. Acupuncture intervention is established under guidelines of Revised STandards for Reporting Interventions in Clinical Trials of Acupuncture (STRICTA)(28). A summary of acupuncture points, locations and insertion details is presented in **Figure 2** and **Table 3**.

According to previous Delphi expert consensus survey [26] and our data mining study [27], participants in the active acupuncture group will receive acupuncture at Baihui (GV20), Sishencong (EX-HN1), Shenting (GV24), and bilateral Neiguan (PC6), Shenmen (HT7), Zusanli (ST36), Taixi (KI3), Sanyinjiao (SP6). Additional acupoints are selected on the basis of traditional Chinese medicine (TCM) syndrome differentiation (**Table 4**): bilateral Xuanzhong (GB39) for syndrome of brain marrow deficiency, Qihai (CV6) for syndrome of dual deficiency of Qi and blood, bilateral Fenglong (ST40) for syndrome of orifices blocked by phlegm, and bilateral Xuehai (SP10) for syndrome of blood stasis. Sterile stainless steel disposable acupuncture needles (size 0.30 mm × 50 mm, Hwato brand, China) are used. After sterilization, a small plastic ring (diameter 10 mm and height 5 mm) will be fixed over the acupuncture point (except for the acupoints on the head) with plaster to facilitate maintenance of blinding for the participant, and then the needle will be inserted through the plaster ring. Acupuncturists will then try to elicit a dull and aching sensation by lifting, thrusting and/or rotating manipulation of needles as well as perceive a ‘‘bait-biting’’ sensation caused by mechanical interaction between the needle and connective tissue [28]. These sensations are indicative of proper needle placement (traditionally termed ‘‘De Qi’’). The needle handles at GV20, GV24, bilateral PC6 and HT7 will be connected to an electroacupuncture apparatus (SDZ-III electroacupuncture apparatus, Hwato brand, China) and stimulated with dilatational wave, 10/50 Hz and tolerable electric current. Needles placed in other acupoints will be manually stimulated by rotation every 15 minutes. Each treatment session lasts for 45 minutes and is conducted in a quiet treatment room with dimmed light. Acupuncture treatment in this study is to be taken 3 times weekly over a period of 14 weeks (42 sessions).

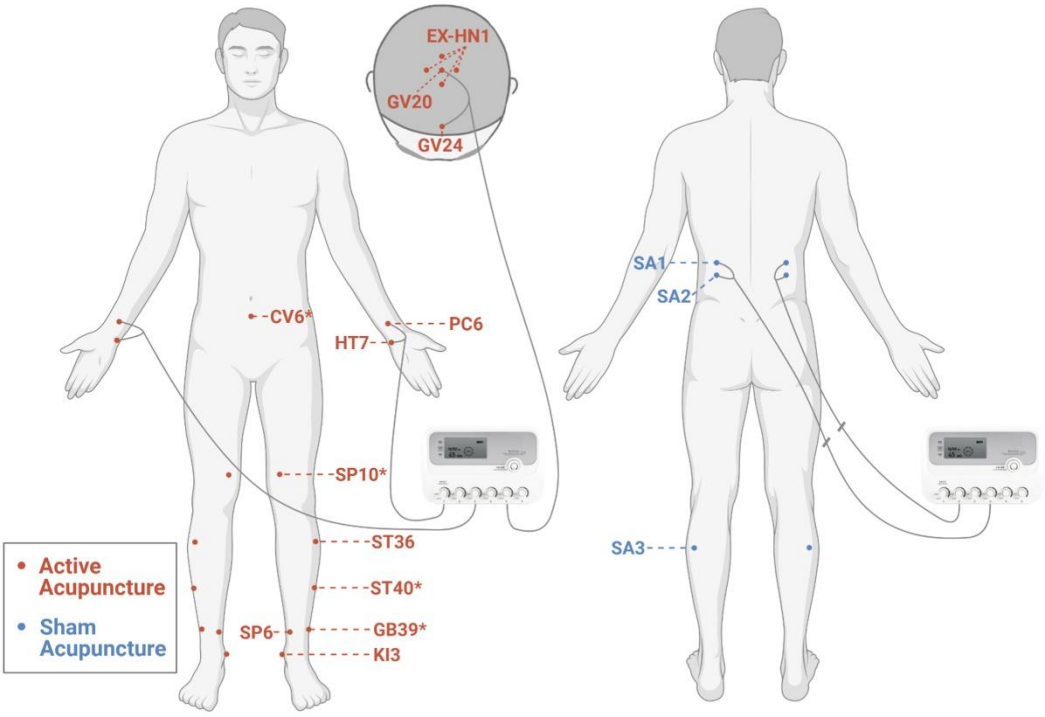

Figure 2 Locations of acupoints for the active and sham acupuncture. *Additional acupoints.

For the sham acupuncture group, non-penetrating sham acupuncture will be performed at bilateral sham acupoints (SA1, SA2 and SA3). After sterilization, same plastic rings are placed at the sham acupoints and pragmatic placebo needles with blunt tip (size 0.30 mm × 50 mm) are used. When the needle tips are pressed against the skin through plastic rings, participants will feel a pricking sensation. The electric stimulator will be applied to bilateral SA1 and SA2 with no current output. The internal electrical wires are disconnected (see **Figure 3**) with a same outlook as the electroacupuncture group. The wave type, frequency and time are presented on the display screen of the device during each treatment, indicating a “running” status. Needles placed in SA3 will be manually rotated every 15 minutes. To best simulate contextual aspects of acupuncture, sham needles are taken out from their packaging right before insertion and cast away in a sharps container after treatment. The number, duration, and frequency of sessions in the sham acupuncture group are the same as in the active acupuncture group.

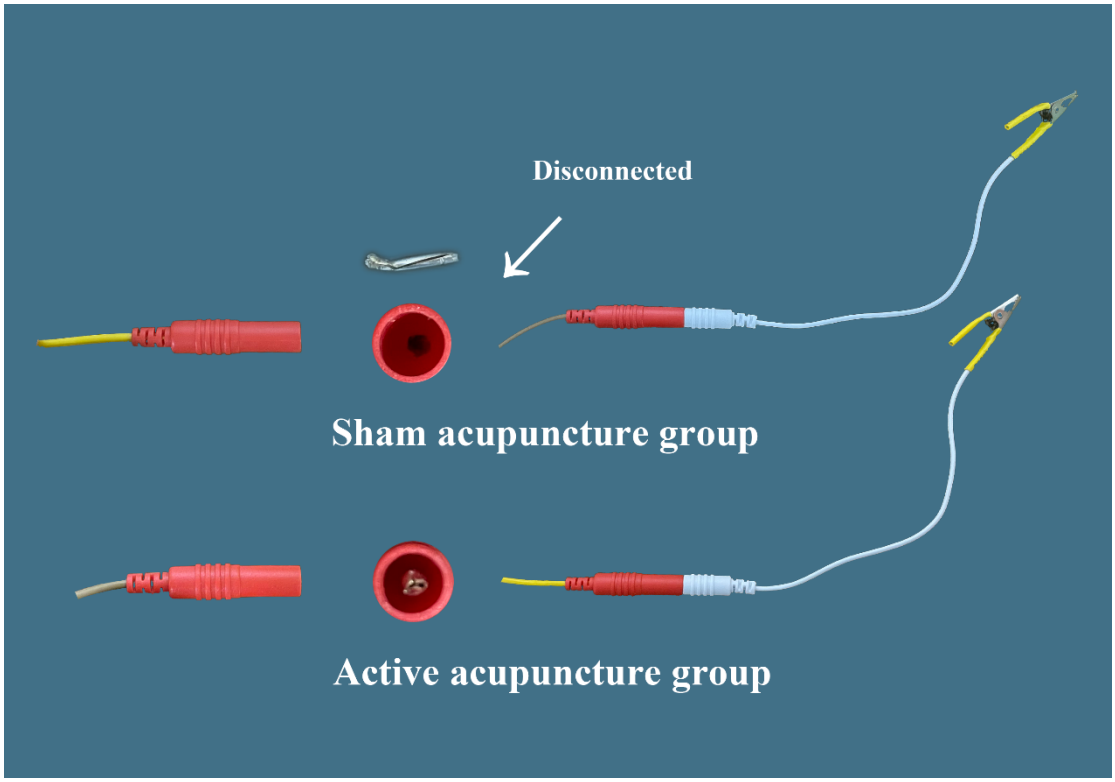

Figure 3 Disconnected electrical wires used in the sham acupuncture group

Table 3 Locations of Real and Sham Acupoints

Acupoint/ Sham Acupoint	Location	Direction of Insertion	Depth of Insertion (mm)
GV20	On the head, 5 cun superior to the anterior hairline, on the anterior median line	Oblique, 10-20°	8-15
EX-HN1	On the head, 1 cun anterior, posterior and lateral to GV20	Oblique, 10-20°	8-15
GV24	On the head, 0.5 cun superior to the anterior hairline, on the anterior median line	Oblique, 10-20°	5-8
PC6	On the anterior aspect of the forearm, between the tendons of tha palmaris longus and the flexor carpi radialis, 2 cun proximal to the palmar wrist crease	Perpendicular	8-15
HT7	On the anteromedial aspect of the wrist, radial to the flexor carpi ulnaris tendon, on the palmar wrist crease	Perpendicular	5-8
ST36	On the anterior aspect of the leg, on the line connecting ST35 with ST41, 3 cun inferior to ST35	Perpendicular	20-40
KI3	On the posteromedial aspect of the ankle, in the depression between the prominence of the medial malleolus and the calcaneal tendon	Perpendicular	8-15
SP6	On the tibial aspect of the leg, posterior to the medial border of the tibia, 3 cun superior to the prominence of the medial malleolus	Perpendicular	15-35
GB39	On the fibular aspect of the leg, anterior to the fibula, 3 cun proximal to the prominence of the lateral malleolus	Perpendicular	8-15
CV6	On the lower abdomen, 1.5 cun inferior to the centre of the umbilicus, on the anterior median line	Perpendicular	20-40
ST40	On the anterolateral aspect of the leg, lateral border of the tibialis anterior, 8 cun superior to the prominence of the lateral malleolus	Perpendicular	20-35
SP10	On the anteromedial aspect of the thigh, on the bulge of the vastus medialis muscle, 2 cun superior to the medial end of the base of the patella	Perpendicular	20-35

SA1	In the lumbar region, 5 cun lateral to GV5	Perpendicular	NA
SA2	In the lumbar region, 5 cun lateral to GV4	Perpendicular	NA
SA3	On the posterior aspect of the leg, 1 cun lateral to BL56	Perpendicular	NA

The locations of acupoints and the acupoints used for locating the sham acupoints are referenced to WHO Standard Acupuncture Point Locations [29]. A cun is a unit of measure that is used in traditional Chinese medicine to measure the length of a certain part of the body surface for the convenience of locating acupoints.

Table 4 Diagnosis basis of TCM patterns of dementia

TCM pattern	**Tongue manifestations**	**Pulse conditions**	**Possible accompanying symptoms**
Brain marrow deficiency	Thin and pale	Deep and thready	Loss of interest, sluggishness in daily activities, slow movements, dizziness, tinnitus, soreness and weakness of the lower back and knees, dry teeth, withered hair
Qi and blood deficiency	Pale with white coating	Thready and weak	Insomnia, reluctance to speak, poor appetite, fatigue and low activity, pale and lusterless complexion and lips, pale nails, loose stools
Orifices blocked by phlegm	Sticky and turbid coating	Wiry and slippery	Mental confusion (silence, occasional singing or laughing), expectoration of phlegm and saliva, poor appetite, nausea, obesity, lethargy
Blood Stasis Obstruction Pattern	Dark purple with stasis spots	Thready and wiry, or deep and slow	Dull complexion, dizziness, headache

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.

4.4.2 Standardized acupuncture procedure in the study

When asked about the differences between the two acupuncture treatments, we referred to the CONSORT recommendations for describing sham acupuncture interventions. We uniformly explained that “both treatment methods have been reported in previous studies to have positive effects. One is based on prior research on acupuncture for AD, while the other serves as a control to clarify the importance of acupoint selection. There is no evidence suggesting that one protocol is more effective than the other, which is also the reason why we are conducting this study.”

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1) Active acupuncture

Preparation

Practitioner greets the participant:

“Hello, we are going to begin your acupuncture treatment, which will take about 45 minutes. You may rest for a moment while I prepare. We’ll start shortly.”

After a brief rest:

“Please lie in a supine position. I will now disinfect the acupoint areas and use sterile pads to mark and stabilize the needles. Please relax.”

Acupoint Prescription

Main acupoints:

Baihui (GV20), Sishencong (EX-HN1), Shenting (GV24), Neiguan (PC6), Shenmen (HT7), Zusanli (ST36), Taixi (KI3), Sanyinjiao (SP6).

Additional points based on pattern differentiation:

- Marrow Sea Deficiency Pattern: Xuanzhong (GB39)
- Qi and Blood Deficiency Pattern: Qihai (CV6)
- Phlegm Obstructing the Orifices Pattern: Fenglong (ST40)
- Blood Stasis Obstruction Pattern: Xuehai (SP10)

Needling Technique

After insertion, the practitioner asks whether the participant feels Deqi sensation (soreness, numbness, distension, or radiation).

Upon achieving Deqi, each acupoint is manipulated by twirling and lifting-thrusting for 10 seconds.

Electrical Stimulation

“Now we will connect some needles to the electroacupuncture device.”

Connections:

- Baihui (GV20) and Shenting (GV24) form one pair
- Bilateral Shenmen (HT7) and Neiguan (PC6) each form one pair

Settings:

- Waveform: dense-disperse wave (10/50 Hz)
- Intensity: adjusted gradually to patient tolerance

Treatment Duration: 45 minutes

- At 15 minutes: repeat manual stimulation (10 seconds per point)
- At 30 minutes: repeat manual stimulation again (10 seconds per point)
- At 45 minutes, when the timer rings, turn off the device and perform final manual stimulation (10 seconds per point)

Needle Removal

Remove needles while applying sterile cotton balls to stop bleeding.
“The treatment is finished. Please get up slowly. Do you feel any discomfort?”
All needles disposed of in a sharps container.

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2) Sham acupuncture
Preparation
Practitioner greets the participant:
“Hello, we are going to begin your acupuncture treatment, which will take about 45 minutes. You may rest for a moment while I prepare. We’ll start shortly.”
After a brief rest:
“Please lie in a prone or lateral position. I will now disinfect the acupoint areas and use sterile pads to mark and stabilize the needles. Please relax.”
Sham Acupoints
Located 5 cun lateral to the lower border of L1 and L2 spinous processes, and 1 cun lateral to Chengjin (BL56) on each side.
Needling Technique
When the needle tip approaches the skin, ask the participant about sensation. Upon reporting sensation, lightly touch the skin and perform superficial rotation for 10 seconds, without actual insertion into muscle.
Sham Electrical Stimulation
“Now we will connect the needles to the electroacupuncture device. When the time is up, a bell will ring.”
Connections are identical in appearance, but internal wires are cut to prevent current flow.
Device settings mimic the EA group:

- Display: dense-disperse wave (10/50 Hz)
- Knob turned to produce sound, creating a similar treatment atmosphere

Treatment Duration: 45 minutes

- At 15 minutes: repeat mock rotation (10 seconds)
- At 30 minutes: repeat mock rotation (10 seconds)
- At 45 minutes, timer rings, perform final mock rotation (10 seconds)

Needle Removal
Remove the needles, press with sterile cotton balls.
“The treatment is finished. Please get up slowly. Do you feel any discomfort?”
Dispose of all materials in the sharps container.

4.5 Study assessments

According to a systematic review [30] which identified 81 outcome measures used across trials in mild-to-moderate dementia, recommended core outcomes for future disease modification trials were cognition as the fundamental deficit in dementia, and cognition should be measured by Alzheimer’s Disease Assessment Scale-Cognitive Subscale (ADAS-cog) or MMSE. Considering that the ADAS-cog is more sensitive to change than the MMSE, we set the primary efficacy measure in the present study as the change from baseline to 28 weeks in scores on the 12-item cognitive subscale of the ADAS-cog [31]. The ADAS is a rater-administered instrument that was designed to assess the severity of dysfunction in the cognitive and noncognitive behavior characteristics of persons with AD. The cognitive subscale of the ADAS consists of 11

items assessing areas of cognitive function most typically impaired in AD: orientation, verbal memory, attention, reasoning, language and praxis. The 12th item "concentration/distractibility" is added. The ADAS-cog12 scale ranges from 0 to 75, with higher scores indicating greater cognitive impairment.

Secondary efficacy measures include changes in the scores of the following assessments: the MMSE (score ranges from 0 to 30; a lower score indicates greater disease severity); the Alzheimer's Disease Cooperative Study-Activities of Daily Living (ADCS-ADL; score ranges from 0 to 78, with lower scores indicating greater functional impairment) and Neuropsychiatric Inventory (NPI; score ranges from 0 to 144, with lower scores indicating fewer behavioral disturbances).

16S rRNA gene sequencing analysis will be performed. Bacterial diversity will be determined by α-diversity (Chao1 richness estimator, Abundance-based Coverage Estimator metric, Shannon diversity index, and Simpson index) and β-diversity (Principle coordinates analysis, PCoA). To further reveal the linking hypotheses of "acupuncture-gut microbiota-AD" axis, the functional profile of Kyoto Encyclopedia of Genes and Genomes (KEGG) Orthology for each sample will be predicted. The participants and their caregivers will be instructed under tutoring video to collect a fresh fecal sample using sample collection kits, packaged within insulated containers and chilled with frozen gel packs. All fecal samples will be dispensed in 2 ml Eppendorf tubes and immediately stored in −80 ℃ until analysis. Total bacterial genomic DNA samples are extracted using the Fast DNA SPIN extraction kits (MP Biomedicals, Santa Ana, CA, USA), following the manufacturer's instruction. DNA quantity is determined using the Nanodrop1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and quality is assessed by 1.0% agarose gel electrophoresis. PCR amplification of the bacterial 16S rRNA gene V3–V4 region is performed in a multiplex approach. The amplicons will be purified using Agencourt AMPure XP Beads (Beckman Coulter, Indianapolis, IN, USA) and quantified using the PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA). Then the final equimolar pool will be sequenced using an Illumina MiSeq platform according to the manufacturer's recommendations. The Quantitative Insights into Microbial Ecology (QIIME, v1.8.0) pipeline is employed to process the sequencing data. Operational taxonomic units (OTUs) are defined by clustering sequences using the UCLUST algorithm and a similarity threshold of 97% against the Greengenes database.

The treatment credibility scale will be assessed at the end of session 1 and session 42 by asking participants to rate their response to four questions on a 5-point scale developed by Borkovec and Nau [32]. The first assessment allows us to test the participants' blinding before sustained efficacy is evident. The second assessment will allow us to detect the association of efficacy with beliefs about treatment received.

The safety of the acupuncture intervention is evaluated by documenting any symptoms possibly related to acupuncture at each acupuncture visit, which includes subcutaneous hematoma, local errhysis at acupoints, sharp pain, palpitation, nausea, dizziness and faint during acupuncture. All adverse events will be carefully documented throughout the study period. For each Data and Safety Monitoring Board

(DSMB) report, a list and summary of the reported adverse events will be presented in a blinded fashion, unless otherwise formally requested.

After the 14-week acupuncture intervention, monthly phone calls will be made to maximize retention and loss to follow up. Participants may withdraw from the study for any reason at any time. Their assessment data up to the timepoint that they withdrew will be used and such patients will not be replaced.

4.6 Adverse events and serious adverse events

4.6.1 Definition of adverse event (AE)

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.

4.6.2 Definition of serious adverse event (SAE)

Events occurring during the clinical trial that result in hospitalization, prolongation of hospital stay, disability, impairment of working capacity, life-threatening conditions, death, or congenital malformations.

4.6.3 Classification of adverse events

4.6.3.1 Severity of events

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).

4.6.3.2 Relationship to study intervention

The possible association between the AE and the investigational drug or medical device shall be assessed using a five-level classification:

- Definitely related
- Probably related
- Possibly related
- Possibly unrelated
- Unrelated

The first three levels are considered related to the investigational drug or medical device. When calculating the incidence of adverse reactions, the number of related cases (sum of the first three levels) is used as the numerator, and the total number of subjects included in the safety analysis is used as the denominator.

Table 5 Criteria for Determining the Relationship Between Adverse Events and Treatment

Criteria	Definitely related	Probably related	Possibly related	Possibly unrelated	Definitely unrelated
Reasonable temporal relationship	Yes	Yes	Yes	Yes	No
Known reaction type of the drug or device	Yes	Yes	Yes	No	No
Improvement after withdrawal of cause	Yes	Yes	Yes/No	Yes/No	No
Recurrence after re-administration or reuse	Yes	No	No	No	No
Alternative explanation possible	No	No	No	Yes	Yes

4.6.3.3 Expectedness

Expected adverse reactions are collected in a standardized format. 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.

4.6.4 Timing of adverse event assessment

Assessments shall be conducted after the patient signs the informed consent form and within 4 weeks after the end of the trial. Follow-up shall be performed according to the severity of the AE to observe its outcome.

4.6.5 Reporting of adverse events

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.

4.7 Date management

An electronic data capture system will be used in this study for data entry management. When the data entry is complete, the database will perform consistency check automatically. On trial-specific documents, except for the signed consent, the participant will be referred to by a unique trial-specific code in any database, not by name. All data sets will be password protected. Modifications to data stored in the electronic database will be documented via a data change system. All paper

documents are to be stored in numerical order and maintained safely in confidential conditions for a period of 3 years after completion of the study. The project investigator will have access to all data sets.

4.8 Sample size calculation

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(10), 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.

4.9 Statistical analysis

All analyses were pre-specified in a statistical analysis plan finalized before database lock. The primary analysis followed a modified intention-to-treat (mITT) principle, including all randomized participants with at least one post-baseline efficacy assessment. A per-protocol (PP) analysis was performed as a sensitivity analysis, excluding participants with major protocol deviations (<85% intervention adherence or violation of inclusion criteria). Changes from baseline in ADAS-cog12 scores were analyzed 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 analyzed 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 using fully conditional specification was conducted for primary and key secondary endpoints as sensitivity analyses. All statistical tests were two-sided with a significance threshold of 0.05. Analyses were performed using SAS software, version 9.4 (SAS Institute, Cary, NC, USA).

16S rRNA gene sequencing analysis are performed using QIIME and R packages (v3.2.0). OTU-level alpha diversity indices are calculated using the OTU table in QIIME. OTU-level ranked abundance curves are calculated to assess OTUs richness and evenness among samples. Beta diversity analysis will be performed to investigate the structural variation of microbial communities across samples using UniFrac distance metrics and visualized via PCoA. The linear discriminant analysis (LDA) effect size method is used to characterize the taxa with statistical significance and biological relevance. The set value of the LDA score is 2. Partial least-squares-latent structure discriminate analysis is performed using Simca-P 14.0 (Umetrics AB, Sweden) to observe the fecal microbiota pattern in different groups based on OTUs of the sequencing data from each sample. Variables with variable importance in projection >1 are identified as important contributors to generation of the model. The functional profile of KEGG

Orthology for each sample will be predicted from 16S rRNA amplicon data with Phylogenetic Investigation of Communities by Reconstruction of Unobserved States. To test the overall correlation between changes in microbiome and altered outcome variables, pairwise Spearman's rank correlations within the active and sham acupuncture groups at baseline, 14 and 28 weeks are calculated and adjustments are performed using the Benjamini–Hochberg procedure and Spearman rho values are filtered with a false discovery rate at 5%.

5 Trial monitoring

An independent DSMB is established to review accumulating study data on a periodic basis and make recommendations to protect the safety of the participants. The members of the DSMB include a recognized expert in the field of AD, a senior acupuncturist, and a statistician. All members are external to our institute. Only the DSMB is authorized to evaluate unblinded interim efficacy and safety analyses. An interim analysis is proposed to be conducted after approximately 50% of participants had completed the week 14 assessments. 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.

Table 6 Data and Safety Monitoring Board Members

Name	Role	Affiliation	Responsibilities
Chunbo Li, MD	Chair	Shanghai Mental Health Center, Shanghai, China	Chair the discussion. Review the the protocol and progress of the trial with respect to ethical and safety standards, monitor the integrity of the data with respect to original study design, and provide advice on study conduct.
Shifen Xu, MD	Member	Shanghai Municipal Hospital of Traditional Chinese Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai, China	Review the the protocol and progress of the trial with respect to ethical and safety standards, monitor the integrity of the data with respect to original study design, and provide advice on study conduct.
Ruiping Wang, MD	Member	Skin Disease Hospital of Tongji University, Shanghai, China	

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Appendix 1 Protocol revised history

Protocol revised history-V2.0
1) P11 The model of electroacupuncture apparatus was revised from SDZ-V to SDZ-Ⅲ.
2) P18 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.

Protocol revised history-V3.0
1) P21-4.8 The original sample size of 143 cases considering 10% dropout rate was revised to 160 cases considering 20% dropout rate.

Protocol revised history-V4.0-30/9/2022 (after the trial started)
1) P21-4.9 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).