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Investigating the efficacy of acupuncture in treating patients with metabolic-associated fatty liver disease: a protocol for a randomised controlled clinical trial

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ABSTRACT

Introduction Acupuncture is widely used for metabolic-associated fatty liver disease (MAFLD) treatment; however, the clinical efficacy has not been confirmed due to the lack of high-level evidence-based clinical practice. The purpose of this study is to design a research protocol that will be used to determine the efficacy of acupuncture versus sham acupuncture (SHA) for MAFLD treatment.

Methods and analysis This will be a multicentre, randomised and sham-controlled trial. Ninety-eight participants with MAFLD will be enrolled in this trial. Participants will be randomly assigned in a 1:1 ratio to receive acupuncture or SHA for 12 weeks. The primary outcome is the rate of patients with a 30% relative decline in liver fat after 12 weeks of treatment in MRI-proton density fat fraction (MRI-PDFF), which will be obtained by quantitative chemical shift imaging such as the multipoint Dixon method at 0, 12 and 24 weeks. Secondary outcomes include the changes in the relative liver fat content measured by MRI-PDFF, magnetic resonance elastography, liver function, lipid metabolism, homeostatic model assessment for insulin resistance (HOMA-IR) and serum high sensitivity C reactive protein, which will be obtained at 0, 6, 12 and 24 weeks. Body measurement indicators (body mass index, waist circumference, hip circumference and waist-to-hip ratio) will be obtained at 0, 3, 6, 9, 12 and 24 weeks. The alteration in the gut microbiota composition and its metabolism will be assessed by 16S ribosomal RNA sequencing and liquid chromatography-mass spectrometry at 0 and 12 weeks.

Ethics and dissemination This study protocol has been approved by the ethics committee of Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine (2023-1347-114-01). The results of this study will be published in a peer-reviewed journal and presented at academic conferences.

Trial registration number ChiCTR2300075701.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This will be the first multicentre study to assess the effects of acupuncture in treating metabolic-associated fatty liver disease (MAFLD) based on MRI-proton density fat fraction.
⇒ Gut microbiota is identified for exploring the efficacy of acupuncture in the treatment of MAFLD.
⇒ Acupuncturists will insert needles into the patients' bodies, so acupuncturists can not be blind to the treatment.

INTRODUCTION

Fat accumulation in the liver is a prevalent disorder affecting up to 40% of the global population.[1] Metabolic-associated fatty liver disease (MAFLD) is characterised by fat accumulation with varying degrees of inflammation and fibrosis, becoming a significant global health.[2] MAFLD is more prevalent in Western (20%–30%) than in Eastern countries (10%–20%) and often leads to cardiovascular diseases, such as hypertension, atherosclerosis and cardiac diseases.[3 4] Additionally, patients with MAFLD with advanced fibrosis and cirrhosis are at increased risk of hepatocellular carcinoma.[5]

The pathogenesis of MAFLD remains unclear. Previous studies have revealed multiple mechanisms involved in MAFLD, including hepatic steatosis, glycolipid metabolism, insulin resistance (IR), gut–liver axis unbalance and metabolites of intestinal microbiota.[6–8] Therefore, effective treatments targeting multiple pathways need to be explored. Although dietary restriction and exercise are generally recommended for MAFLD, patients often find it difficult to persist.[9] New treatment strategies for MAFLD are necessary.

Acupuncture, an alternative therapy of traditional Chinese medicine (TCM), has been practised for thousands of years in China and other Asian countries, such as Japan and Korea. Recent research has demonstrated that acupuncture may effectively mitigate hepatic steatosis, improve glycolipid metabolism and IR and regulate intestinal microbiota composition, suggesting that acupuncture

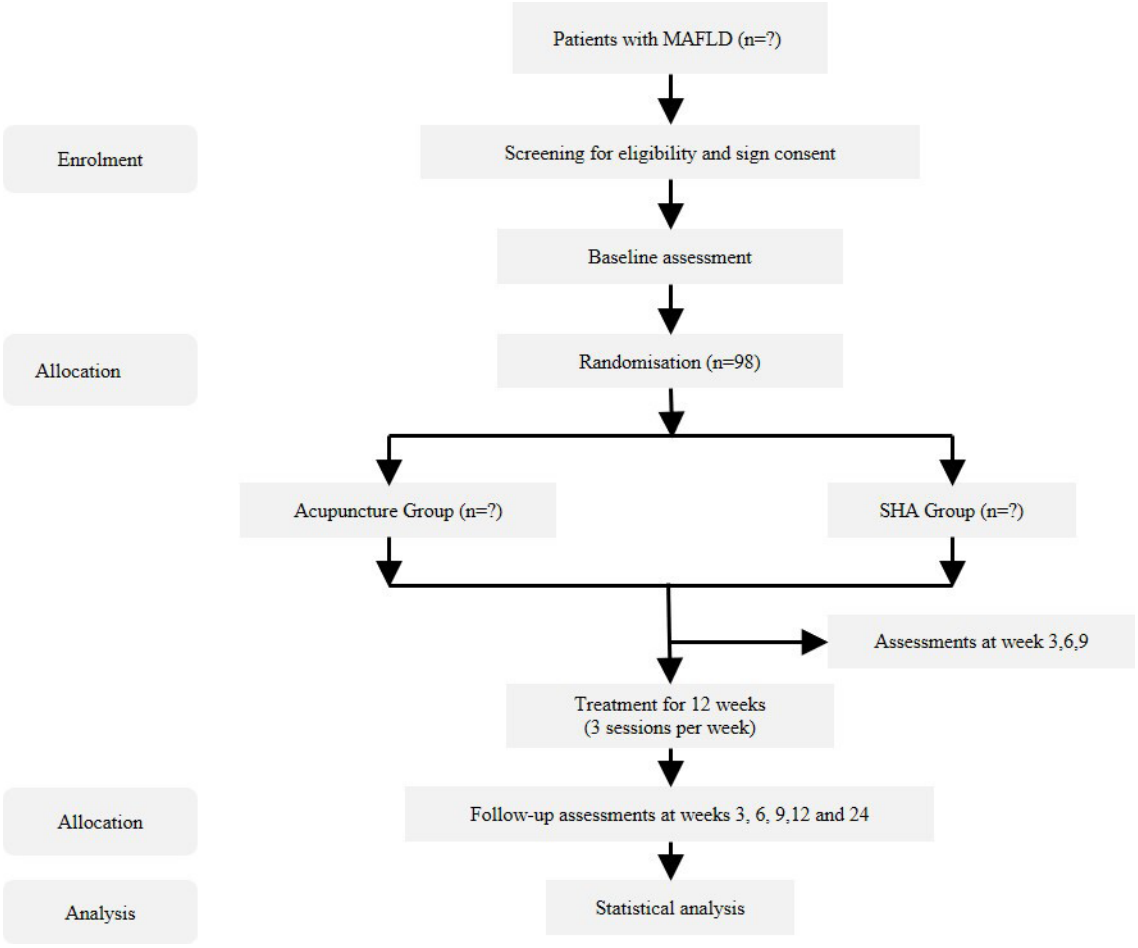

Figure 1 Flow diagram of the trial. MAFLD, metabolic-associated fatty liver disease; SHA, sham acupuncture.

is a potentially effective therapy for MAFLD.[10–12] Many studies have focused on non-pharmacologic therapies in clinical practice, with acupuncture increasingly being used as a treatment for MAFLD.[13] However, there are no high-quality clinical studies to determine the efficacy of acupuncture for the MAFLD treatment. Therefore, we design a multicentre, randomised and controlled trial that will be used to evaluate the effects of acupuncture versus sham acupuncture (SHA) in treating MAFLD.

METHODS/DESIGN

Study design

The multicentre, randomised and sham-controlled trial will be conducted at three hospitals in Shanghai: (1) Shuguang Hospital Affiliated to Shanghai University of TCM, (2) Longhua Hospital Affiliated to Shanghai University of TCM and (3) Yueyang Hospital Affiliated to Shanghai University of TCM. The study protocol has been approved by the ethics committees of all hospitals. The study will be conducted according to the Declaration of Helsinki and reported following the Standard Protocol Items: Recommendations for Interventional Trials guidelines (online supplemental additional file 1).[14] The flow diagram of the clinical trial approach is shown in figure 1.

Patient and public involvement

Patients and/or the public will not be involved in the design, practice, reporting, or dissemination of this research.

Population and recruitment

MAFLD is diagnosed according to The Asian Pacific Association for the Study of the Liver clinical practice guidelines for the diagnosis and management of MAFLD.[15] Once informed consent is obtained, randomisation will be performed (online supplemental materials 1 and 2). The schedule of enrolment, intervention and assessments is shown in table 1.

Inclusion criteria

1. Aged 18–70 years (either sex).
2. Confirmation of MAFLD according to guidelines.[15]
3. Stagnation of dampness and turbidity, accumulation of dampness and heat, and mutual accumulation of phlegm and blood stasis confirmed by TCM syndrome classification.[16]
4. Body mass index (BMI)≥23 kg/m^2.
5. MAFLD with MRI-proton density fat fraction (MRI-PDFF)≥11%.
6. Written informed consent.

Table 1 The schedule of enrolment, intervention and assessments

	Study period						
	Enrolment	Allocation	Post allocation				Closeout
Time point (week)	−2	0	3	6	9	12	24
Enrolment							
Eligibility screen	X						
Informed consent	X						
Randomisation		X					
Intervention							
Acupuncture			X	X	X	X	
Sham acupuncture			X	X	X	X	
Assessments							
General information	X	X					
MRI-PDFF	X					X	X
Liver function		X		X		X	X
Lipids metabolism		X		X		X	X
HOMA-IR		X		X		X	X
hs-CRP		X		X		X	X
Adipocytokines		X		X		X	X
BMI, WC, HC, WHR		X	X	X	X	X	X
MRE		X				X	X
Gut microbiota		X				X	
Adverse events		X	X	X	X	X	X

BMI, body mass index; HC, hip circumference; HOMA-IR, homeostatic model assessment for insulin resistance; hs-CPR, high sensitivity C reactive protein; MRE, magnetic resonance elastography; MRI-PDFF, MRI-proton density fat fraction; WC, waist circumference; WHR, waist-to-hip ratio.

Exclusion criteria

1. Hepatic and biliary diseases induced by other causes, including but not limited to hepatitis B or C virus infection, alcohol-associated liver disease, drug-induced liver disease, autoimmune hepatitis, cirrhosis, primary sclerosing cholangitis, Wilson's disease, α-1 antitrypsin deficiency and liver cancer (or a family history of liver cancer).
2. Severe liver function injury, defined as alanine transaminase (ALT)≥2.5 times the upper limit of normal.[17–19]
3. Clinical evidence of the presence of cirrhosis or decompensation of liver function.
4. Severe renal insufficiency.
5. Primary diseases such as cardiovascular, cerebrovascular, urinary and hematopoietic systems, malignant tumours, other serious comorbidities, or psychiatric disorders.
6. Combined with type 1 diabetes or uncontrolled type 2 diabetes.
7. Combined with thyroid disease, including hyperthyroidism, hypothyroidism and subclinical hypothyroidism.
8. Contraindications to MRI, including but not limited to severe claustrophobia, inner ear implants, pacemakers or other implanted rhythm management equipment, intracranial aneurysm clips incompatible with MRI, any other metal, non-MRI compatible implantation equipment (such as insulin pump, medullary joint replacement), medical history of orbital metal fragments that have not been removed and weight or waist circumference (WC) that exceeds the scanner function.
9. Those who have received acupuncture treatment before enrolment within at least 1 year.
10. Pregnant and lactating women, as well as women who are at risk of pregnancy refuse to maintain contraceptive measures recognised by the researchers.
11. Participated in other clinical trials in the past 3 months.
12. Those who are unable to follow medical advice for lifestyle interventions.
13. Other situations that are not suitable for participation in this study were identified by the researchers.

Randomisation and allocation concealment

Eligible patients will be randomly assigned to the acupuncture group or SHA group in a 1:1 ratio. A random allocation sequence number will be generated using the blockrand package of R V.4.1.3 by the relevant statisticians.

Each random number will be written on letter paper and sealed in an opaque envelope. Participants will draw an envelope to obtain their random number, which will then be recorded in a case report form (CRF).

Masking

Patients, outcome assessors and statisticians who perform the statistical analyses will be blind to group assignment. To ensure the implementation of blinding, patients in different groups will be arranged to be treated and followed up in separate rooms. Throughout the study, patients from different groups will not meet or converse with each other. On the completion of the study, questionnaires are distributed to participants, asking them whether they are aware of their assigned group and the reasons for their beliefs.[20]

Sample size

Sample size was calculated based on the formula:

$$n_C = \frac{(Z_{1-\alpha}+Z_{1-\beta})^2 \times (\pi_T(1-\pi_T)+\pi_C(1-\pi_C))}{(\varepsilon-\Delta)^2}$$

where n_C represents the number of participants in each group; π_T represents the overall rate of the test group (55%), π_C represents the overall rate value of the control group (25%) and ε represents the difference between the two groups (30%).[21] Superiority margin Δ is 5%.[22] Based on a previous report with the p value of 0.05 and power of 80%, the expected difference in MRI-PDFF is 30%.[23 24] Through calculation, the sample size in each group is 45. The sample size is inflated to allow for a 10% dropout rate over the course of the study, and finally, the calculated sample size in each group is approximately 49.

Interventions

Following a 2-week washout period, the subjects will receive 30 min of acupuncture treatment three times weekly for 12 weeks. The practice of conducting multiple treatments within a single day will be expressly prohibited. The treatment protocol has been mastered by all acupuncturists. The acupuncture point locations are shown in figure 2.

Acupuncture group

The fixed acupoints include Zhongwan (RN12), Shuifen (RN9), Guanyuan (CV4), Qihai (RN6), Huaroumen (ST24), Tianshu (ST25), Daheng (SP15), Daimai (GB26), Fenglong (ST40), Zusanli (ST36) and Sanyinjiao (SP6), which are used to treat patients with MAFLD in published papers.[25] In addition, according to TCM syndrome differentiation, the syndrome of stagnation of dampness and turbidity will be treated with Hegu (LI4), the syndrome of accumulation of dampness and heat will be treated with Yinlingquan (SP9) and the syndrome of mutual accumulation of phlegm and blood stasis will be treated with Xuehai (SP10).[16 26] After sterilisation, the 0.25 mm×60 mm needles (Hwato, Suzhou, China) will be inserted vertically into acupoint sites and then the manipulations of twirling will be practised. The location of acupoints is shown in table 2.

SHA group

In this group, the SHA needles (0.25 mm × 60 mm) featuring flat tips (Hwato, Suzhou, China) will be inserted into the same acupoints as the acupuncture group.[27 28]

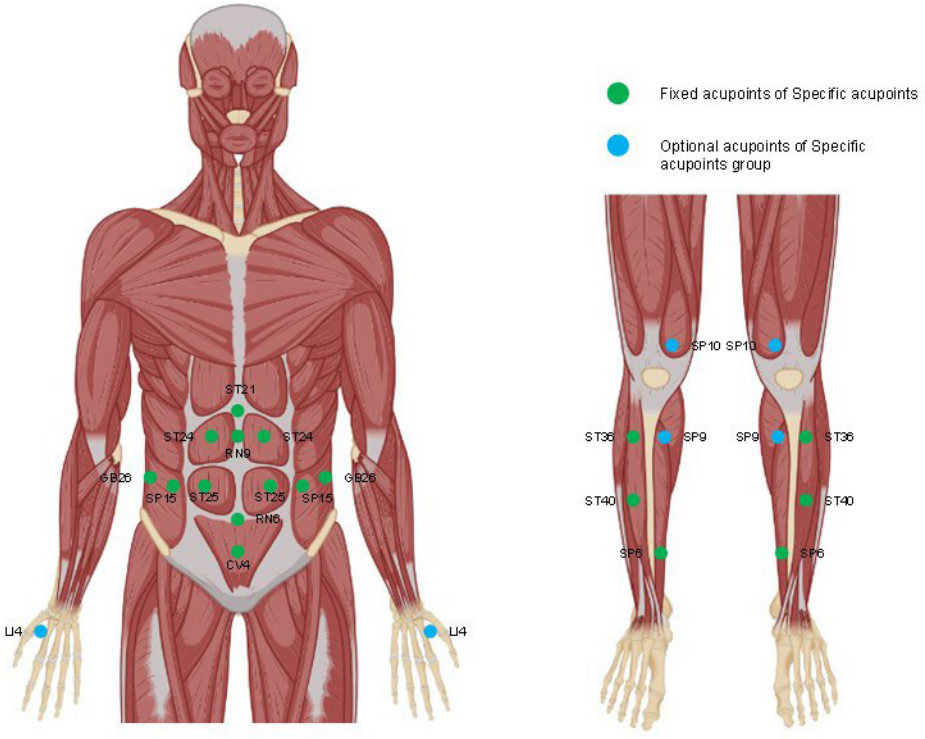

Figure 2 Locations of acupoints.

Table 2 The location of the acupoints

Acupoints	Location
Fixed acupoints	
Zhongwan (RN12)	On the anterior midline, 4 cun above the umbilicus.
Shuifen (RN9)	On the anterior midline, 1 cun above the umbilicus.
Guanyuan (CV4)	On the anterior midline, 3 cun below the umbilicus.
Qihai (RN6)	On the anterior midline, 1.5 cun below the umbilicus.
Huaroumen (ST24)	On the anterior midline, 1 cun above the umbilicus and 2 cun lateral to the anterior midline.
Tianshu (ST25)	On the same level of the umbilicus and 2 cun lateral to the anterior midline.
Daheng (SP15)	On the same level of the umbilicus and 4 cun lateral to the anterior midline.
Daimai (GB26)	At the intersection of the perpendicular line below the free edge of the 11th rib and the horizontal line of the umbilical cord.
Fenglong (ST40)	One finger-breadth lateral to Tiaokou and at the midpoint of the line joining Dubi and the tip of the external malleolus.
Zusanli (ST36)	3 cun directly below Dubi and 1 finger-breadth lateral to the anterior border of the tibia.
Sanyinjiao (SP6)	Posterior to the mesial border of the tibia and 3 cun above the tip of the medial malleolus.
Matching acupoints	
Hegu (LI4)	Between the first and second metacarpal bones and in the midpoint of the radial side of the second metacarpal bone; or, the transverse crease of interphalangeal joint of one thumb are placed on the stretched hukou between the thumb and index finger of the other hand, and the acupoint is where the tip of the thumb touches.
Yinlingquan (SP9)	Posteroinferior to the medial condyle of the tibia.
Xuehai (SP10)	2 cun above the upper border of the medial patella; or the acupoint is where the tip of the thumb when the doctor puts his right palm on the left knee of the patient and with the centre of the palm pointing to the centre of patella of patient and the patient flexes his knee joint and makes it to be a right angle (right Xuehai is measured by the left hand)

Diet and exercise

Based on the guidance for prescribing exercise reported in *Med Sci Sports Exerc* 2011, subjects are recommended to keep 30 min of moderate-intensity exercise per day, like jogging, swimming, Tai Chi and cycling.[29] In addition, a hypocaloric diet (500–1000 kcal deficit) is recommended for all subjects, such as 100 g of cake, 200 g of fried chicken, 400 g of rice, 120 g of pork and any vegetables.[15] During the treatment, all subjects are asked to complete the questionnaire concerning the dietary pattern. This questionnaire encompasses a prescribed low-fat and low-carbohydrate plan. Response options include none, <50% of the time or ≥50% of the time. Additionally, participants will be also asked to report their levels of daily physical activity. Specifically, they will be asked about the frequency of engaging in exercise sessions lasting at least 30 min during the study. The response alternatives include exercising less than once a week, engaging in physical activity once to two times per week or participating in such activities three times a week or more, as shown in table 3.

Outcomes

Primary outcome

The primary outcome is the rate of patients with a 30% relative decline in liver fat assessed by MRI-PDFF after 12 weeks of treatment.[23 24]

Secondary outcomes

The secondary outcomes include the changes as follows: (1) liver fat content measured by MRI-PDFF at 12 weeks, (2) the degree of liver fibrosis assessed by magnetic resonance elastography (MRE), (3) hepatic enzymes including ALT, aspartate transaminase and γ-glutamyl transferase levels in serum, (4) indicator of lipid profiles (triglycerides, total cholesterol, low-density lipoprotein cholesterol and high-density lipoprotein cholesterol, (5) serum high sensitivity C reactive protein levels, (6) serum adipocytokines including leptin, adiponectin and resistin, (7) anthropometric measurements including BMI, WC, hip circumference and waist-to-hip ratio and (8) HOMA-IR.

Table 3 Diet and exercise record table

Name	ID	Date
Have you followed dietary recommendations this week, such as a low-fat and low-carbohydrate diet?		► Completed ► Partially completed ► Not completed
Have you followed exercise recommendations this week, such as 30 min/day of jogging for ≥5 days/week?		► Completed ► Partially completed ► Not completed

Faecal microbiota assessment

The count and composition of operational taxonomic units within the gut microbiota will be tested by 16S ribosomal RNA sequencing. We will also measure the α-diversity indices, including Sobs, Chao1, Ace, Shannon, Simpson and Coverage, to gauge the diversity within the microbial population. In order to probe the differences in community composition, β-diversity analyses will be conducted using the unweighted and weighted UniFrac metrics. In addition, the untargeted metabolomics analysis will be conducted by using liquid chromatography-mass spectrometry to investigate the impact of acupuncture on gut microbiota metabolism.

Statistical analysis

The data will be analysed by using SPSS (V.22.0.0). Descriptive analyses will be performed for all baseline variables. Continuous variables under an assumption of normality are described as mean±SD, and those with a non-normal distribution are described as medians and quartiles (Q1; Q3). Categorical variables are represented as absolute values with percentages.

Baseline demographic and clinical data will be analysed using the t-test (normally distributed) or Mann-Whitney U test (non-normally distributed) for continuous variables, or the χ2 test for categorical variables.

Intention-to-treat set will be used for the analysis of the controlled trial. The χ^2 test will be used to compare the differences in the primary outcome between the acupuncture group and the SHA group. In instances of missing or inconsistent data, the last observation carried forward method or multiple imputation method will be done. Differences in the secondary outcomes between the acupuncture group and the SHA group will be assessed by using the linear mixed model.

In the per-protocol set, a sensitivity analysis will be conducted. All statistical analyses will be performed using SAS V.9.4 and R V.4.1.2 (https://www.r-project.org/).

Permutational multivariate analysis of variance will be used to analyse the correlation between gut microbiome and clinical characteristics. This analysis will be done on the Bray-Curtis dissimilarity.[30] The non-parametric Mann-Whitney U test will be performed to analyse the alteration of gut microbiota's relative abundance before and after treatment. Pearson's correlation analysis will be done between changes in metabolites originating from the gut microbiota and enhancements in clinical variables.

In the aforementioned statistical analyses, a p value<0.05 is regarded as indicative of statistical significance.

Adverse events

During the trial, all adverse events will be observed by the investigator and reported by the subjects. The possible treatment-related adverse events may include subcutaneous haematoma, postneedling discomfort, pruritus at the needle insertion sites and sensations of dizziness, which will be analysed using a χ^2 test or Fisher's exact test.[31 32]

Data management

All data will be initially recorded on CRF and inputted into the data management system of this study by investigators. Pertinent characteristics of all patients will be recorded as an anonymised version, such as names, ID card numbers and telephone numbers.

Records containing personal information on patients will be held securely for a minimum of 5 years. In each clinical centre, the data are evaluated by controllers. Controllers will monitor the progress of the trial and evaluate the relevant adverse events, which will be independent of the investigators.

Quality control

The design of this trial has been evaluated and refined by experts specialising in acupuncture, gastroenterology, statistics and methodology. All modifications made to this trial will be reported to the ethics committee of Shuguang Hospital Affiliated to Shanghai University of TCM. Any alteration made to our database will be recorded on CRF.

ETHICS AND DISSEMINATION

This trial was registered in the Chinese Clinical Trial Registry (ChiCTR-2300075701). Furthermore, the study protocol has been approved by the ethics committee of Shuguang Hospital Affiliated to Shanghai University of TCM (No. 2023-1347-114-01). Patients who meet the inclusion criteria in 3 clinical centres will be enrolled after obtaining written informed consent from patients. If the protocol amendments are required, all pertinent materials will be reported to the ethics committee.

The independent data monitoring committee will be used to review the progression and the collected data during the study in Shuguang Hospital Affiliated to Shanghai University of TCM. The committee holds the authority to initiate necessary modifications or even terminate the trial.

Study outcomes will be disseminated via peer-reviewed journals and presented at academic conferences.

DISCUSSION

Previous research has indicated that acupuncture may have benefits in reducing liver fat content among individuals with MAFLD.[21 25] However, multicentre randomised controlled clinical trials are essential to properly evaluate the role of acupuncture in treating MAFLD. Therefore, our study will provide high-quality evidence to assess the clinical effectiveness of acupuncture for MAFLD.

Blunt-tipped placebo needles have been used in previous studies to identify the efficacy of acupuncture in treating MAFLD.[20 31] The selection of acupoints is considered one of the most pivotal factors influencing the effectiveness of acupuncture.[20]

Although liver biopsy is the gold standard for diagnosing MAFLD, its invasive nature limits its use in clinical studies. In December 2018, the Food and Drug

Administration (FDA) issued guidelines suggesting the adoption of MRI-PDFF for assessing therapeutic efficacy in early-phase clinical trials for non-alcoholic steatohepatitis.[2 3 33] Research shows that a relative decrease in MRI-PDFF≥30% is significantly correlated with histological improvement in MAFLD, making it a useful criterion for evaluating clinical effects.[33]

There is a growing body of evidence that the gut microbiota are involved in the onset and progression of MAFLD.[34–36] Alterations in gut microbiota can disrupt beneficial microbial populations, affecting the integrity of the intestinal mucosal barrier.[37] A high-fat diet can alter gut microbiota, leading to increased intrahepatic lipids, liver enzymes and histologically confirmed steatosis in patients with MAFLD.[38]

Metabolites originating from the gut microbiota can modulate the intestinal morphology and immune response, changing with the severity and fibrosis stage of MAFLD. Perturbation in amino acid metabolism, especially aromatic amino acids, glutamate–serine–glycine index and branched-chain amino acids, has been implicated in the pathogenesis of MAFLD and MASH.[39 40] To further explore the potential mechanism of acupuncture in treating MAFLD, faecal samples will be collected before and after treatment to analyse gut microbiota composition. These alterations may shed light on the effective mechanisms of acupuncture.

However, there are some limitations in this study. First, only the obese patients with MAFLD are enrolled in this study, therefore we cannot provide evidence of acupuncture's effectiveness for treating lean patients with MAFLD. Second, as liver biopsy is not used as a therapeutic indicator, we cannot compare the sensitivity and specificity of MRE and liver biopsy in evaluating the clinical effects of acupuncture. Finally, the treatment course is short, and future studies should explore the long-term clinical efficacy.

Trial status

At the time of manuscript submission, recruitment work for the trial is ongoing. The protocol version is MAFLD V.8.16, 16 August 2023. The recruitment of this study began on 15 September 2023 and will be ongoing until 31 January 2025.

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Contributors ML is the corresponding author and responsible for the overall content as guarantor. LF and LH contributed equally to this paper. YG and MY participated in the conception and design of the trial. LF and ML drafted the manuscript. LH, JL, JD and JP are in charge of the recruitment and treatment of patients. WZ is responsible for data curation. YC and SW analysed the data and made the table. All authors approved the submitted version of the manuscript.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Consent obtained directly from patient(s).

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