PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Protocol for a randomized controlled clinical trial to investigate the efficacy of acupuncture in treating patients with metabolic-associated fatty liver disease
AUTHORS	Fu, Lihong; Huang, Lingying; Gao, Yue; Zhu, Wanchun; Cui, Yu; Wang, Shihao; Yan, Meihua; Li, Jing; Duan, Junyi; Pan, Jielu; Li, Man

VERSION 1 - REVIEW

REVIEWER NAME	Zhao, Jingjie
REVIEWER AFFILIATION	Capital Medical University, Department of Traditional Chinese Medicine
REVIEWER CONFLICT OF INTEREST	None.
DATE REVIEW RETURNED	04-Dec-2023

GENERAL COMMENTS	This is a protocol to evaluate the efficacy of acupuncture for MAFLD. The design is completed, however, there are some comments to the paper. 1. About the primary outcome The primary outcome includes the proportions of patients with≥10% and ≥30% relative changes in the MRI-PDFF. This is a composite endpoint, the sample size needs to be calculated separately, and then the largest sample size will be chosen. 2. About the intervention The description of two interventions in the method, one is acupuncture, the other is sham acupuncture, however, three interventions are in Table 1. Please clarify this part. 3. About exclusion criteria Line 133. Severe liver function injury, defined as alanine transaminase (ALT) ≥ 2.5 times the upper limit of normal value, please ensure the upper 2.5 times is defined the severe liver function injury. Line 153. Those who have received acupuncture before treatment, please ensure how long before treatment. Line 157. Those who are unable to follow medical advice for lifestyle interventions, how to assess the criteria? Some recent studies showed the efficacy of improving the fatty liver with some hypoglycemic agents, such as GLP-1 receptor agonist, and SGLT2 inhibitor. Please consider whether the patient used the drug to affect the result. 4. About sample size Line176. According to the part, I think the study is the superiority trial, please explain why the superiority margin is 5%.

	5. About the blind assessment The study is single-blinded, please set about the blinded question for whether the blinded method is broken. 6. About the figures The sample size needs to be shown in Figure 1. 7. About the statistics Line 262-292: The statistical analysis section needs to be logical, please recognize this section. There are some issues, such as: starting first with statistical principles, data description analysis methods for different types of data, etc...

REVIEWER NAME	Draz, Ramy Salama
REVIEWER AFFILIATION	Cairo University
REVIEWER CONFLICT OF INTEREST	Competing interests Alternative therapy Rehabilitation therapy
DATE REVIEW RETURNED	25-Dec-2023

GENERAL COMMENTS	In page 15, the optional acupoints What do you mean by optional? Would it be applied for some patients and not fot the others?

VERSION 1 – AUTHOR RESPONSE

To Reviewer #1:

Comment:

1. About the primary outcome

The primary outcome includes the proportions of patients with≥10% and ≥30% relative changes in the MRI-PDFF. This is a composite endpoint, the sample size needs to be calculated separately, and then the largest sample size will be chosen.

Reply: We eliminate the outcome specifying 10%, because recent clinical observations have revealed that the MRI-PDFF in all patients who receive these therapies can decrease by more than 30%. Furthermore, we have reclassified " The changes in relative liver fat content measured by MRI-PDFFat week 12" as a secondary endpoint. (It can be seen in the revised manuscript, Line 245-246, 249, Page 17).

2. About the intervention

The description of two interventions in the method, one is acupuncture, the other is sham acupuncture, however, three interventions are in Table 1. Please clarify this part.1

Reply: The part “INTERVENTION” in Table 1 has been corrected.

	STUDY PERIOD

	Enrolment	Allocation	Post allocation				Closeout
TIME POINT (WEEK)	-2	0	3	6	9	12	24
ENROLLMENT							
Eligibility screen	X						
Informed consent	X						
Randomization		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

3. About exclusion criteria

(1) Line 133. Severe liver function injury, defined as alanine transaminase (ALT) ≥ 2.5 times the upper limit of normal value, please ensure the upper 2.5 times is defined the severe liver function injury.

(2) Line 153. Those who have received acupuncture before treatment, please ensure how long before treatment.

(3) Line 157. Those who are unable to follow medical advice for lifestyle interventions, how to assess the criteria?

Some recent studies showed the efficacy of improving the fatty liver with some hypoglycemic agents, such as GLP-1 receptor agonist, and SGLT2 inhibitor. Please consider whether the patient used the drug to affect the result.

Reply (1) Given the absence of specific threshold regulations, we have referred to several clinical studies for guidance[1,2,3].

[1]. Jasiewicz M, Siedlaczek M, Kasprzak M, et al. Elevated serum transaminases in patients with acute coronary syndromes: Do we need a revision of exclusion criteria for clinical trials? Cardiology Journal 2021. doi: 10.5603/CJ.a2021.0081.

[2]. Lebovitz HE, Kreider M, Freed MI. Evaluation of liver function in type 2 diabetic patients during clinical trials: evidence that rosiglitazone does not cause hepatic dysfunction. Diabetes care 2002;25:815-821. doi: 10.2337/diacare.25.5.815.

[3]. Lee WM, Larrey D, Olsson R, et al. Hepatic findings in long-term clinical trials of ximelagatran. Drug safety 2005;28:351-370. doi: 10.2165/00002018-200528040-00006.

(2). To ensure the implementation of blinding, we hope that the patients have never undergone acupuncture treatment before.

(3). We will collect data using questionnaires, directly calculating the compliance ratio based on the collected data, and employing statistical methods to analyze which factors may be associated with non-compliance, such as age, gender, education level, etc. In the analysis of the results, these patients will only appear in the ITT (Intention to Treat) analysis and will not be included in the PP (Per Protocol) analysis. In the final research report, we will provide a detailed account of compliance and its potential impact on the study outcomes.

We have prohibited patients from taking any medication for treating fatty liver during the study period, including GLP-1 receptor agonist, and SGLT2 inhibitor.

4. About sample size

Line176. According to the part, I think the study is the superiority trial, please explain why the superiority margin is 5%.

Reply: In the referenced literature[1], the efficacy rates were 53.3% and 25.9%, respectively. Based on past experience, for comparisons between two groups, the maximum superiority should not exceed one-fifth of the control group's sample ratea[2]. Therefore, we propose that a superiority threshold of 5% be deemed indicative of superior efficacy.

[1]. Zhao J, Wang Q, Zhao X, et al. Electro-acupuncture reduced steatosis on MRI-PDFF in patients with non-alcoholic steatohepatitis: a randomized controlled pilot clinical trial. Chinese Medicine 2023;18:1-12. doi: 10.1186/s13020-023-00724-w.

[2]. Liu Y X, Yao C, Chen F, et al. Statistical methods for clinical non-inferiority/equivalence evaluation. Chinese Jounal of Clinical Pharmacology and Therapeutics 2000;5:344-348. (in Chinese).

5. About the blind assessment

The study is single-blinded, please set about the blinded question for whether the blinded method is broken.

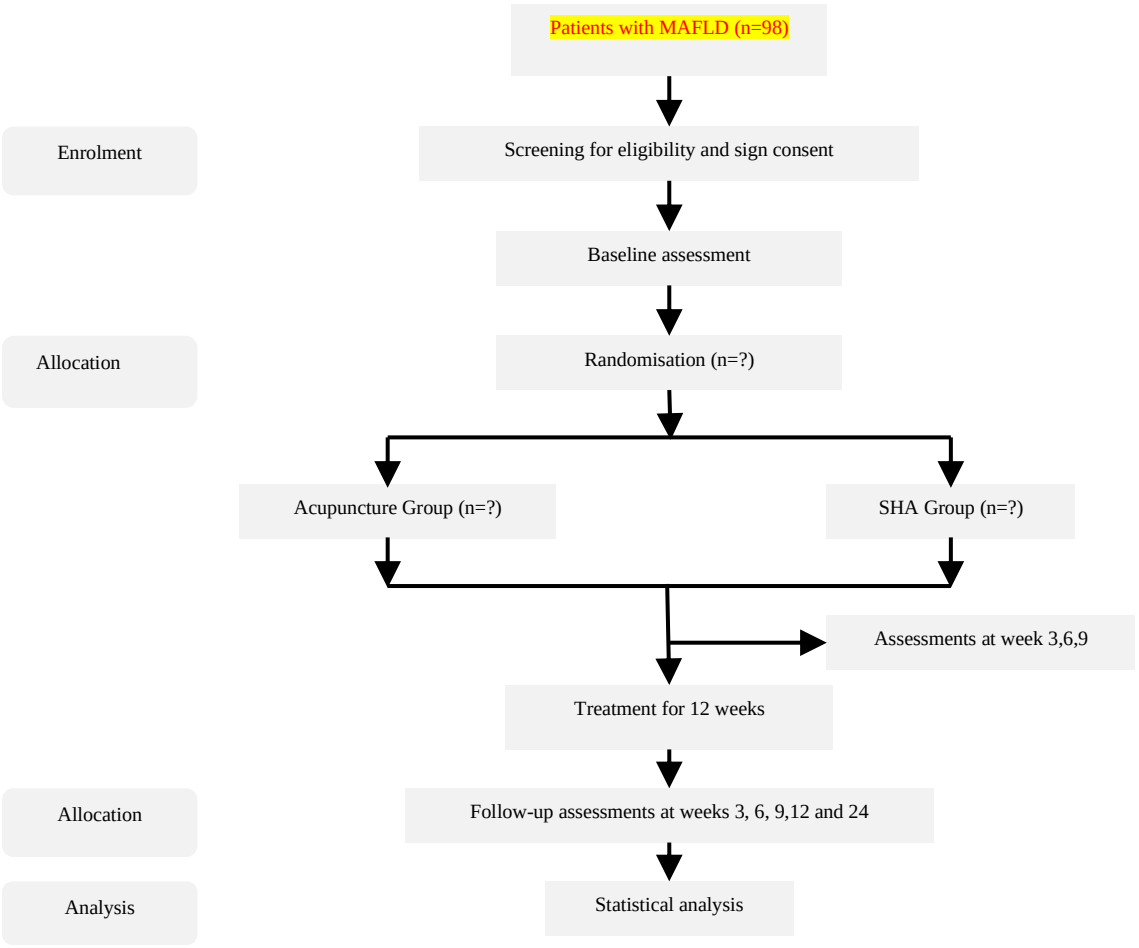
Reply: To ensure the implementation of blinding, we will arrange for patients in different groups to be placed in separate rooms. Throughout the entire course of the study, patients from different groups will not meet or converse with each other. To assess whether participants believe they are aware of their group assignment, we employ a questionnaire survey to furnish evidence regarding whether the blinding has been compromised[1].

[1]. Qi L-Y, Yang J-W, Yan S-Y, et al. Acupuncture for the Treatment of Diarrhea-Predominant Irritable Bowel Syndrome: A Pilot Randomized Clinical Trial. JAMA Network Open 2022;5:e2248817-e2248817. doi: 10.1001/jamanetworkopen.2022.48817.

6. About the figures

The sample size needs to be shown in Figure 1.

Reply: The sample size in Figure 1 has been replenished .


Patients with MAFLD (n=98)
Enrolment
Screening for eligibility and sign consent
Baseline assessment
Allocation
Randomisation (n=?)
Acupuncture Group (n=?)
SHA Group (n=?)
Assessments at week 3,6,9
Treatment for 12 weeks
Allocation
Follow-up assessments at weeks 3, 6, 9,12 and 24
Analysis
Statistical analysis

7. About the statistics

Line 262-292: The statistical analysis section needs to be logical, please recognize this section. There are some issues, such as: starting first with statistical principles, data description analysis methods for different types of data, etc...

Reply: We have revise this section for in Line 269-297, as shown in the following sentences.

The data will be subjected to analysis employing SPSS (version 22.0.0, SPSS Inc., Chicago, IL, USA). Descriptive analyses were performed on all baseline variables. Continuous variables whose distribution met normality assumptions were given as mean ± standard deviation or else given as medians and quartiles (quartile 1 [Q1], quartile 3 [Q3]); categorical variables were presented as the absolute value and relative frequency. The demographic and baseline clinical data will be compared using the t-test or Mann-Whitney U test and $\chi 2$ test, where appropriate.

Intention-to-treat (ITT) set will be used in all efficacy analyses, which consists of all patients who have been randomized into groups. $\chi 2$ test will use to compare the differences between the acupuncture and sham acupuncture groups regarding the primary and secondary outcomes. In instances of missing outcome data, these gaps will be addressed by applying either the last observation carried forward (LOCF) methodology or multiple imputation techniques.

A sensitivity analysis will be conducted within the per-protocol (PP) set, which will encompass all participants who completed the treatment with at least 80% of the designated sessions and maintained compliance with the follow-up procedures, devoid of any major breaches or deviations. The analyses will be carried out by using SAS version 9.4 (SAS Institute Inc., Cary, NC) and R version 4.1.2 (https://www.r-project.org/).

To examine the correlation between the comprehensive gut microbial community and clinical conditions, a permutational multivariate analysis of variance (PERMANOVA) will be conducted. This analysis will involve the utilization of the Bray-Curtis dissimilarity method 24. Differences in the alteration of gut microbiota's relative abundance before and after treatment will be computed by applying the non-parametric Mann-Whitney U-test. Furthermore, Pearson’s correlation between changes in metabolites originating from the gut microbiota and enhancements in clinical variables will be computed within each respective group.

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

To Reviewer #2:

Comment:

1.In page 15, the optional acupoints

What do you mean by optional?

Reply: Following the Traditional Chinese Medicine guidelines for the diagnosis and treatment of Non-Alcoholic Fatty Liver Disease based on syndrome differentiation[1], patients was categorized into three groups,which corresponding to three syndrome types, namely accumulation of dampness and heat, syndrome of retention of dampness, and syndrome of blood stasis and have selected appropriate acupoints for each group that can enhance therapeutic efficacy. The utilization of these optional acupoints provides additional means of adjustment in acupuncture therapy, allowing for a more detailed and personalized treatment plan, thereby enhancing therapeutic efficacy.

[1]. Zhao W, Xu E, Wang X, et al. Guidelines for Traditional Chinese Medicine Diagnosis and Treatment of Non alcoholic Fatty Hepatitis. Chinese Journal of Integrated Traditional and Western Medicine on Liver Diseases 2023;32:1059-1062.

2. Would it be applied for some patients and not fot the others

Reply: Thank you for your inquiry, which has enlightened us.

At present, we do not know which population is suitable for this therapy. In order to get an answer to the question, we will stratify or group the patient population based on relevant characteristics such as age, gender, and disease stage. Statistical methods will be utilized to evaluate the relationship between different patient characteristics and treatment outcomes.

In addition, we have inclusion and exclusion criteria for enrolling patients. Each individual is informed of the treatment they will receive and the potential risks involved, and they have signed an informed consent form. All untoward incidents will be meticulously documented during the trial, utilizing a dedicated questionnaire, by patients, outcome assessors, and acupuncturists. In the event of an adverse event, researchers may take necessary measures based on the condition, such as temporarily suspending treatment, and decide whether to terminate the study.

VERSION 2 – REVIEW

REVIEWER NAME	Zhao, Jingjie
REVIEWER AFFILIATION	Capital Medical University, Department of Traditional Chinese Medicine
REVIEWER CONFLICT OF INTEREST	None.
DATE REVIEW RETURNED	15-Mar-2024

GENERAL COMMENTS	1. The author answer "to ensure the implementation of blinding, we hope that the patients have never undergone acupuncture treatment before." Please ensure the word “never”, the patients have never undergone acupuncture treatment. 2.In Figure 1, the total samples should be randomized, instead of enrolled samples. Please ensure the sample size in this trial.

VERSION 2 – AUTHOR RESPONSE

To Reviewer #1:

Comment:

1. The author answer "to ensure the implementation of blinding, we hope that the patients have never undergone acupuncture treatment before."

Please ensure the word “never”, the patients have never undergone acupuncture treatment.

Reply: Thank you for your reminder. We will make sure the patients have never received acupuncture treatment before enrollment within at least one year.

The Exclusion criteria has been revised on page 9, as follows: Those who have received acupuncture treatment before enrollment within at least one year.

2. In Figure 1, the total samples should be randomized, instead of enrolled samples.

Please ensure the sample size in this trial.

Reply: The sample number in Figure 1 has been revised.

The sample size of this study has been evaluated by the statistician.