Author’s response to reviews

Title: Acupuncture Treatment for Carotid Atherosclerotic Plaques: Study Protocol for a Pilot Randomized, Single Blinded, Controlled Clinical Trial

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Reviewer reports:

Reviewer #1: The manuscript is generally well written and describes a study protocol for a randomized controlled trial of acupuncture treatment for carotid atherosclerotic plaques. There are several areas that need to be addressed.

1.Line 104, "another object is to establish acupuncture treatment for carotid atherosclerotic plaques", you may change another expression or delete it, since your sample size trial couldn't carry its point.

A: Delete “The secondary objective is to establish acupuncture as an adjuvant therapy” in the Line 104.

2.Line 163, you mentioned "four questions", there seems only three. Besides, If the results are concluded that the blinding is not sufficient, what will you deal with that? Because many Chinese patients have acupuncture experience, they are familiar with the needling sensation.
A: The wording of line 186 "four questions" has been modified into "three questions". During the study, we will explain the acupuncture methods to the patients. The purpose of selecting the blunt needle is to maintain the stability of the needle and reduce the doubts in the patients. In previous studies, this method of sham acupuncture was also used in the clinical research. If there is an intergroup difference at the end, we will conduct a subgroup analysis based on the difference and assess the possible reasons for the difference.

3. Table 1, there is a spelling mistake of "YINTANG", and what is the acupuncture manipulation for this acupoint, straightly or oblique insertion? If using oblique insertion, maybe the angle is 15 degree, will the pedestal affect the acupuncture manipulation for the true acupuncture group?

A: Spelling error has been corrected in Table 1. The YINTANG will use straightly insertion.

4. Line 467, there are several kinds of statins, why you choose atorvastatin? According to the clinical guideline?

A: Atorvastatin has higher bioavailability and fewer side effects than other statins. Atorvastatin is suitable for patients with hypercholesterolemia and mixed hyperlipidemia, and atorvastatin has a shorter time to peak plasma concentration, a longer half-life period, and has a longer-lasting lipid-lowering effect. In clinical applications, atorvastatin is more widely used, and the subject's acceptance is higher. Therefore, atorvastatin was selected in this study.

Reviewer #2: Peer review on manuscript
"Acupuncture treatment for carotid atherosclerotic plaques: protocol for a randomized, blind clinical trial"

26th May, 2020
This is an interesting study protocol that provide knowledge about safety and efficacy of acupuncture treatment for carotid atherosclerotic plaques. In brief, in order to achieve this goal, the authors are conducting a randomized clinical trial of 105 cervical atherosclerotic plaque patients in to three groups (TA, SA, and MC) that will be allocated at a 1:1:1 ratio. Participants in the TA group (Acupuncture group) will receive acupuncture related intervention by skilled acupuncturist on the location of acupoints called Neiguan (PC6), Yanglingquan (GB34), Renying (ST9), Baihui (DU20) and Yintang (EX-HN3). In SA (Sham-acupuncture group), they will be provided non-acupuncture treatment on the same acupoints as those used in the TA group. Participants randomized in MC (Medication group) group will be given aspirin enteric-coated tablets and atorvastatin calcium tablets. The treatment will last for 12 weeks. This paper mentioned that the total plaque volume (PV) and the grey-scale median (GSM) are primary outcomes. Furthermore, secondary outcomes consist of intima-media thickness (IMT), lipid levels, apolipoprotein A-IV level, platelet count (PLT), fibrinogen (FIB) and platelet aggregation rate (PAR).
Generally, the paper is quite well written and there are number of general problems with the manuscript that need some revision. I recommended Below are my comments by section.

Title and abstract
☐ The title is appropriate for the content of the article. However, I suggest the present new title instead of previous one: Safety and Efficacy Assessment of Acupuncture Treatment for Carotid Atherosclerotic Plaques: Study Protocol for A Randomized, Single Blinded, Controlled Clinical Trial.

A: The title has been modified. The title will highlight the effectiveness and safety of acupuncture.

☐ Although the abstract summarized the content of the article very well, it need some minor revision to include enough information. In the background section line 23, mention the importance of the present study and how it differs from previous works. In addition, give a summary of what you want to do. Furthermore, in line 29, it is better to add the setting of study: "This is a three-arm randomized clinical trial in China".

A: The summary section(“The study objective is to evaluate the efficiency and reliability of acupuncture to relive CAD and provide a new therapeutic idea for the clinical treatment of CAD”) has been added in the abstract. "In China" has been added in Line 29.

Background
☐ Generally, the first part of the introduction should be summarized: I suggest that authors reform it into three main paragraphs: First, a brief definition of atherosclerotic disease, with some statistics in China, and its burden. Second, a brief description of possible primary, secondary, and tertiary preventions. Third, the studied role of acupuncture in atherosclerotic disease.

A: The part of introduction has been adjusted according to the recommendations. Summarize according to the above three points. The epidemiology and drug treatment have been reduced. And we had increased Chinese epidemiological statistics in the manuscript.

☐ Line 73, the Study was performed in China. So, it's necessary to talk about statistics in the Chinese population, after referring to American population.

A: A study has counted the disease burden in China from 1990 to 2010. Among them, the main cause of death was stroke, and 1.7 million patients died of stroke in 2010. Ischemic heart disease ranks second, with 948,700 cases of death from ischemic heart disease.


☐ Line 89, a bit more information about acupuncture and acupoints would be needed and useful and provide more details.
A: Studies provide acupuncture robust protection against transient cerebral ischemic injury and promotes the proliferation of neural stem/progenitor cells in response to ischemia via the Wnt/β-catenin pathway. Electroacupuncture can reduce vasoconstriction and improved blood supply in ischemic region, and ultimately conferred beneficial effects on cerebral ischemia. Studies have found that acupuncture may promote neurological functional recovery by modulating retinoic acid expression in the post-ischemic brain is one of the mechanisms by which EA can be effective in the treatment of ischemic stroke. In terms of acupuncture points, the acupuncture points for angina pectoris are bilateral acupoints PC6 and HT5. Acupuncturists often choose BAIHUI (DU20), YINTANG (EX-HN3) and YANGLINGQUAN (GB34) to treat stroke.

□ Line 93, please mention how your study will advance medical practice and the health of humankind. In other word, highlight the gaps in current understanding, why the research will be carried out and the novelty of the manuscript.

A: Acupuncture is widely used in the rehabilitation treatment of atherosclerotic diseases, but acupuncture is mostly used after cardiovascular and cerebrovascular events occur. The purpose of this study is to provide data support for acupuncture in the treatment of atherosclerotic plaques, and to provide a new idea for the early prevention of atherosclerosis. The relevant content has been supplemented in the article Line 94.

□ At the end of this section (line 95), authors talked about study method, which is not acceptable here. Please omit this part, this could be visited in method part.

A: This section has been removed from the research background.

Methods
□ In study design section, please explain the design, which I think it should be parallel. Also describe why the study design proposed is the best way to answer the research question.

A: This study is designed as a parallel control study to explore the effect of acupuncture in improving carotid atherosclerotic plaque. Therefore, the positive drug group is set for the positive control to evaluate the difference in the curative effect between acupuncture and drugs. At the same time, sham acupuncture group is set for placebo control to evaluate the clinical efficacy of acupuncture. The corresponding part has been supplemented in the "Study Design".

□ In line 109 mention the total sample size.
A: The sample size has been added to Line 109.

□ In intervention section, authors mentioned moxibustion, what was the exact type of this procedure? Direct or indirect? Please define it in the acupuncture method. Was it possible not to
use moxibustion with acupuncture, as it may skew the results? The authors did not explain it in interventions.

A: In this study, moxibustion therapy was not used in the acupuncture intervention methods, which was the author's mistake in translation. The part of moxibustion written in "Study Design" and "intervention section" has been deleted.

☐ In SA group part, did the authors asked patients about the history of performing acupuncture, especially in SA group? As a known history of acupuncture might affect the blinding procedure.

A: Among patients in SA group, the researchers will not deliberately ask the patients to mention the previous acupuncture process. However, in the actual treatment, the patients may inquire about the acupuncture method of this study. In the study, the methods of the TA and SA groups are similar from an intuitive observation, and the pain of acupuncture is also similar. For the SA patients, the only difference is the acupuncture sensation after the acupuncture pierces the skin. However, in the actual normal acupuncture operation, when no acupuncture is done, the phenomenon of no acupuncture is common. The researchers will also explain to the SA patients that this kind of acupuncture method is to ensure the stability of the acupuncture points near the arteries. Because the acupuncture points near the blood vessels, for the safety of the acupuncture, we will not pursue strong feeling of deqi during the treatment.

☐ Line 152, how did you decide on the number of subjects in each group? Also how will the subjects be found?

A: In the part of the sample size, in reviewing previous acupuncture studies, we had not found the similar studies. Previous acupuncture studies were focuse on the sequelae of stroke and hypertension. There are only a few clinical studies in Chinese that discuss the effect of acupuncture on carotid plaque, but the quality of these studies are low and the evaluation indicators are different from this study. Therefore, it cannot be used as the basis for estimating the sample size of this study. This study is a prospective study, considering the difficulty of case screening, research funding and manpower constraints, and in order to ensure a certain sample size, only 105 cases will be selected as the sample size of this study.

   In terms of patient recruitment, the study site is in a large hospital in China with multiple hospital branch courts. The hospital acupuncture research team has many years of clinical research experience. We have many publicity channels. The hospital physical examination center, cooperative unit, cardiovascular center, neurological rehabilitation center, acupuncture rehabilitation center and other departments carried out publicity to strengthen recruitment.

☐ Line 118, you need to cite a reference.

A: Related references has been added to Line 118

☐ Line 124, Please add gender along with the age of the patients.
A: There is no restriction on the gender of the subjects in this study, the relevant content has been added in Article 5 of "Inclusion criteria"

According to safety assessment part, what will you do to reduce risk and what will happen if adverse events occur.

A: To reduce the risk of adverse events. Firstly, acupuncturists should have years of work experience. Before the study begins, all acupuncturists need an unified training to standardize the acupuncture operation and increase the safety of acupuncture. Secondly, during the research process, the cardio-cerebrovascular doctor will evaluate the patient's condition to ensure that the patients with severe cardio-cerebrovascular disease during the study period can be treated in time. During the treatment, the acupuncturist will also evaluate the subject's condition to reduce the occurrence of adverse events. Finally, this study will be conducted in Guangdong Provincial Hospital of Traditional Chinese Medicine. The hospital has a strong medical level and ability to handle crisis events, which can guarantee the progress of this study. For patients with serious adverse reactions, we will send them for professional treatment, and report to the ethics committee.

According to trial status part, I checked the information in the Chinese clinical trial registry (protocol number ChiCTR1800019259) there are some differences, so please confirm the details in this protocol. In inclusion criteria, you state that low-desity lipoprotein (LDL) should be from 2.59 mmol/L-8.0 mmol/L (line 122), also you mention more than 5 plaques in exclusion criteria; they are different to the information of Chinese clinical trial registration. Furthermore, you mention hyperlipidemia (>8.00mmol/L), Triglyceride greater than 10.0mmol/L and allergies as exclusion criteria in the clinical trials registery - please add to the protocol if this is correct. In protocol, age older than 70 years is an exclusion criterion which is different to the information in the clinical trials registery (older than 60 years).

A: It has been modified according to the information of the clinical registration center. In Article 4 of the inclusion criteria, “LDL” has changed to “Cholesterol”. In Article 2 of the exclusion criteria, patients with more than 5 plaques should be excluded. The exclusion criteria have been revised in the text. In Article 3 of the exclusion criteria, “Hyperlipidemia patients with cholesterol greater than 8.00mmol/L” and “Triglyceride is greater than 10.0mmol/L” have been added. The age in Article 5 is revised to 60 years.

Reviewer #3: In this manuscript, Zhou et al. report that will use Acupuncture treatment for carotid atherosclerotic plaques. However, I have questions and suggestions for revision.

1. Attention to detail with grammar throughout the paper.
A: Some grammatical errors in the article have been corrected. The manuscript has been submitted to a professional English retouching agency for retouching. The retouching certificate has been uploaded in the attachment.

2. I think that the author needs to include one more group: TA group (receiving acupuncture and moxibustion therapy) and the medication group (taking aspirin and statins).
A: In this study, three groups of acupuncture, drugs, and sham acupuncture are selected for comparison to evaluate the efficacy of acupuncture. The effect of acupuncture combined with drugs has not been determined. Perhaps the combination of the two will achieve better results, or it may be counterproductive. This will be the focus of our next research, to explore whether adding acupuncture on the basis of drugs can achieve better results.

3. The author needs to improve in Figure 1.
A: Figure 1 has been modified and related files have been uploaded to the system.

4. Is important include the informed consent form.
A: Informed consent has been uploaded in the upload attachment.

5. "All participants can receive treatment unrelated to atherosclerotic disease." Please explain in the METHODS section.
A: Because the age of the population included in this study is generally older, the middle-aged and elderly people are often accompanied by other symptoms of physical discomfort. In order to ensure the rights and interests of the patients and meet the ethical requirements, the subjects are allowed to use the treatment of diseases not closely related to atherosclerosis (such as cervical spondylosis, pain, insomnia, fever, etc.) during the study period. It should be noted that the treatment received by the patients cannot interact with the drugs taken in the study, and the patients cannot receive any acupuncture treatments, so as not to interfere with the evaluation of the efficacy of acupuncture interventions.

6. There is a need for greater control over the severity of the disease to ensure that all patients have the same degree of seriousness.
A: Although AS contributes severely to CVD events and stroke, the seriousness of the disease is hard to measure. Instead, we attach more emphasis on risk than degrees. In addition, the size and number of plaques only mean that the patient attends to have higher risks to suffer CVD events or stroke, but it is not definite and absolute. Though not exhaustively, we have excluded subjects with higher risks, such as Carotid aortic stenosis, larger than five plaques, severe hyperlipidemia patients, history of stroke and so forth.

7. Specifics points:
Correct:
on a voluntary basis to voluntarily, in line 31.
The treatment will last for 12 weeks, with 2 treatments. Consider adding a comma (after weeks) and spelling out the number (2).
This trial to trial, in line 41.
Arteritis to arthritis, in line 57.
metabolic diseases to metabolic disorders, line 58.
highest morbidity to highest morbidities, line 67.
Remains to remain, line 70.
aspirin, combined with statins, can reduce.. Consider adding a comma, line 76.
not good to not suitable, line 80.
Stenosis to stenoses, line 84.
As an adjuvant to as adjuvant, line 104.
than 5 plaques; spelling out the number, line 121.
than 5 plaques; spelling out the number, line 131.
have serious adverse to have severe adverse, line 143.
on a voluntary basis to voluntarily, line 154.
2 week to two weeks …spelling out the number and plural; line 169.
a tiny to a little, line 234.
Detailed to individual situations, line 244.
On carotid to on the carotid, line 302.
least 1 treatment to least one treatment, line 361.
course of the disease, line 368.
Trial, remove the, line 383.
both the ethics, include the article, line 386.
provide 3 months, spelling out the number, line 395.
The formation and rupture of carotid atherosclerotic plaque ARE; correct, line 413.
the way in which to how.
A: The above mentioned places have been modified in the manuscript.