PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Efficacy and safety of High-Dose Xueshuantong injection (lyophilized) in reducing the incidence of major adverse cardiovascular events in patients with unstable angina: A protocol of a randomized, parallel-arm, controlled, double-blind, and multicenter clinical trial based on dual antiplatelet therapy
AUTHORS	Long, Wenjie; Liao, Huili; Huang, Xi; Liu, Qingqing; Tang, Yaqing; Lu, Liming; Liu, Jianhong; Yuan, Tianhui; Ling, Yan; Hong, Yu; Duan, Jiao; Lin, Weiji; Xian, Shaoxiang; Yang, Zhongqi

VERSION 1 - REVIEW

REVIEWER	Jingyuan Mao First Teaching Hospital of Tianjin University of Traditional Chinese Medicine, Tianjin, China.
REVIEW RETURNED	08-Mar-2020

GENERAL COMMENTS	The protocol is clear about major aspects of the clinical trial; however, I have some questions about the process and suggest the authors to revise the manuscript with the supplementary answers. 1. As a UA patient, physician should evaluate the circumstances by the GRACE risk score, is there any evaluating process in this trial? And are there any influences on enrollment, especially for patients who should take PCI? 2. Please clarify the precise randomization time and when the patient takes first intervention? 3. For the control group, patients treat via an intravenous drip with 25 mg XST, is there any previous experiment or trail to confirm the dosage is invalid for UA? In addition, please revise the grammars and expressions of written English in the manuscript.

REVIEWER	Akihiko Narisada Aichi Medical University, Japan
REVIEW RETURNED	06-Apr-2020

GENERAL COMMENTS	Thank you for the opportunity to review. This randomized control trial study protocol is to examine the effects of Xueshuantong injection (lyophilized) in reducing the major adverse cardiovascular events in patients with unstable angina. First of all, Reference section is incomplete in this manuscript. Thus, the authors should complete it. Additionally, my major concern is about

	“control group”, as below. 1. (Page 17, Line 17) The authors referred 14, there is no 14 in Reference section. 2. (Page 17, Line 18) The authors referred 15, there is no 15 in Reference section. Major comment 3. (Page 12, Line 15-20) This study is to assess the effects of XST. I do not understand why the patients in “control group” will be treated via interventions drip with, not 0 mg, but 25 mg. Explain is needed.

REVIEWER	Anggoro Budi Hartopo Department of Cardiology and Vascular Medicine, Faculty of Medicine, Public Health and Nursing Universitas Gadjah Mada, Yogyakarta, Indonesia
REVIEW RETURNED	08-Apr-2020

GENERAL COMMENTS	Authors of this protocol manuscript perform the study of Efficacy and safety of Xueshuantong injection (lyophilized) in reducing the incidence of major adverse cardiovascular events in patients with unstable angina. The authors address an interesting study of a randomized, parallel-arm, controlled, double-blind, and multicenter clinical trial. Reviewer raises concern and comment as follows: 1. The biological mechanism of the investigational medicine product (XST) in reducing MACE among unstable angina should be clearly defined in Introduction section 2. In methods, it should be explained the reason of using 1/5 dose of XST in controlled subjects? Why is it not placebo to be used for the control? 3. In methods, the subjects are patients with UA. Patients with UA is considered an emergency situation and need an intensive cardiac care unit with intravenous UFH. The recruitment of the subjects is not clear, "1200 patients will be recruited at the 17 centers mentioned earlier through the official website of the hospitals, posters, and networks" this statements contradict the condition of UA patients which needed intensive hospitalisation. The consecutive enrollment should be more suitable for subjects enrollment. 4. In methods, the timing of investigational drugs administered to patients is unclear. Please give information about timing of administration of investigational drug (H-0 hospitalisation, et cetera). 5. In methods, page 12 : "Patients who continue to use the drug for more than 7 days until discharge will end the medication and move directly to the follow-up period". This sentence is unclear, because the drug administration is between 7 - 14 days. Please also explain whether the subjects are hospitalised during treatment (7-14 days) ? 6. Please clearly define the primary outcome in more detail. Operational definition of each outcome is necessary. 7. Please clearly defined the secondary outcome. The efficacy of angina pectoris --> how to measure this outcome? What modalities do the authors use to assess the efficacy of angina pectoris? 8. The secondary outcome: Myocardial injury markers [serum creatine kinase MB; cardiac troponin T/cardiac troponin I) ... this statement is unclear what is a clinical condition is assessed by myocardial injury markers? Is it myocardial infarction ? 9. The adverse event (AE) should be clearly defined what condition

	constitute an AE. 10. The inclusion criteria in table 1 : Patients diagnosed with coronary heart disease who met at least one of the following diagnostic criteria. The criteria is contradicted the subjects of UA diagnosis. 11. Figure 1 : there is two weeks time for participant recruitment before randomisation. The subjects are patients with UA, which should be managed in intensive care. Why two weeks time is needed for participant recruitment ? This is not in the range of therapy for UA, instead the patients already in the state of stable coronary heart disease because the acute events already subsided after two weeks.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1:
The protocol is clear about major aspects of the clinical trial; however, I have some questions about the process and suggest the authors to revise the manuscript with the supplementary answers.
1) As a UA patient, physician should evaluate the circumstances by the GRACE risk score, is there any evaluating process in this trial? And are there any influences on enrollment, especially for patients who should take PCI?
Response to comment 1): The primary evaluating assessment process is determined by two or more physicians through the patients' clinical manifestations, ECG, and laboratory examination. Taking into account the patient's benefit, for patients with a moderate to high GRACE risk score, we recommend actively considering interventional therapy, especially for patients who require PCI, we have described this in the exclusion criteria (Table 1).

2) Please clarify the precise randomization time and when the patient takes first intervention?
Response to comment 2): Patients will be randomized and initiated treatment on the day of enrollment. We have modified in the revision manuscript.
Section Randomization, Paragraph 1, Sentence 1.

3) For the control group, patients treat via an intravenous drip with 25 mg XST, is there any previous experiment or trail to confirm the dosage is invalid for UA? In addition, please revise the grammars and expressions of written English in the manuscript.
Response to comment 3): Thanks for the comment. In our study, the XST is a lyophilized powder form, which is a white and light-yellow amorphous powder, or a loose solid substance. Before using it, it will be dissolved with an appropriate amount of sodium chloride injection, so there will be color Changes and a small amount of powder precipitation. To better implementing the blind method, the extremely low dose 25mg is used as the control group. Extremely low dose 25mg was only used as a control drug, will not increase the drug efficacy of the experimental group, so we choose extremely low dose 25mg XST as a control group. In addition, from our previous pharmacokinetic experiment and trials, we confirm that extremely low dose 25mg XST is invalid for UA. The reason extremely low dose 25mg was chosen as the control group, we will explain in the Blinding section.
We will revise the grammars and expressions of written English in the manuscript. We also provide proof of English editing.
Section Blinding, Paragraph 1.

Reviewer 2:
This randomized control trial study protocol is to examine the effects of Xueshuantong injection (lyophilized) in reducing the major adverse cardiovascular events in patients with unstable angina. First of all, Reference section is incomplete in this manuscript. Thus, the authors should complete it. Additionally, my major concern is about “control group”, as below.

1) (Page 17, Line 17) The authors referred 14, there is no 14 in Reference section.
Response to comment 1): We apologize for this mistake, we have corrected it in the reference part and marked it with red font.
Page 19 Line 12

2)(Page 17, Line 18) The authors referred 15, there is no 15 in Reference section.
Response to comment 2): We apologize for this mistake, we have corrected it in the reference part and marked it with red font.
Page 19 Line 16
3)(Page 12, Line 15-20) This study is to assess the effects of XST. I do not understand why the patients in “control group” will be treated via interventions drip with, not 0 mg, but 25 mg. Explain is needed.
Response to comment 3): Thanks for the comment. In our study, the XST is a lyophilized powder form, which is a white and light-yellow amorphous powder, or a loose solid substance. Before using it, it will be dissolved with an appropriate amount of sodium chloride injection, so there will be color Changes and a small amount of powder precipitation. To better implement the blind method, the extremely low dose 25mg is used as the control group. In addition, from our previous pharmacokinetic experiment and trials, we confirm that extremely low dose 25mg XST is invalid for UA. The reason 25mg was chosen as the control group, we will explain in the Blinding section.
Section Blinding, Paragraph 1.

Reviewer 3 :
Authors of this protocol manuscript perform the study of Efficacy and safety of Xueshuantong injection (lyophilized) in reducing the incidence of major adverse cardiovascular events in patients with unstable angina. The authors address an interesting study of a randomized, parallel-arm, controlled, double-blind, and multicenter clinical trial. Reviewer raises concern and comment as follows:
1)The biological mechanism of the investigational medicine product (XST) in reducing MACE among unstable angina should be clearly defined in Introduction section
Response to comment 1): Thanks for the suggestion. We have added a description of the biological mechanism of the XST in reducing MACE among unstable angina in the Introduction section.
Section Introduction, Paragraph 2, Sentence 4 to 5. (Page 5 line 23-28, page 6 line 1-4.)
2)In methods, it should be explained the reason of using 1/5 dose of XST in controlled subjects? Why is it not placebo to be used for the control?
Response to comment 2): Thanks for the comment. In our study, the XST is a lyophilized powder form, which is a white and light-yellow amorphous powder, or a loose solid substance. Before using it, it will be dissolved with an appropriate amount of sodium chloride injection, so there will be color Changes and a small amount of powder precipitation. To better implement the blind method, the extremely low dose 25mg is used as the control group. In addition, from our previous pharmacokinetic experiment and trials, we confirm that extremely low dose 25mg XST is invalid for UA. The reason 25mg was chosen as the control group, we will explain in the Blinding section.
The reason 25mg was chosen as the control group, we will explain in the Blinding section
Section Blinding, Paragraph 1.

3)In methods, the subjects are patients with UA. Patients with UA is considered an emergency situation and need an intensive cardiac care unit with intravenous UFH. The recruitment of the subjects is not clear, "1200 patients will be recruited at the 17 centers mentioned earlier through the official website of the hospitals, posters, and networks" this statements contradict the condition of UA patients which needed intensive hospitalisation. The consecutive enrollment should be more suitable for subjects enrollment.
Response to comment 3): Thanks for the comment. We have indicated in the exclusion criteria that the middle-high-risk stratified UA patients were excluded, so the enrolled patients were treated in the

general wards. For clarity, we have added that recruitment will be conducted in outpatient or inpatient Settings and marked it in red font.
Section Study setting and recruitment, Paragraph 1, Sentence 1.

4)In methods, the timing of investigational drugs administered to patients is unclear. Please give information about timing of administration of investigational drug (H-0 hospitalisation, et cetera).
Response to comment 4): We apologize for this unclear description. Patients will be admitted to the hospital on the day of registration for the first intervention.
Section Interventions, Paragraph 5, Sentence 1.

5) In methods, page 12: "Patients who continue to use the drug for more than 7 days until discharge will end the medication and move directly to the follow-up period". This sentence is unclear, because the drug administration is between 7 - 14 days. Please also explain whether the subjects are hospitalised during treatment (7-14 days)?
Response to comment 5): We apologize for this mistake. We have modified as: “Patients who discharge will move directly to the follow-up period”. The patients included are hospitalized during treatment.
Section Interventions, Paragraph 5, Sentence 1.

6)Please clearly define the primary outcome in more detail. Operational definition of each outcome is necessary.
Response to comment 6): The major adverse cardiovascular events (MASE), as the primary outcome, is a commonly used indicator to evaluate the prognosis of patients with coronary heart disease or other cardiovascular disease.([1]. Association of Initial and Serial C-Reactive Protein Levels With Adverse Cardiovascular Events and Death After Acute Coronary Syndrome: A Secondary Analysis of the VISTA-16 Trial. JAMA Cardiol. 2019;4(4):314-320. [2]. Reductions in Atherogenic Lipids and Major Cardiovascular Events A Pooled Analysis of 10 ODYSSEY Trials Comparing Alirocumab With Control. Circulation. 2016 Dec 13; 134(24): 1931–1943. [3]. Use of High-Risk Coronary Atherosclerotic Plaque Detection for Risk Stratification of Patients With Stable Chest Pain. JAMA Cardiol. 2018 Feb; 3(2): 144–152.) It is including cardiovascular death, nonfatal myocardial infarction, and revascularization, which was already mentioned in the section Primary Outcomes, and we will add more explanation.
Section Outcome measurements, Paragraph 1, Sentence 1

7)Please clearly defined the secondary outcome. The efficacy of angina pectoris --> how to measure this outcome? What modalities do the authors use to assess the efficacy of angina pectoris?
Response to comment 7): We evaluate the efficacy of angina mainly by the frequency of angina attacks, and also focus on patients’ clinical manifestations, ECG, and laboratory examination.
Section Secondary Outcomes, Paragraph 1, Sentence 1.

8)The secondary outcome: Myocardial injury markers [serum creatine kinase MB; cardiac troponin T/cardiac troponin I) ... this statement is unclear what is a clinical condition is assessed by myocardial injury markers? Is it myocardial infarction?
Response to comment 8): Thanks for the comment. Since Myocardial injury markers were elevated in some UA patients, the dynamic changes of myocardial enzymes were used to evaluate the efficacy and safety of the experiment. We have add more explanation in the revision.
Section Secondary outcomes, Paragraph 2, Sentence 1.

9)The adverse event (AE) should be clearly defined what condition constitute an AE.
Response to comment 9): Thanks for the suggestion. We have added in the manuscript about AE.
Section Safety assessment, Paragraph 1, Sentence 2 to Paragraph 2.

10)The inclusion criteria in table 1: Patients diagnosed with coronary heart disease who met at least one of the following diagnostic criteria. The criteria is contradicted the subjects of UA diagnosis.
Response to comment 10): Thanks for the comment. The patient should be diagnosed with coronary heart disease (CAD) before diagnosis as UA. So, Patients diagnosed with coronary heart disease who met at least one of the following diagnostic criteria in table 1, which was necessary for the patient included. What's more, the diagnosis of patients with UA was made and confirmed using the "Guideline Update for the Management of Patients with Chronic Stable Angina" (ACC/AHA, 2002) and "Diagnosis and treatment recommendations of UA" (Chinese Society of Cardiology, 2000). (Zheng XY. Chinese Medicine New Drug Clinical Guidelines. 2002; Beijing, China: China Pharmaceutical Science and Technology Press, 392.) So it is not contradicted the subjects of UA diagnosis.

11)Figure 1: there is two weeks time for participant recruitment before randomisation. The subjects are patients with UA, which should be managed in intensive care. Why two weeks time is needed for participant recruitment ? This is not in the range of therapy for UA, instead the patients already in the state of stable coronary heart disease because the acute events already subsided after two weeks.
Response to comment 11): We apologize for this mistake. We have modified the enrollment time point as "day -3~0" in the Table 2. and the Figure 1.

VERSION 2 – REVIEW

REVIEWER	Jingyuan Mao First Teaching Hospital of Tianjin University of Traditional Chinese Medicine, Tianjin, China.
REVIEW RETURNED	22-May-2020

GENERAL COMMENTS	The responses of reviewers' comments are appropriate, and the interesting study is recommened to publish.

REVIEWER	Akihiko Narisada Institute for Occupational Health Science, Aichi Medical University, Japan
REVIEW RETURNED	03-Jun-2020

GENERAL COMMENTS	Thank you for the opportunity to review. I understand why an "extremely low dose (25mg)" was chosen as the control group in this study. This study is to examine the effect of high dose Xueshuantong (XST), compared to low dose XST, in reducing the major adverse cardiovascular events in patients with UA. This study protocol should be changed so. Major comment 1. (Title and Manuscript) This randomized control trial study protocol is to examine, not the effects of XST, but the effect of high dose XST (compared to low dose), in reducing the major adverse cardiovascular events in patients with unstable angina. Thus, the authors should change Title and Manuscript so. 2. (Page 43, Line 39) Although the authors mentioned that an extremely low dose (25mg) was invalid for UA from their previous experiment, more explains about its biological and pharmacological background in Introduction section are needed.

REVIEWER	Anggoro Budi Hartopo Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia
REVIEW RETURNED	01-Jun-2020

GENERAL COMMENTS	The authors have made substantial amendment of the research protocol. It is much clearer for readers. However there are still more confirmations needed : 1. The criteria of UA is based on: The diagnosis of patients with UA was made and confirmed using the “Guideline Update for the Management of Patients with Chronic Stable Angina” (ACC/AHA, 2002) and “Diagnosis and treatment recommendations of UA” (Chinese Society of Cardiology, 2000). (Zheng XY. Chinese Medicine New Drug Clinical Guidelines. 2002; Beijing, China: China Pharmaceutical Science and Technology Press, 392.) " However, the authors did not mention this reference clearly in the manuscript and the reference of their manuscript. There are may be different diagnosis criteria of UA between AHA, ESC and Chinese Society of Cardiology Guideline because UA have many subsets : post infarction angina, new onset angina (CCS III), CCS III and IV angina pectoris, angina at rest e.t.c. Therefore the description of what guideline and diagnosis criteria use to determine UA in the study is important. Authors should put the guideline references in the manuscript. 2. The stratification of UA : mild, moderate, high risk is unclear... again which guideline(s) the authors use to define the stratification? Based on ESC and GRACE scoring, all subjects with UA is considered NSTEACS with low risk. So the definition of stratification of UA should be clearly define in the manuscript. TIMI risk score > 3 is the inclusion criteria, is it also contradict with mild UA ? https://jamanetwork.com/journals/jama/fullarticle/192996

VERSION 2 – AUTHOR RESPONSE

Reviewer 1:
The responses of reviewers' comments are appropriate， and the interesting study is recommened to publish.
Thanks for your comments.

Reviewer 3 :
The authors have made substantial amendment of the research protocol. It is much clearer for readers. However there are still more confirmations needed :
1） The criteria of UA is based on: The diagnosis of patients with UA was made and confirmed using the “Guideline Update for the Management of Patients with Chronic Stable Angina” (ACC/AHA, 2002) and “Diagnosis and treatment recommendations of UA” (Chinese Society of Cardiology, 2000). (Zheng XY. Chinese Medicine New Drug Clinical Guidelines. 2002; Beijing, China: China Pharmaceutical Science and Technology Press, 392.) " However, the authors did not mention this reference clearly in the manuscript and the reference of their manuscript. There are may be different diagnosis criteria of UA between AHA, ESC and Chinese Society of Cardiology Guideline because UA have many subsets : post infarction angina, new onset angina (CCS III), CCS III and IV angina pectoris, angina at rest e.t.c. Therefore the description of what guideline and diagnosis criteria use to determine UA in the study is important. Authors should put the guideline references in the manuscript.
Response to comment 1):Thanks for the comment.We apologize for this mistake.We have added the guideline and diagnosis criteria references in the manuscript to determine UA in the study.

2） The stratification of UA : mild, moderate, high risk is unclear... again which guideline(s) the authors use to define the stratification? Based on ESC and GRACE scoring, all subjects with UA is considered NSTEACS with low risk. So the definition of stratification of UA should be clearly define in the manuscript. TIMI risk score > 3 is the inclusion criteria, is it also contradict with mild UA ?
Response to comment 1):Thanks for the comment.We through the patients' clinical manifestations, ECG, and laboratory examination to clarify the stratification of UA.And we already clarify that the high-risk patients will be excluded.Taking into account the patient's benefit, for patients with a moderate to high GRACE risk score, we recommend actively considering interventional therapy, especially for patients who require PCI, we have described this in the exclusion criteria (Table 1).

Reviewer 2:
I understand why an "extremely low dose (25mg)" was chosen as the control group in this study. This study is to examine the effect of high doseXueshuantong (XST), compared to low dose XST, in reducing the major adverse cardiovascular events in patients with UA. This study protocol should be changed so.
1) (Title and Manuscript) This randomized control trial study protocol is to examine, not the effects of XST, but the effect of high dose XST (compared to low dose), in reducing the major adverse cardiovascular events in patients with unstable angina. Thus, the authors should change Title and Manuscript so.
Response to comment 1):Thanks for the comment.We have corrected the title to "Efficacy and safety of High-Dose Xueshuantong injection (lyophilized) in reducing the incidence of major adverse cardiovascular events in patients with unstable angina: A protocol of a randomized, parallel-arm, controlled, double-blind, and multicenter clinical trial based on dual antiplatelet therapy", and modified our description in the manuscript.
2) (Page 43, Line 39) Although the authors mentioned that an extremely low dose (25mg) was invalid for UA from their previous experiment, more explains about its biological and pharmacological background in Introduction section are needed.
Response to comment 2):Thanks for the comment.In the Introduction section, we have added the description of the biological and pharmacological background of extremely low dose XST for UA.

VERSION 2 – REVIEW

REVIEWER	Akihiko Narisada Institute for Occupational Health Science, Aichi Medical University, Japan
REVIEW RETURNED	16-Jul-2020

GENERAL COMMENTS	Thank you for the opportunity to review this article. I have no additional comments on this article. I hope the authors' works will go well.

REVIEWER	Anggoro Budi Hartopo Faculty of Medicine, Public Heath and Nursing Universitas Gadjah Mada - Dr. Sardjito Hospital, Yogyakarta, Indonesia
REVIEW RETURNED	12-Jul-2020

GENERAL COMMENTS	The authors have addressed my review.