PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Association of High-Sensitivity Cardiac Troponin T with Mortality and Cardiovascular Events in a Community-Based Prospective Study in Beijing
AUTHORS	Xiao, Wenkai; Cao, Ruihua; Liu, Yuan; Wang, Fan; Bai, Yongyi; Wu, Hongmei; Ye, Ping

VERSION 1 - REVIEW

REVIEWER	Zhivko Zhelev University of Exeter Medical School, UK
REVIEW RETURNED	08-Sep-2016

GENERAL COMMENTS	This is well-designed and reported study. I have only two suggestions: - First, the authors state that 181 participants were lost to follow up and excluded from the analysis. If baseline data on these subjects is available, this should be compared to the included patients and reported in the paper, to show if any significant differences existed. - Second, minor revision is needed to address some grammar/style issues.

REVIEWER	Razvan T Dadu, MD Baylor College of Medicine, USA
REVIEW RETURNED	04-Oct-2016

GENERAL COMMENTS	1. Excellent written paper by Xiao et al. 2. The association between hs-cTnT and outcomes which includes mortality, CHD, HF and brain disease has been previously shown in multiple populations of healthy individuals (eg ARIC study publications) therefore commenting on how these results add to already existing publications would definitely strengthen the manuscript. 3. hs-cTnT has been shown to be associated with subclinical brain disease in ARIC population (study not cited here) which shows that there may be other mechanisms that may explain the association between hs-cTnT and brain disease. 4. Most manuscripts divided the cohort in quartiles of hs-cTnT. Commenting on why these cutoffs were chosen would improve the manuscript.

REVIEWER	Ndrepepa, Gjin German Heart Center Munich, Technical University, Munich, Germany
REVIEW RETURNED	17-Nov-2016

GENERAL COMMENTS	The authors of this manuscript investigated the prognostic value of high-sensitivity cardiac troponin T (hs-cTnT) for long-term major adverse cardiovascular events (MACE) in subjects living in the Pingguoyuan area of the Shijingshan district in Beijing, China. Subjects were recruited for the study between September 2007 and January 2009 and were followed up for a median of 4.8 years. Overall 1680 subjects were initially recruited and 1499 subjects with complete data (181 subjects were lost to follow-up) were included in the study. In brief, hs-cTnT was detectable in 820 subjects (54.7%). There were 52 deaths (3.5%), 154 MACE (10.3%) and 99 coronary events of new onset (6.6%) over the follow-up. Subjects with a hs-cTnT level ≥ 14 ng/L (the upper reference limit of the assay) had a higher risk of mortality (adjusted hazard ratio [HR] 2.07 [1.05-3.01], MACE (adjusted HR=3.27 [1.88-5.70] coronary events of new onset (adjusted HR=4.50 [2.26-9.02] but not a higher risk of stroke. The hs-cTnT change over time was also associated with the increased risk of adverse events. The authors concluded that hs-cTnT was associated with subsequent risk for all-cause mortality and major cardiovascular events in a community-based population cohort. Although, several studies have investigated the prognostic value of newly developed high-sensitivity troponin assays in general population, this study may be the first one in Chinese population (at least in literature outside China) and as such the study is reassuring as regards the prognostic value of hs-TnT in various populations. Intuitively, the study findings are accurate. Furthermore, the number of subjects included, length of follow-up and assessment of baseline and longitudinal changes of hs-cTnT concentration in association with outcome are impressive. In my knowledge, the study has an appropriate methodology and has no flaws that can invalidate its main findings. I have some several concerns that I believe are addressable by the authors: 1 The quality of English is less than optimal which is particularly evident in the introduction and discussion sections of the manuscript. Some terms also need correction; e.g., the term “coronary artery insufficiency” is not in use for almost 40 years; the log-rank test is not for the trend, as stated. 2. The authors state to have performed a prospective population-based prospective observational study. However it remains unclear how subjects are recruited for the study. Does it mean that all adults in the district were included in the study? The authors give a list of exclusions but do not mention the inclusion criteria for the study. Please clarify this aspect and offer information on the age range of the subjects. An 11% rate of lost to follow-up is also of concern. 3. Definition of the study outcomes is incomplete. Specifically, definitions of nonfatal myocardial infarction and cardiac death are not provided. Mentioning of Cox analysis under the subheading Defintion of end points is inappropriate, 4. Division of subjects into 4 groups is acceptable. However, groups 2 and 3 (subjects with hs-cTnT between 3 and 14 ng/L) may be divided based on median value of hs-cTnT in this range. 5. Hs-cTnT in population has a nonGaussian distribution. One solution is to use logarithmic scale (which was done by the authors). However, throughout the material all risk estimates (hazard ratios) should be calculated per unit of log hs-cTnT. In each of models, the

	authors have to clarify whether hs-cTnT was entered as raw continuous data or after log transformation. 6. Kaplan-Meier analyses (and curves) are correct but information on KM estimates in each of the hs-cTnT subgroups (for all outcomes) was not provided. Thus I advise the authors to make a table with numbers events (plus KM estimates) for each outcome and for each of the 4 hs-cTnT subgroups. Furthermore, all figures need numbers of subjects at risk for each of the subgroups. 7. Although, hs-cTnT showed an independent association with outcomes of interest, it was not tested whether hs-cTnT improves the discriminatory power of the models for each of the outcomes. Thus I advise the authors to calculate the C statistic of the models before and after inclusion of tropoinin, and compare them to find whether troponin improves the discriminatory power of the models as regards the prediction of the outcome of interest. Other tests such as IDI or NRI may also be used. 8. Data on hs-TnT change (delta hs-cTnT) over the follow-up was not reported. This should be done for each of the subgroups. There may be, however a reverse relationship in which events increased hs-cTnT and not vice versa (reverse causality). Did the authors account for this likelihood?

REVIEWER	Peder Myhre Akershus University Hospital and University of Oslo, Norway
REVIEW RETURNED	24-Nov-2016

GENERAL COMMENTS	Wenkai Xiao et al have explored the prognostic value of cTnT levels in a large population-based study from Beijing, China. The study is carefully conducted and the statistical methods are applied in a satisfactory manner. Still, there are some shortcomings of the manuscript that reduce the clinical impact. Also, there is a need for improved phrasing and grammar, especially the introduction (i.e. the first sentence). I would suggest that a native English speaker reviews the manuscript before submitting the revised version. Major: 1. The authors have explored cTnT in association to end-points: MACE, coronary event, stroke and all-cause mortality. However, development of heart failure or heart failure worsening is not explored as an end point. I think this would be of great interest as the increased levels found in the general population most of all seems related to left ventricular remodelling (cardiac structure) and less related to atherosclerosis. This point should also be more clearly addressed in the discussion, although it is mentioned at the bottom of page 18. Moreover, ECG-recordings of the patients, especially with regards to left ventricular hypertrophy, would be of great interest. If this is not available it should be reported in “limitations”. 2. The multivariate analysis used to adjust for confounders are done well. Still, I miss stratification of the results according to gender, as this has been shown to significantly influence the prognostic value of cTnT (Omland, Clin Chem 2015).

	Also, there has been recent focus on the paradoxical impact of smoking on troponin levels (Lyngbakken, Circulation 2016). Your study is in support of this with a trend towards lower values in the highest quartiles of cTnT. This should be discussed. Also, the results should be discussed in relation to ethnicity. Finally, I would recommend the use of c-statistics in future studies like this to evaluate whether cTnT provides *additional* information to established models. Minor: 1. I miss the coefficient of variation (CV) for the different concentrations of cTnT. Please provide this if available. 2. Table 1: I think it is more appropriate to provide p-values for Group 2-4 compared to Group 1 (reference) instead of using p-value for trend. 3. Page 8, line 3 from the bottom: Please remove “serially” and use incrementally. 4. Alcohol consumption: Do you have information on this, especially in relation to stroke? 5. Page 17, line 13: "It has been proposed that such levels may be physiological, reflecting normal myocardial cell turnover and apoptosis within the senescent heart tissue." I disagree with the statement and also do not think the reference (23) used supports this. To my knowledge, previous studies found no lower limits of cardiac troponins in risk assessment. 6. Reference 14-15 is not reported correctly, and the sentence should as an example be: ”…can be detectable and give information about the plaque characteristics and mortality…” 7. In general I think some of the references used are not the by original studies in the field. i.e. Omland, NEJM 2009 in stable coronary disease and deLemos, JAMA 2010 in the general population. 8. Inconsistent use of cTnT and hs-cTnT. cTnT is preferred, as “hs” refers to an analytical method and not what is measured. Cardiac should also always be used before troponin. 9. Ref # 27 is referred to the authors first name (Christopher) 10. Methods: endocrinical is not a word I have seen before. 11. Page 13, line 4 from the bottom: disclosed is used incorrect.

REVIEWER	Arnold von Eckardstein University of Zurich, Switzerland
REVIEW RETURNED	04-Dec-2016

GENERAL COMMENTS	The study is well done. However, there is already a considerable

	number of publications on the prognostic values of cardiac troponins in healthy populations. The first one was the Dallas City Heart study. Other examples are MAlmö Study, Rotterdam Study, EPIC studies. The results are hence of limited novelty. The discussion does not reflect this situation and should be adapted accordingly. The special aspect may be the investigation of a Chinese population

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
This is well-designed and reported study. I have only two suggestions:
- First, the authors state that 181 participants were lost to follow up and excluded from the analysis. If baseline data on these subjects is available, this should be compared to the included patients and reported in the paper, to show if any significant differences existed.
Response: I have compared the baseline data between the included and lost subjects, please see supplementary Table1. Compared with the included participants, the lost to follow up group were younger, had lower postprandial blood glucose, eGFR and NT-proBNP levels. Significant differences were not presented in other characteristics.
- Second, minor revision is needed to address some grammar/style issues.
Response: Thanks for your professional suggestion. I have rewritten some sentences to improve the clarity and accuracy.

Reviewer: 2
1. Excellent written paper by Xiao et al.
Response: Thanks.
2. The association between hs-cTnT and outcomes which includes mortality, CHD, HF and brain disease has been previously shown in multiple populations of healthy individuals (eg ARIC study publications) therefore commenting on how these results add to already existing publications would definitely strengthen the manuscript.
Response: Just as what you mentioned, though the association between hs-cTnT and outcomes has been previously shown in multiple populations, our study extends the prognostic implications to Asians. I have commented the significance of this manuscript in discussion section.
3. hs-cTnT has been shown to be associated with subclinical brain disease in ARIC population (study not cited here) which shows that there may be other mechanisms that may explain the association between hs-cTnT and brain disease.
Response: Just as what you mentioned, elevated plasma cTnT concentrations are associated with increased risk of cardioembolic and other nonlacunar ischemic but not with hemorrhagic strokes in ARIC population. While in our study hemorrhagic stroke accounts for more than one half of all strokes, I have analyzed the potential mechanisms of this difference.
4. Most manuscripts divided the cohort in quartiles of hs-cTnT. Commenting on why these cutoffs were chosen would improve the manuscript.
Response: Thanks for your professional suggestion. Unlike other biomarkers, a substantial portion of hs-cTnT can not be detected in the general population, it was only detectable in 54.7% of our enrollment. what value are assigned in these undetectable population are controversial. Thus, for the categorical analyses, the 54.7% with undetectable levels were the reference group (group 1). With respect to elevated troponin (group 4), using the definition of ‘‘elevated’’ being, exceeds the 99th percentile value of a healthy reference population, for the hs-cTnT assay this threshold is specified by Roche to be 14ng/L. The remaining were split into two groups based on the median value of hs-cTnT in this range. To my knowledge, most manuscripts have taken this classification method.
Reviewer: 3
1 The quality of English is less than optimal which is particularly evident in the introduction and discussion sections of the manuscript. Some terms also need correction; e.g., the term “coronary artery insufficiency” is not in use for almost 40 years; the log-rank test is not for the trend, as stated.

Response: I am very sorry for my imprecise English expression. I have rewritten and corrected some terms and sentences. Moreover, the revision has been edited by native English-speaking experts, I hope it could express my real intention more clearly and professionally.
2. The authors state to have performed a prospective population-based prospective observational study. However it remains unclear how subjects are recruited for the study. Does it mean that all adults in the district were included in the study? The authors give a list of exclusions but do not mention the inclusion criteria for the study. Please clarify this aspect and offer information on the age range of the subjects. An 11% rate of lost to follow-up is also of concern.
Response: Thanks for your professional suggestion. I have supplied more details on recruitment of study population in the methods section. Please see the revision about the full details of the inclusion criteria and age range of the subjects
3. Definition of the study outcomes is incomplete. Specifically, definitions of nonfatal myocardial infarction and cardiac death are not provided. Mentioning of Cox analysis under the subheading Defintion of end points is inappropriate,
Response: According to your advice, I have provided the definitions of nonfatal myocardial infarction and cardiac death in revision. Also the definition of survival time has been adjusted position.
4. Division of subjects into 4 groups is acceptable. However, groups 2 and 3 (subjects with hs-cTnT between 3 and 14 ng/L) may be divided based on median value of hs-cTnT in this range.
Response: My inappropriate expression (two equal-sized groups) leads to this ambiguity. In fact the remaining subjects were split into two groups based on the median value of hs-cTnT between 3 and 14 ng/L. I have corrected the expression in revision.
5. Hs-cTnT in population has a nonGaussian distribution. One solution is to use logarithmic scale (which was done by the authors). However, throughout the material all risk estimates (hazard ratios) should be calculated per unit of log hs-cTnT. In each of models, the authors have to clarify whether hs-cTnT was entered as raw continuous data or after log transformation.
Response: In this manuscript we modeled hs-cTnT as both a categorical and a continuous variable. For the analyses of hs-cTnT as a categorical variable, we divided participants into 4 categories: those with undetectable hs-cTnT were placed in the first category as the reference group.
When hs-cTnT was regarded as a continuous variable, we analyzed the relationship between baseline and change in hs-cTnT levels with end points. In this analysis, the hs-cTnT levels were natural logarithm transformed and hazard ratios were calculated as per unit of log hs-cTnT increment.
6. Kaplan-Meier analyses (and curves) are correct but information on KM estimates in each of the hs-cTnT subgroups (for all outcomes) was not provided. Thus I advise the authors to make a table with numbers events (plus KM estimates) for each outcome and for each of the 4 hs-cTnT subgroups. Furthermore, all figures need numbers of subjects at risk for each of the subgroups.
Response: The event numbers and event rate for each outcome across the 4 hs-cTnT subgroups had been provided in Table2, please see Table2. According to your advice, I have added the numbers of subjects at risk for each of the subgroups in Kaplan-Meier curves. For detailed information, see Figure1 in revision.
7. Although, hs-cTnT showed an independent association with outcomes of interest, it was not tested whether hs-cTnT improves the discriminatory power of the models for each of the outcomes. Thus I advise the authors to calculate the C statistic of the models before and after inclusion of tropoinin, and compare them to find whether troponin improves the discriminatory power of the models as regards the prediction of the outcome of interest. Other tests such as IDI or NRI may also be used.
Response: Thanks for your professional suggestion. According to your advice, I have added the C statistic in revision (Table4). The area under the receiver operating characteristic curve summarized the diagnostic discrimination. The result shows that addition of troponin T levels to clinical variables led to significant increases in risk prediction with significant improvement of the c-statistic.
8. Data on hs-TnT change (delta hs-cTnT) over the follow-up was not reported. This should be done for each of the subgroups. There may be, however a reverse relationship in which events increased hs-cTnT and not vice versa (reverse causality). Did the authors account for this likelihood?
Response: Thanks for your professional suggestion. Because in each of the subgroups, the hs-TnT

may increase, decrease or unchanged, so we didn't analyzed the kinetic changes of hs-TnT for each of the subgroups. In view of the markedly skewed distribution of hs-cTnT, changes in concentrations over time were calculated as the difference between the natural logarithm of the concentrations at follow-up and at baseline.

Reviewer: 4

Wenkai Xiao et al have explored the prognostic value of cTnT levels in a large population based study from Beijing, China. The study is carefully conducted and the statistical methods are applied in a satisfactory manner. Still, there are some shortcomings of the manuscript that reduce the clinical impact. Also, there is a need for improved phrasing and grammar,
especially the introduction (i.e. the first sentence). I would suggest that a native English speaker reviews the manuscript before submitting the revised version.

Major:

1. The authors have explored cTnT in association to end-points: MACE, coronary event, stroke and all-cause mortality. However, development of heart failure or heart failure worsening is not explored as an end point. I think this would be of great interest as the increased levels found in the general population most of all seems related to left ventricular remodelling (cardiac structure) and less related to atherosclerosis. This point should also be more clearly addressed in the discussion, although it is mentioned at the bottom of page 18. Moreover, ECG-recordings of the patients, especially with regards to left ventricular hypertrophy, would be of great interest. If this is not available it should be reported in "limitations".

Response: Just as what you mentioned, heart failure is an important end point in the general population, the association between hs-cTnT and heart failure development has been previously shown in multiple populations. Unfortunately, due to ECG and echocardiogram were not available in our community survey, we failed to investigate the incidence of heart failure. I have discussed this aspect in "limitations".

2. The multivariate analysis used to adjust for confounders are done well. Still, I miss stratification of the results according to gender, as this has been shown to significantly influence the prognostic value of cTnT (Omland, Clin Chem 2015). Also, there has been recent focus on the paradoxical impact of smoking on troponin levels (Lyngbakken, Circulation 2016). Your study is in support of this with a trend towards lower values in the highest quartiles of cTnT. This should be discussed. Also, the results should be discussed in relation to ethnicity.

Response: Thanks for your professional suggestion. First, associations of cTnT level with all-cause mortality and major adverse cardiovascular event were consistent in stratification analysis defined by sex in our study. Second, our study population was restricted to Chinese Han origin inhabitant. Therefore, extrapolation of our results to other ethnic groups should be done with caution. I have discussed this in "limitations".

3. Finally, I would recommend the use of c-statistics in future studies like this to evaluate whether cTnT provides additional information to established models.

Response: According to your advice, I have added the C statistic in revised version (Table4). The result shows that addition of troponin T levels to clinical variables led to significant increases in risk prediction with significant improvement of the c-statistic.

Minor:

1. I miss the coefficient of variation (CV) for the different concentrations of cTnT. Please provide this if available.

Response: Sorry, the coefficient of variation for the different concentrations of cTnT is not available. Some data have reported that hs-cTnT by Roche have an interassay coefficient of variation of 8% at 10 pg/mL and 2.5% at 100 pg/mL.

2. Table 1: I think it is more appropriate to provide p-values for Group 2-4 compared to Group 1 (reference) instead of using p-value for trend.

Response: Thanks for your suggestion. We refer to some similar articles, they generally use the p-value for trend in the comparison of clinical variables across cTnT categories.

3. Page 8, line 3 from the bottom: Please remove "serially" and use incrementally.

Response: According to your advice, I have corrected it in revision.
4. Alcohol consumption: Do you have information on this, especially in relation to stroke?
Response: Although we collected information on alcohol consumption at baseline, we found that there were significantly different in alcohol category, alcohol content for each drinkers. So we think it is difficult to evaluate the effect of alcohol objectively.
5. Page 17, line 13: "It has been proposed that such levels may be physiological, reflecting normal myocardial cell turnover and apoptosis within the senescent heart tissue." I disagree with the statement and also do not think the reference (23) used supports this. To my knowledge, previous studies found no lower limits of cardiac troponins in risk assessment.
Response: Thanks for your suggestion. The risk assessment of hs-cTnT in apparently healthy individuals are not presented in all detectable levels, minimally detectable cTnT levels were not significantly associated with long-term outcomes. Three large cohort studies in general population have drawn the similar conclusions. In the Atherosclerosis Risk in Communities (ARIC) Study, only cTnT values in the highest category (≥14 pg/mL) were associated with incident CHD events. In the Cardiovascular Health Study (CHS), the detectable cTnT values (3.00–5.44 pg/mL) were not associated with heart failure events. Also in the Dallas Heart Study (DHS), only cTnT in the fourth category (6.6-<14 pg/mL) and fifth category (≥14 pg/mL) remained independently associated with all-cause mortality in the fully adjusted models.
6. Reference 14-15 is not reported correctly, and the sentence should as an example be: ”…can be detectable and give information about the plaque characteristics and mortality…”
Response: According to your advice, I have corrected it in revision.
7. In general I think some of the references used are not the by original studies in the field. i.e. Omland, NEJM 2009 in stable coronary disease and deLemos, JAMA 2010 in the general population.
Response: According to your advice, I have corrected it in revision.
8. Inconsistent use of cTnT and hs-cTnT. cTnT is preferred, as “hs” refers to an analytical method and not what is measured. Cardiac should also always be used before troponin.
Response: I am very sorry for my inaccurate English expression, which leads to the confusing. According to your advice, I have corrected it in revision.
9. Ref # 27 is referred to the authors first name (Christopher)
Response: According to your advice, I have corrected the name of author to “DeFilippi”.
10. Methods: endocrinical is not a word I have seen before.
Response: I have revised it in the methods section.
11. Page 13, line 4 from the bottom: disclosed is used incorrect.
Response: I have revised the word to “found”.
Reviewer: 5
The study is well done. However, there is already a considerable number of publications on the prognostic values of cardiac troponins in healthy populations. The first one was the Dallas City Heart study. Other examples are MAlmö Study, Rotterdam Study, EPIC studies. The results are hence of limited novelty. The discussion does not reflect this situation and should be adapted accordingly. The special aspect may be the investigation of a Chinese population
Response: Just as what you mentioned, the association between hs-cTnT and outcomes has been previously shown in multiple populations, our study extends the prognostic implications to Chinese population. I have commented this situation and discussed the meaning of this manuscript in the discussion section.

VERSION 2 – REVIEW

REVIEWER	Zhivko Zhelev University of Exeter Medical School, UK
REVIEW RETURNED	20-Jan-2017

GENERAL COMMENTS	As far as I can see, all issues identified in the previous version have

	been addressed.

REVIEWER	Razvan Dadu, MD Baylor College of Medicine, Houston, TX, USA
REVIEW RETURNED	11-Jan-2017

GENERAL COMMENTS	The authors have responded adequately to reviewers comments.

REVIEWER	Peder Langeland Myhre Akershus University Hospital and University of Oslo, Oslo, Norway
REVIEW RETURNED	13-Jan-2017

GENERAL COMMENTS	The revised manuscript by Xiao et al has improved substantially and all my comments have been adequately answered. I thank the authors for this. Just a few more comments regarding the definition of end points. I suggest to change "ischemic heart disease" to "newly diagnosed coronary heart disease". Also, I don't understand the sentence: "...or mortality from other atherosclerotic and CVD including HF".

VERSION 2 – AUTHOR RESPONSE

Reviewer: 4

- The revised manuscript by Xiao et al has improved substantially and all my comments have been adequately answered. I thank the authors for this. Just a few more comments regarding the definition of end points. I suggest to change "ischemic heart disease" to "newly diagnosed coronary heart disease". Also, I don't understand the sentence: "...or mortality from other atherosclerotic and CVD including HF"..

Response: Thanks for your professional suggestion. My inappropriate expression leads to this ambiguity. I have revised the definition of end points in the revision according to your suggestion.