PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Role of HDL cholesterol in anthracycline-induced subclinical cardiotoxicity: a prospective observational study in patients with diffuse large B-cell lymphoma treated with R-CHOP
AUTHORS	Ou, Wenxin; Jiang, Tiantian; Zhang, Nan; Lu, Kai; Weng, Yue; Zhou, Xi; Wang, Dong; Dong, Qian; Tang, Xiaoqiong

VERSION 1 – REVIEW

REVIEWER	Furuhashi, Masato
REVIEW RETURNED	15-Jul-2023

GENERAL COMMENTS	The authors addressed all issues which this reviewer raised [when reviewing for a previous journal].

REVIEWER	Xiao, Qingzhong QMUL
REVIEW RETURNED	11-Sep-2023

GENERAL COMMENTS	In this observational prospective study, Ou and colleagues reported that a high level of HDL-C may be associated with a lower risk of AISC in DLBCL patients treated with R-CHOP, and suggested HDL-C could be a cardio-protective target. This study is interesting and clinical relevant. The authors have also adequately addressed almost all the concerns raised by the previous two reviewers, which significantly improved the quality of this study as described in the revised manuscript. I only have several minor suggestions as detailed below for the authors to further improve their manuscript prior to publication. 1. The authors reported HR values in abstract, but the corresponding 95% confidence intervals are missing. Please provide them in abstract. 2. The authors briefly mentioned that there is a significant increase in CVD prevalence and mortality in cancer patients. However, it is well-known that the relationship between CVD and cancer is not one-way system, and growing epidemiologic data revealed a high cancer risk in CVD patients such as Takotsubo cardiomyopathy (PMID: 28318661, PMID: 29722868, PMID: 31311438). It would be more informative if the authors also discuss such bidirectional links between cancers and CVDs in the 'Introduction' or 'Discussion'. 3. Did the authors regularly perform ECG examination on patients? Please mention it. 4. There are some overstated sentences that should be modified to reflect the current study. For instance, page 16, line 361-363: 'Although there was no statistically significant difference, it appears that patients with pre-existing hypertension were less

	likely to experience AISC.' Since only four patients with pre-existing hypertension were reported in this study, it is inappropriate for the authors to draw the abovementioned assumption. 5. Moreover, it appears that there are two duplicated sentences on same page (line 367-370). 6. Full name(s) should be given for any aberrations when used for the first time, for instance R-CHOP, BMI and E, HR within abstract.

REVIEWER	Jorgensen, Andrea University of Liverpool, Health Data Science
REVIEW RETURNED	16-Oct-2023

GENERAL COMMENTS	1. It is not necessary to state 'both sex' as an inclustion criteria, since it does not narrow down the target population in any way. 2. It is unnecessary to state 'None of the patients in our study received lipid-lowering treatment' since this was part of the exclusion criteria. 3. Please clarify how you are defining the timepoint 'baseline'. 4. How are you defining the variable 'diabetes mellitus' ? Is it a binary variable ? 5. How are you defining the variable 'hypertension' ? 6. How are smoking and drinking history assessed and represented in the analysis ? 7. What is justification for dichotomising HDL ? As it is a continuous measure, information is lost when it is dichotomised. I would recommend that the analysis is repeated with HDL-C as a continuous variables. 8. What is justification for the two different multivariable Cox regression models fitted ? What hypotheses are being tested when fitting these ? 9. You mention using 'Bonferroni correction' but then state the p-value threshold for statistical significantce as being 0.05 - which is a contradiction. What was your Bonferroni corrected p-value threshold ? 10. In the statistical analysis methods section you mention calculating the cutoff point of ApoA1. What is purpose of this, and how does it feed into the analysis ? How does it fit into the hypotheses being tested ? 11. I am confused by the attention paid to ApoA1 in the results section. The introduction and methods sections allude to HDL-C as being the covariate of interest, so therefore please justify this additional focus on ApoA1 in the results section. 12. You state that in the survival analysis, 24 patients experienced AISC during follow up whilst 10 did not. You then go on to state that 36 were censored. In a survivial analysis, those not experiencing the event of interest (in this case AISC) during follow-up are usually censored. Please clarify why the 10 without the outcome were not censored here ? 13. Please ensure that you adhere to the reporting guidelines for observational studies, and attach the completed reporting checklist as an appendix.

VERSION 1 – AUTHOR RESPONSE

Responses to Reviewer #1

Comments to the Author

The authors addressed all issues which this reviewer raised [when reviewing for a previous journal]

Answer:

Thank you for taking the time to review our revised manuscript. We appreciate your approval of our responses.

Responses to Reviewer #2

Comments to the Author

In this observational prospective study, Ou and colleagues reported that a high level of HDL-C may be associated with a lower risk of AISC in DLBCL patients treated with R-CHOP, and suggested HDL-C could be a cardio-protective target. This study is interesting and clinical relevant. The authors have also adequately addressed almost all the concerns raised by the previous two reviewers, which significantly improved the quality of this study as described in the revised manuscript. I only have several minor suggestions as detailed below for the authors to further improve their manuscript prior to publication.

Comments:

1. The authors reported HR values in abstract, but the corresponding 95% confidence intervals are missing. Please provide them in abstract.

Answer:

It's very kind of you to point out our oversight. We have included the 95% CIs for the HRs in the Abstract as suggested.

2. The authors briefly mentioned that there is a significant increase in CVD prevalence and mortality in cancer patients. However, it is well-known that the relationship between CVD and cancer is not one-way system, and growing epidemiologic data revealed a high cancer risk in CVD patients such as Takotsubo cardiomyopathy (PMID: 28318661, PMID: 29722868, PMID: 31311438). It would be more informative if the authors also discuss such bidirectional links between cancers and CVDs in the 'Introduction' or 'Discussion'.

Answer:

We appreciate the reviewer's insightful feedback. It's very kind of you to point out this kind of cardiomyopathy, Takotsubo cardiomyopathy (TTS), and provide us with some relevant references. TTS represents an acute heart failure condition, which can occur in the setting of severe psychological or

physical stress, and the typical clinical presentation is chest pain or dyspnea(1). TTS can be diagnosed by electrocardiogram (ECG), plasma cardiac troponin, and echocardiography. The ECG commonly demonstrates acute dynamic changes at presentation, resembling those of acute coronary syndrome(2). There is strong association of the occurrence of TTS and malignant diseases, and the diagnosis of malignant disease was timely closely related to the occurrence of TTS(3, 4).

Bidirectional links between cancer and cardiovascular disease (CVD) refer to the complex relationship where the presence of one condition can increase the risk of the other, and vice versa. These connections can be attributed to various shared risk factors, biological mechanisms, and treatment-related effects. Many risk factors for cancer and CVD overlap. Lifestyle factors like smoking, poor diet, physical inactivity, and obesity can increase the risk of both cancer and CVD(5). Chronic inflammation plays a crucial role in the development of both cancer and cardiovascular diseases(6). Certain cancer treatments like anthracyclines can lead to cardiovascular complications(7). And there are some interactions between some CVD and cancers, such as TTS(3, 4).

However, it's important to note that the primary focus of this article is to explore the cardiovascular effects of chemotherapy drugs on cancer patients. While discussing these other factors would undoubtedly enrich the article, it could potentially complicate the overall structure and focus of the manuscript. Therefore, we have to express our apologize to the reviewer that we decide not to discuss the bidirectional link between cancers and cardiovascular diseases in this article.

3. Did the authors regularly perform ECG examination on patients? Please mention it.

Answer:

We appreciate your comments. As elaborated in the inclusion and exclusion criteria, patients with normal LVEF and without severe cardiac disease were enrolled in our study. Every patient had a normal ECG at baseline, and every patient received ECG examination before every cycle of chemotherapy to ensure the safety of the treatment. We have mentioned this in the **Method** section as suggested.

Anthracycline-induced cardiotoxicity includes acute events, such as ECG changes, arrythmias, and pericarditis and myocarditis syndromes, as well as chronic conditions, such as systolic and diastolic left ventricular dysfunction(8). However, the happening of early acute cardiotoxicity is rare(9, 10). It was reported that cardiotoxicity and heart remodeling would precede conduction abnormality and arrythmias, so the value of repeated ECG testing in detecting cardiotoxicity may be limited(11). And a systematic review has reported that multiple ECG abnormalities were described in cardiotoxic treatment cancer patients in several studies, but all ECG abnormalities were found after a long-term follow-up (≥2 years)(12). So, in our study, we didn't focus on the changes of ECG. In fact, the relationship between ECG and early stage of cardiotoxicity is rarely investigated, and we will pay more attention to it in our further research, and maybe ECG can be a potential method to detect AISC.

4. There are some overstated sentences that should be modified to reflect the current study. For instance, page 16, line 361-363: 'Although there was no statistically significant difference, it appears that patients with pre-existing hypertension were less likely to experience AISC.' Since only four patients with pre-existing hypertension were reported in this study, it is inappropriate for the authors to draw the abovementioned assumption.

Answer:

We thank you for your valuable comments very much. We agree with you that this sentence was overstated. Accordingly, we have promptly revised the relevant section to accurately reflect the current study's findings.

5. Moreover, it appears that there are two duplicated sentences on same page (line 367-370).

Answer:

Thank you for your carefully review. We have amended relative part of this paragraph.

6. Full name(s) should be given for any aberrations when used for the first time, for instance R-CHOP, BMI and E, HR within abstract.

Answer:

Thank you for your comment regarding the full names of abbreviation. We have revised the full names of R-CHOP and HR in the Abstract as suggested. But due to the limitation word number of Abstract, we removed some words including BMI and E as necessary. Furthermore, we checked the full text again to avoid such mistakes. Your attention to detail is greatly appreciated and has undoubtedly improved the clarity of our work.

Responses to Reviewer #3

Comments to the Author

1. It is not necessary to state 'both sex' as an inclusion criteria, since it does not narrow down the target population in any way.

Answer:

Thank you for your feedback. We appreciate your clarification regarding the inclusion criteria 'both sex'. We have removed 'both sex (male and female)' from the inclusion criteria as suggested.

2. It is unnecessary to state 'None of the patients in our study received lipid-lowering treatment' since this was part of the exclusion criteria.

Answer:

Thank you for your comments. This sentence is indeed redundant since it was explicitly part of the exclusion criteria. We have removed this sentence as suggested.

3. Please clarify how you are defining the timepoint 'baseline'.

Answer:

Thank you for your inquiry regarding the definition of the timepoint 'baseline' in our study. In our study design, 'baseline' refers to the initial assessment conducted before the commencement of the first cycle of chemotherapy. We have elucidated the definition of 'baseline' in the **Method** section. We appreciate your request for clarification, and we hope this explanation provides the necessary context.

4. How are you defining the variable 'diabetes mellitus'? Is it a binary variable?

Answer:

Thank you for your question. Yes, whether a patient has diabetes mellitus is indeed a binary variable. In this study, the determination of patients' diabetes status was determined based on whether they had been formally diagnosed with diabetes by a specialist prior to commencing chemotherapy.

5. How are you defining the variable 'hypertension'?

Answer:

Thank you for your review. Hypertension is also a binary variable in our study. Patients' hypertension status was determined based on whether they have a confirmed diagnosis of hypertension from a specialist physician prior to commencing chemotherapy.

6. How are smoking and drinking history assessed and represented in the analysis?

Answer:

We would like to sincerely thank the reviewer for the carefully review. According to SNOMED CT standard, smoking history is defined as an adult who has smoked at least 100 cigarettes in their lifetime. According to the lifetime drinking history (LDH) questionnaire, drinking history is defined as an adult who has consumed more than 20 drinks in lifetime, with each drink is considered to have an average alcohol content of 12 g. The definition of smoking and drinking history have been added in **Methods** section.

7. What is justification for dichotomising HDL? As it is a continuous measure, information is lost when it is dichotomised. I would recommend that the analysis is repeated with HDL-C as a continuous variable.

Answer:

We greatly appreciate the valuable input from the reviewer regarding the statistical methodology. We acknowledge the concern that dichotomizing a continuous variable can result in information loss.

In our analysis, we initially considered the option of using HDL-C as a continuous variable for the Cox regression analysis. However, during the preliminary linear correlation analysis, we observed that HDL-C did not exhibit a linear relationship with the outcome event. This non-linearity violates a fundamental assumption for Cox regression analysis, making it unsuitable for direct Cox regression analysis.

Moreover, we carefully weighed the interpretation of using HDL-C as a continuous variable in our model. When introduced as a continuous variable, its interpretation would be the change in the outcome for

every one-unit increase in the independent variable. In some instances, this change might be so minor that it lacks clinical significance and practical utility.

In light of these considerations, we made the decision to transform HDL-C into a dichotomous variable for our regression analysis. We believe it enhances the clinical relevance and practical applicability of the results.

We hope our reply can provide some clarity about the statistical methodology.

8. What is justification for the two different multivariable Cox regression models fitted? What hypotheses are being tested when fitting these?

Answer:

We appreciate the reviewer's question regarding the use of two different multivariable Cox regression models in our study. The rationale for fitting these models is to investigate the influence of a range of covariates on the outcome of interest, providing a more comprehensive understanding of the relationships at play.

Model 1 includes HDL-C group, age, and gender as covariates. The primary hypothesis being tested with this model is whether HDL-C, after adjusting for age and gender, is independently associated with the outcome event AISC. This model helps us assess the specific impact of HDL-C group while controlling for the potential effects of age and gender on the outcome.

Model 2 extends the analysis by incorporating additional variables that are potentially related to the outcome event, which has a P-value <0.15 in univariable Cox regression analysis, including age and gender. In this model, we aim to explore a broader set of potential predictors and assess their combined effects. The hypothesis tested in this case is whether, when considering a wider range of covariates, HDL-C still retains its significance as a predictor of the outcome, and how it interacts with other variables in the model.

We appreciate the reviewer's interest in our methodology.

9. You mention using 'Bonferroni correction' but then state the p-value threshold for statistical significance as being 0.05 - which is a contradiction. What was your Bonferroni corrected p-value threshold?

Answer:

We apologize for any confusion caused by our previous statement regarding the use of Bonferroni correction. Upon thorough review of our data and analysis, we have realized that we did not employ Bonferroni adjustment in our study.

In Table S1 and Table S3, particularly for the ECoG performance status, where we had three groups, we utilized Fisher's exact test for the analysis. The mention of Bonferroni correction was indeed superfluous, and we have removed this extraneous information from the manuscript.

We appreciate the reviewer's careful evaluation of our work and their valuable feedback.

10. In the statistical analysis methods section you mention calculating the cutoff point of ApoA1. What is purpose of this, and how does it feed into the analysis? How does it fit into the hypotheses being tested?

Answer:

We appreciate the reviewer's question and would like to clarify the purpose of calculating the cutoff point for ApoA1 in our study.

In this research, we initially considered using the cut-off point of ApoA1 to categorize patients into high and low ApoA1 groups. The intention behind this was to analyze the association between ApoA1 levels with AISC, aligning the grouping approach with that of HDL-C for consistency in our analytical methods.

However, as pointed out in the comment #11 from the reviewer, we acknowledge that introducing ApoA1 analysis would introduce complexity and potentially shift the focus away from our primary research objective, which is to investigate the relationship between HDL-C and AISC. To maintain clarity and maintain the primary focus on HDL-C, we have made the decision to remove the analysis related to ApoA1 from the manuscript.

11. I am confused by the attention paid to ApoA1 in the results section. The introduction and methods sections allude to HDL-C as being the covariate of interest, so therefore please justify this additional focus on ApoA1 in the results section.

Answer:

We appreciate the reviewer's input and agree that there was some inconsistency in the focus of our analysis. This manuscript indeed emphasizes HDL-C as the primary covariate of interest, and we acknowledge that the additional attention to ApoA1 in the results section may have caused confusion. In light of the reviewer's feedback and to maintain the clarity and coherence of our study, we have removed the additional analysis of ApoA1 from the results section. The reasons for this decision are as follows:

1) Our study's sample size and design were primarily calculated based on the relationship between HDL-C and AISC. Thus, the sample size and study design were not optimized for investigating ApoA1. 2) Furthermore, there was no statistically significant difference between AISC and ApoA1 at the primary analysis, and there was insufficient evidence to support this aspect of the analysis.

We want to clarify that the adjustments made to our manuscript do not alter the core emphasis of our study, and the removal of the ApoA1 analysis was done to enhance the overall coherence of our work.

We sincerely thank the reviewer for their constructive feedback, which has contributed to the refinement of our manuscript.

12. You state that in the survival analysis, 24 patients experienced AISC during follow up whilst 10 did not. You then go on to state that 36 were censored. In a survival analysis, those not experiencing the event of interest (in this case AISC) during follow-up are usually censored. Please clarify why the 10 without the outcome were not censored here?

Answer:

We appreciate the reviewer's feedback. We would like to clarify that 36 patients were lost of follow-up, and those 10 patients who did not experience the AISC during the follow-up period were indeed treated

as "censored" in the survival analysis. We have corrected this kind of word in relative part of the manuscript. We apologize for any confusion and appreciate the reviewer's comments.

13. Please ensure that you adhere to the reporting guidelines for observational studies, and attach the completed reporting checklist.

Answer:

Thank you for your comment. We have thoroughly reviewed our manuscript and are pleased to confirm that we have followed the recommended reporting guidelines for observational studies. We will attach a completed STROBE checklist as a separate document to ensure transparency and clarity in our reporting when we re-submit our manuscript.

Finally, we thank the reviewers again for the thoughtful and constructive suggestions and comments. We hope our reply can provide some clarity and clear up any doubts.

Reference

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VERSION 2 – REVIEW

REVIEWER	Xiao, Qingzhong QMUL
REVIEW RETURNED	03-Nov-2023

GENERAL COMMENTS	My comments have been fully addressed by the authors. Well-done!

VERSION 2 – AUTHOR RESPONSE