Author’s response to reviews

Title: Plasma fibrinogen and mortality in patients undergoing peritoneal dialysis: a prospective cohort study

Authors:

Jing Yu (yujing19910320@163.com)
Tong Lin (lintong323@163.com)
Naya Huang (huangnaya@163.com)
Xi Xia (xiaxi1990@126.com)
Jianbo Li (edmend2010@163.com)
Yagui Qiu (250558219@qq.com)
Xiao Yang (yangxsysu@126.com)
Haiping Mao (haipingmao@126.com)
Fengxian Huang (hfxyl@163.net)

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Author’s response to reviews:

Acting Editor-in-Chief
BMC Nephrology

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Re: Manuscript BNEP-D-19-00833

Dear Acting Editor,

Thank you for your review of our entitled “High plasma fibrinogen and mortality in patients undergoing peritoneal dialysis: a prospective cohort study”. We appreciate your comments and suggestions and those of the other reviewers.

Indicated below in red font are responses to all your comments. We believe the revised manuscript and supplement address your concerns and are hopeful that you will find the article of sufficient interest to the readership of the BMC Nephrology.
Please let me know if you have any questions or if we can be of any further assistance.

Sincerely,

Fengxian Huang, MD, PhD,
Department of Nephrology, The First Affiliated Hospital, Sun Yat-sen University,
No.58, Zhongshan Road Ⅱ, Guangzhou 510080, China.
Email: hfxyl@163.net

-------------------------------1. COMMENTS FROM EDITOR-------------------------------
Editor:
Comments to the author:
It is important that your manuscript gives a clear and complete account of your study, and BMC supports reporting initiatives that contribute to this. Please adhere to the appropriate STROBE guideline for your methodology, and include a completed checklist with your revision as a supplementary file. You can see more information here: https://www.biomedcentral.com/getpublished/writing-resources/reporting-guidelines.

The quality of the English used throughout your manuscript does not currently meet our requirements, as there are several incorrect sentence constructions and grammatical errors throughout obscuring the message the authors want to convey. We recommend that you ask a native English speaking colleague to help you copy-edit the paper.

We operate a policy of open peer review for this journal, which means that you will be able to see the names of the reviewers who provided the reports via the online peer review system. We encourage you to also view the reports there, via the action links on the left-hand side of the page, to see the names of the reviewers.

Reply: We thank the editor for these comments. We’ve revised the manuscript and adhere to the STROBE guideline for our methodology. We’ve uploaded a completed checklist with our revision as a supplementary file.
On the other hand, we’ve asked American Journal Experts (http://bit.ly/AJE_BS) to help us re-edit the manuscript. We hope the manuscript is improved sufficiently and now is ready for publication.
Reviewer comments

Reviewer: 1
General comments
The authors describe the association between plasma fibrinogen levels and both cardiovascular and all-cause mortality in a large population of peritoneal dialysis patients. Not much is known on this topic and the paper is therefore of interest. The study population is very large considering that it is a single center study.
The study is on an etiological research question, i.e. whether fibrinogen is causally related to (cardiovascular) mortality. Therefore, terminology like ‘predictor’ and ‘prognostic’ should be avoided as it suggests a prediction study.

Reply: We thank the reviewer for these comments. We’ve revised the manuscript using terminology ‘association’ or ‘relationship’ instead of ‘predictor’ or ‘prognostic’ to describe the subject and addressed your comments below.

Specific comments

Introduction
Most of the cited literature is quite old, also the more general literature on CKD and fibrinogen, Please check for more recent papers (e.g. Gackler et al, 2019, Schuett et al, 2017 and Brophy et al 2013).

Reply: We thank the reviewer for the suggestions and read the literatures provided by the reviewer carefully. We’ve updated some of the introduction according to the referred literatures. Thanks.

Methods
Participants
It remains unclear whether the study involves incident or prevalent patients. In the section on data collection it is mentioned that all data (including fibrinogen) is collected before PD characterization. However, in the discussion it is mentioned that prevalent patients were included. If I understood correctly incident PD patients were included with measurements available before the start of PD. Follow-up time only starts at the time patients received PD for 3 months (and therefore patients that died before were excluded). Is this correct? Please make this more clear throughout the paper. In the case I misunderstood and it involves also patients that were treated with PD for longer periods, information should be provided on dialysis vintage.

Reply: Unfortunately we did not clarify this issue in the data collection section. We have revised the participant and data collection sections of the manuscript. The study involves only incident patients who started maintenance PD in our center. Therefore, patients with PD less than three months were not included in this study. Baseline demographic data, including age, sex, smoking, a history of cardiovascular events, diabetes, and hypertension, were collected at the initiation of PD therapy. Clinical data, including BMI, blood pressure, and medication use, and biochemical
data, were collected three months after PD therapy initiation. We’ve re-collected the clinical and biochemical data.

Statistical analyses
-Fibrinogen is treated both as a categorical exposure variable and continuously. Please mention this clearly in the statistics section.

Reply: Thanks for this comment. We’ve revised the manuscript and explained in detail in the statistics section.

- The study population is large and I would therefore suggest to divide the study population in three groups based on fibrinogen levels. This would provide more insight into the dose-response association.

Reply: Thanks for this suggestion. We’ve divided the study population into quartiles according to plasma fibrinogen levels and re-analyzed the data. We found that the relationship between plasma fibrinogen and CV and all-cause mortality was nonlinear and an elevated plasma fibrinogen level was significantly associated with an increased risk of CV and all-cause mortality.

- In the statistics section it is only mentioned which P value was considered for interactions. It is however not clear which variables were tested in this respect. A significant interaction was found with diabetes. But were other characteristics tested as well (and turned out to be non-significant)? There should be a clear rationale to perform these kind of analyses and it should be made clear if these analyses were pre-specified.

Reply: Thanks for the questions and comments. In fact, we treated fibrinogen as a continuous exposure, we first conducted a simple linear regression to explore which are the associated factors of fibrinogen levels. The results are as follows. We found that diabetes was the strongest associated factor of fibrinogen (B = 0.79), whereas it attenuated the significance in the model of fibrinogen and CV and all-cause mortality most. Therefore, we performed interaction between diabetes and fibrinogen. Meanwhile, we performed interaction between antiplatelet agent usage, lipid-lowering drug usage, a history of CV events and fibrinogen (B values were 0.64, 0.63, and 0.60, respectively), but we only found a significant interaction between diabetes and fibrinogen. The P values of the interaction between diabetes, antiplatelet agent usage, lipid-lowering drug usage, a history of CV events and fibrinogen were 0.03, 0.22, 0.20, and 0.63, respectively. We re-collected and re-analyzed the data based on the opinions of the two reviewers, and further found that the relationship between fibrinogen and CV and all-cause mortality was nonlinear. It is not rational to treat fibrinogen as a continuous exposure for Cox regression analysis. Therefore, in the revised manuscript, we did not conduct interaction analyses.
Table R1. Associated factors of plasma fibrinogen levels in a simple linear regression

<table>
<thead>
<tr>
<th>Variables</th>
<th>Unstandardized coefficients</th>
<th>Standardized coefficients</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>Standard error</td>
<td>β</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.02</td>
<td>0.003</td>
<td>0.17</td>
<td>6.85</td>
</tr>
<tr>
<td>Sex (female/male)</td>
<td>-0.21</td>
<td>0.08</td>
<td>-0.07</td>
<td>-2.63</td>
</tr>
<tr>
<td>Smoker (yes/no)</td>
<td>0.37</td>
<td>0.10</td>
<td>0.09</td>
<td>3.74</td>
</tr>
<tr>
<td>History of CV events (yes/no)</td>
<td>0.60</td>
<td>0.10</td>
<td>0.15</td>
<td>6.06</td>
</tr>
<tr>
<td>Diabetes (yes/no)</td>
<td>0.79</td>
<td>0.09</td>
<td>0.22</td>
<td>8.87</td>
</tr>
<tr>
<td>Hypertension (yes/no)</td>
<td>-0.13</td>
<td>0.13</td>
<td>-0.03</td>
<td>-1.00</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>0.10</td>
<td>0.01</td>
<td>0.20</td>
<td>8.07</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>0.004</td>
<td>0.002</td>
<td>0.07</td>
<td>2.67</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>-0.003</td>
<td>0.003</td>
<td>-0.03</td>
<td>-1.01</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>0.003</td>
<td>0.002</td>
<td>0.03</td>
<td>1.21</td>
</tr>
<tr>
<td>Blood platelet count (per 10×109/L greater)</td>
<td>0.06</td>
<td>0.005</td>
<td>0.29</td>
<td>11.87</td>
</tr>
<tr>
<td>Serum potassium (mmol/L)</td>
<td>-0.16</td>
<td>0.05</td>
<td>-0.09</td>
<td>-3.39</td>
</tr>
<tr>
<td>Serum albumin (g/L)</td>
<td>-0.06</td>
<td>0.008</td>
<td>-0.18</td>
<td>-7.30</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>0.17</td>
<td>0.03</td>
<td>0.15</td>
<td>6.04</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>0.20</td>
<td>0.04</td>
<td>0.13</td>
<td>5.15</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>0.23</td>
<td>0.04</td>
<td>0.15</td>
<td>6.22</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>-0.34</td>
<td>0.12</td>
<td>-0.07</td>
<td>-2.79</td>
</tr>
<tr>
<td>hs-CRP (mg/L)</td>
<td>0.04</td>
<td>0.005</td>
<td>0.22</td>
<td>8.38</td>
</tr>
<tr>
<td>eGFR</td>
<td>-0.003</td>
<td>0.02</td>
<td>-0.005</td>
<td>-0.21</td>
</tr>
<tr>
<td>(mL/min/1.73 m²)</td>
<td>Use of antiplatelet agents (yes/no)</td>
<td>0.64</td>
<td>0.13</td>
<td>0.13</td>
</tr>
<tr>
<td>Use of lipid-lowering drugs (yes/no)</td>
<td>0.63</td>
<td>0.13</td>
<td>0.13</td>
<td>5.03</td>
</tr>
</tbody>
</table>

Abbreviations: CV cardiovascular, BMI body mass index, SBP systolic blood pressure, DBP diastolic blood pressure, TC total cholesterol, TG triglycerides, LDL-C low-density lipoprotein cholesterol, HDL-C high-density lipoprotein cholesterol, hs-CRP hypersensitive C-reactive protein, eGFR estimated glomerular filtration rate

Results

- The study population is quite young. Is this representative of the overall dialysis population in China?

  Reply: Thanks for the question. Unfortunately, we don’t have a nation-wide PD cohort study yet, so we’re not sure whether the study population is representative of overall patients in China, but they are representative of PD population in South China as our patients are from South China, especially from Guangdong Province. But to our knowledge, the age of PD patients in China are generally younger than those in Western countries.

- The results of the analyses with fibrinogen as a continuous exposure are presented in the column 'total cohort'. From the text I understood that these HR's are for a 1 g/L increase in fibrinogen. This should however also be clear from the table.

  Reply: Thanks for the comments and suggestions. We examined the shape of the relationship between plasma fibrinogen and mortality and found that the relationship between fibrinogen and CV and all-cause mortality was nonlinear, so it is not very rational to conduct analyses with fibrinogen as a continuous exposure. We’ve removed this part from the results.

- Please provide number of events in the 2 subgroups (Table 3 and Table 4).

  Reply: We’ve re-collected the data and divided the study patients into quartiles according to plasma fibrinogen levels. The number of events in the quartiles are provided in Figure 1.

- Please provide also the results of cardiovascular mortality in Table 4.

  Reply: After re-collecting and re-analyzing the data, we’ve found that it is not rational to treat fibrinogen as a continuous exposure, so we did not conduct interaction analyses and stratified analyses.
- Table S1: these numbers are also mentioned in the main text. This table can therefore be omitted.

Reply: Thanks for the suggestion, we’ve deleted this table.

- Figure S2. To my opinion these figures can be omitted as they do not provide information on the research question.

Reply: Thanks for the suggestion, we’ve deleted this figure.

Discussion

The main conclusion is now on the observed interaction with diabetes for all-cause mortality. However, the primary outcome was cardiovascular mortality and in addition the rationale for the stratified analyses is unclear. To my opinion this finding should thus be presented as an (interesting) secondary finding. It remains unclear how these different associations might be explained. I am thus not convinced this is not a chance finding.

Reply: Thanks for the comments. We’ve re-collected and re-analyzed the data based on the opinions of the two reviewers. The main finding of this study was that an elevated plasma fibrinogen level was significantly associated with an increased risk of mortality in PD patients and the relationship between fibrinogen and CV and all-cause mortality was nonlinear, exhibiting approximate J-shaped curves. Therefore, we’ve revised the discussion section of the manuscript, focusing on the possible causes of the association between fibrinogen and mortality and discussing the difference between our study and other studies. In addition, we did not find an association between lower fibrinogen levels and mortality, we also discussed the possible causes of this result to a certain degree in the discussion section.

Reviewer 2

General comments

Well written overall. Interesting research question. Methods and study design seem appropriate for the research question.

Reply: We thank the reviewer for these comments. We’ve revised the manuscript and addressed your comments below. We hope the manuscript is improved sufficiently and is now ready for publication.

Requested revisions

Revisions/clarifications:

1) Abstract: awkward phrasing "independent prognostic power"--the investigators were examining for an independent association, not a predictive score per se.

Reply: Thanks for the comments. We’ve revised the manuscript using terminology ‘association’ or ‘relationship’ instead of ‘predictor’ or ‘prognostic’ to describe the subject.

2) Provide absolute measures in the Abstract (% death by low/high plasma fibrinogen levels).
Reply: We’ve re-collected and re-analyzed the data, dividing the study population into quartiles according to plasma fibrinogen levels. Due to space limitations, we failed to exhibit absolute measures in the Abstract. However, the specific CV and all-cause mortality in the quartiles are provided in Figure 1.

3) I would not include the subgroup findings in the main conclusion (diabetes subgroup mortality).

Reply: Thanks for the suggestion. In order to analyze the association of plasma fibrinogen with CV and all-cause mortality more comprehensively, we re-collected and re-analyzed the data. When we treated fibrinogen as a continuous exposure, we found that the relationship between fibrinogen and CV and all-cause mortality was nonlinear, so it is not rational to treat fibrinogen as a continuous exposure for Cox regression analysis. Therefore, in the revised manuscript, we did not conduct interaction analyses and further stratified analyses and removed this part from the original manuscript.

4) It appears the fibrinogen measure was obtained pre-dialysis initiation; if so, this should be clearly stated in the Methods (cross-sectional pre-dialysis measure).

Reply: Thanks for the comments and suggestions. Unfortunately we did not clarify this issue in the data collection section. We have revised the participant and data collection sections of the manuscript. The study involves only incident patients who started maintenance PD in our center. Therefore, patients with PD less than three months were not included in this study. Baseline demographic data, including age, sex, smoking, a history of cardiovascular events, diabetes, and hypertension, were collected at the initiation of PD therapy. Clinical data, including BMI, blood pressure, and medication use, and biochemical data, were collected three months after PD therapy initiation. We’ve re-collected the clinical and biochemical data.

5) Fibrinogen may demonstrate a more complex relationship with mortality (for example, U-shaped); I suggest also examining smaller groups such as quartiles.

Reply: Thanks for this suggestion. We’ve divided the study population into quartiles according to plasma fibrinogen levels and re-analyzed the data. We found that the relationship between plasma fibrinogen and CV and all-cause mortality was nonlinear and an elevated plasma fibrinogen level was significantly associated with an increased risk of CV and all-cause mortality.

6) How was missing data handled?

Reply: First, in the participants section, patients who lacked the baseline data of plasma fibrinogen were excluded from the study. Second, our study was a prospective cohort study with very few missing values. Those missing values were replaced with mean value in the statistical analysis.

7) The follow-up time for all survival models must start at day 90 (definition of chronic prevalent PD) or else an immortal time bias may have occurred.
Reply: Thanks for the comments. We’ve revised the Methods section of the manuscript in detail and made it clear that our cohort was composed of incident patients who started maintenance PD in our center.