Author's response to reviews

Title: Elevated levels of plasma D-dimer predict a worse outcome in patients with nasopharyngeal carcinoma

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Author's response to reviews: see over
Annie Lyn Bravo
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BMC cancer

Dear Dr. Bravo,

We are pleased to submit a revised manuscript entitled “Elevated levels of plasma D-dimer predict a worse outcome in patients with nasopharyngeal carcinoma” (Revision MS: 1465528363128895). We greatly appreciate that you and your colleagues have taken time to comment on its contents. We feel that the reviewers offered helpful and constructive suggestions, and we appreciate their comments. All authors have read and approved the revised manuscript, which was prepared in accordance with the BMC Cancer revised manuscript checklist. There are no financial or other relationships that might lead to a conflict of interest.

NPG Language Editing has edited and formatted the revised manuscript.
according to BMC Cancer guidelines (the editorial certificate is attached). We hope that the revised manuscript is acceptable for publication. Below, we have restated the comments of each reviewer and provided point-by-point responses to the reviewer’s concerns; the changes are highlighted in red in the revised manuscript. We feel that these revisions have substantially strengthened our paper, and we greatly appreciate the time and effort of the reviewers.

Point-by-point response to comments of the Reviewers and the Editor

Reviewer#1

Major Compulsory Revisions

1. In the Materials and Methods section, the authors claimed: “Plasma D-dimer concentrations <1.0 µg/ml were considered normal”, however, no reference was cited about how they make the determination. More importantly, in the following group division, the 1st quartile of plasma D-dimer is 0.0-0.3µg/mL, the 2nd group is 0.3-0.5µg/mL and the 3rd group is 0.5-0.8µg/mL, all considered to be normal according to the above criteria, it would then be hard to speculate what is the clinical value for a specific maker which would be normal in the majority of the patient.

Reply: Thank you for your constructive suggestions. We apologise for not including the reference on which we based our choice to use 1.0 µg/ml as the normal cut-off point. This cut-off point was chosen according to the guidelines
of the clinical laboratory of Japan (Takeshi Endo et al.: Guide to Clinical Laboratory Tests 2003-2004, p.698). In this study, the cut-off point of 1.0 µg/ml was probably used to for cardiovascular disease and stroke, so it was not appropriate for cancer patients. Moreover, one study also reported that a plasma D-dimer level below 1.0 µg/ml still presented worse disease-free survival, distant metastasis-free survival, and overall survival for lung, breast, lower gastrointestinal tract, pancreas, stomach, kidney, prostate, and brain cancer patients. (Ay C, Dunkler D, Pirker R, Thaler J, Quehenberger P, Wagner O, Zielinski C, and Pabinger. High D-dimer levels are associated with poor prognosis in cancer patients. Haematologica 2012;97(8):1158-1164). Thus, even when the D-dimer level was below 1.0 µg/ml in our study, an elevated D-dimer level was correlated with worse survival. Another possible reason for this is that the function of the plasma D-dimer in cancer patients may be different from other patients even when the D-dimer levels are at normal levels, but the mechanism underlying this possibility requires further study. Therefore, because the 1.0 µg/ml cut-off point was likely not suitable for cancer patients in this study, the following sentence was deleted for clarification purposes, “Plasma D-dimer concentrations <1.0 µg/ml were considered normal.”

2. In the Discussion section, the authors did not make a comparison of their results with previous published data. A lot of papers have been published
about the role of plasma D-dimer in cancers, for example, gastric cancer, the authors should cite some of these important references to make the discussion more sound.

Reply: Recently, the role of D-dimer has been reported in several other cancers, including lung, prostate, lower gastrointestinal tract, colorectal, gastric, breast, pancreatic, and kidney cancer. To improve the discussion, we have added references to note these important studies.

Revised on Page 13, Para 2, Lines 278-284;

3. In previous studies, a great number of molecular alterations and clinical parameters were reported to be able to predict the prognosis for nasopharyngeal carcinoma patients, it is necessary for the authors to make a discussion of the advantages of plasma D-dimer testing in comparison to other prognostic markers.

expensive and complicated procedures, and rapid clinical implementation was difficult to achieve in a short time. To date, the routine prognostic risk assessment of NPC patients still relies on traditional clinico-pathological prognostic variables and EB virus-associated blood tests. Plasma D-dimer levels are established, routinely measured blood-based parameters that are reproducible and do not require additional labour. Thus, plasma D-dimer is a promising biomarker for NPC patients. We have included an additional discussion of this topic and added new references to the manuscript.

Revised on Page 15, Para 2, Lines 324-331

4. Thorough out the paper, it looks like that the copy of EBV DNA would contribute more in predicting the prognosis than plasma D-dimer, the authors should try to make an explanation for this result.

Reply: Thank you for your comment and suggestion. Elevated levels of circulating cell-free Epstein-Barr virus (EBV) DNA have been detected in plasma and serum samples from nasopharyngeal cancer (NPC) patients using quantitative real-time PCR (qPCR). Plasma EBV DNA is useful for the clinical management of NPC patients, and it is considered the most attractive potential biomarker. High EBV DNA load at disease onset or detectable viral load post-treatment are associated with poor survival and frequent relapse in NPC patients. According to our results, although the magnitude of the predictive value of EBV DNA was superior to that of D-dimer, the use of plasma EBV
DNA alone to predict NPC patient prognosis was not sufficient due to patient heterogeneity. Notably, in the high EBV DNA subgroup, a high plasma D-dimer level was associated with a worse disease-free survival, distant metastasis-free survival, and overall survival. Therefore, plasma D-dimer could play a complementary role with EBV DNA for predicting the prognosis of NPC patients. We discuss this topic in the revised manuscript.

Revised on Page 14, Para 2, Lines 299-301 and Lines 304-309; Reference 37.

5. Since the level of plasma D-dimer could be affected by many other factors, I recommend the authors make an estimation of its value with the clinicopathological parameters.

Reply: We appreciate the reviewer’s suggestion, and we agree that the relationship between plasma D-dimer and clinicopathological parameters should be evaluated. To this end, we performed Spearman relationship analyses between D-dimer and other variables. The Spearman correlation analysis indicated that plasma D-dimer levels correlated with age, serum CRP level, LDH level, EBV DNA level, tumour TNM stage, and distant metastasis (Supplemental table 1). These results are included in Supplemental table 1 and in the manuscript.

Revised on Page 11, Para 1, Lines 223-226, and a new Supplemental table 1 was added.
Supplemental Table 1. D-Dimer Relationships

<table>
<thead>
<tr>
<th>Variable</th>
<th>Spearman Correlation Coefficient</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.123</td>
<td>0.001</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>-0.088</td>
<td>0.019</td>
</tr>
<tr>
<td>Histology, WHO type (II/III)</td>
<td>0.069</td>
<td>0.065</td>
</tr>
<tr>
<td>ECOG (0/1/2)</td>
<td>0.038</td>
<td>0.315</td>
</tr>
<tr>
<td>Clinical stage (1/2/3/4)</td>
<td>0.139</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Tumour stage (1/2/3/4)</td>
<td>0.13</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Node stage (0/1/2/3)</td>
<td>0.03</td>
<td>0.423</td>
</tr>
<tr>
<td>EBV DNA (copies/ml)</td>
<td>0.106</td>
<td>0.005</td>
</tr>
<tr>
<td>VCA-IgA</td>
<td>0.037</td>
<td>0.325</td>
</tr>
<tr>
<td>EA-IgA</td>
<td>0.038</td>
<td>0.314</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>0.106</td>
<td>0.004</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>0.195</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>WBC (10^9/L)</td>
<td>0.044</td>
<td>0.245</td>
</tr>
<tr>
<td>Neutrophil (10^9/L)</td>
<td>0.05</td>
<td>0.183</td>
</tr>
<tr>
<td>HGB (g/L)</td>
<td>-0.205</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>PLT (10^9/L)</td>
<td>0.03</td>
<td>0.419</td>
</tr>
<tr>
<td>Smoking (yes/no)</td>
<td>-0.01</td>
<td>0.799</td>
</tr>
<tr>
<td>Chronic HBV Infection (yes/no)</td>
<td>-0.012</td>
<td>0.749</td>
</tr>
<tr>
<td>Cardiovascular disease (yes/no)</td>
<td>0.023</td>
<td>0.534</td>
</tr>
<tr>
<td>Diabetes mellitus (yes/no)</td>
<td>0.016</td>
<td>0.664</td>
</tr>
<tr>
<td>Family history of NPC (yes/no)</td>
<td>-0.033</td>
<td>0.38</td>
</tr>
<tr>
<td>Distant metastasis</td>
<td>0.113</td>
<td>0.002</td>
</tr>
<tr>
<td>Local/regional recurrence</td>
<td>-0.009</td>
<td>0.816</td>
</tr>
</tbody>
</table>

6. In Table 1, the total number of the patients in some items is not 717, the authors should check if there are some mistakes. In Table 2, the authors should mark specifically when P value is <0.05, also in Table 2, the ECOG in DMFS is not correct.

Reply: We apologise for making these careless mistakes. We carefully revised the entire manuscript and all tables. All mistakes were revised as necessary,
and the $P$ values <0.05 were noted in the tables.

Revised in table 1 and table 2

**Minor Essential Revisions**

1. Normal control is very important and necessary in the study, the authors failed to present such a group in the research. And then, of course, it is impossible to draw conclusions on what are the sensitivity, specificity and accuracy of plasma D-dimer in predication of the prognosis in these patients.

   **Reply:** We appreciate the reviewer’s suggestion, and we agree that the normal control is important and necessary for the study. We have retrospectively collected D-dimer data from 126 healthy volunteers (average age of 45, ranging from 19 to 75 years old) who submitted to D-dimer testing as part of a routine physical examination in our hospital during the same time period. This group was considered a normal control group. Interestingly, the median D-dimer level (ug/mL) was higher in patients with nasopharyngeal carcinoma than healthy volunteers ($P<0.002$, new Figure 1), with values of 0.4 ug/mL (25th-75th percentile: 0.3-0.5) and 0.50 (25th-75th percentile: 0.3-0.8), respectively. These results are presented in the manuscript, and new information was added to figure 1.

   Revised on Page 6, Para 3, Lines 123-126; Page 8, Para 2, Lines 163-164 and Page 10, Para 2, Lines 210-212, and Figure 1

2. The writing of the manuscript should be improved, all the abbreviation, units,
as well as the references should be consistent throughout the manuscript and
meet the requirements of BMC Cancer, and some grammar mistakes should
be avoided.

Reply: We appreciate the reviewer’s advice, and we apologise for the poor
grammar in the manuscript. We have revised the entire manuscript carefully to
ensure that all abbreviation, units, and references are consistent. According to
BMC Cancer guidelines, manuscript editing and formatting were performed by
NPG Language Editing to improve the language.

Reviewer#2

1. The title should accurately reflect the content of the article. A better title
might be something like: Elevated levels of plasma D-dimer predict a worse
outcome in patients with nasopharyngeal carcinoma. Or: Elevated plasma
D-dimer levels is an independent adverse prognostic factor for
nasopharyngeal carcinoma patients.

Reply: We appreciate the reviewer’s constructive suggestion. We agree that
the title should accurately reflect the content of the article. Therefore,
according to the suggestion of the reviewers, the title has been revised as
follows: “Elevated levels of plasma D-dimer predict a worse outcome in
patients with nasopharyngeal carcinoma.”

Revised in title page
2. Generally, there are censored observations in the data after a period of follow-up. However, there are no censored data in Fig2-5. Did the authors get in touch with all the patients?

Reply: We apologise for deleting the censored points when the Figure was plotted. We plotted the Figure again, and all the censored points in the curve and the risk numbers were added to the new figure.

Revised in Figure 2-5

3. Small textual errors should be corrected. Some examples:

Page 5, line 93: … are liable...

Page 13, line 281: … for recurrence

Reply: We appreciate the reviewer’s advice. We have revised the entire manuscript carefully to correct any errors in the text. To improve the language, NPG Language Editing has edited and formatted the manuscript to ensure that no grammatical mistakes or textual errors remain.

We look forward to your favourable reply.

Sincerely yours,

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