Author’s response to reviews

Title: Prognostic value of secreted phosphoprotein-1 in pleural effusion associated with non-small cell lung cancer

Authors:

He Zhang (anzai1983@sina.com)
Hongbing Liu (netlhb@126.com)
Dongmei Yuan (yuandongmei1163.com)
Zhaofeng Wang (wangzhaofeng8811@163.com)
Yunfen Wang (wangyunfen2010@163.com)
Yong Song (yong_song6310@yahoo.com)

Version: 2  Date: 9 January 2014

Author’s response to reviews: see over
Dear editor:

Thank you very much for your letter and advice. The comments from you and the reviewers have helped to strengthen this manuscript significantly. We have addressed the comments raised by the reviewers, and the amendments are highlighted in red in the revised manuscript. We hope that the revision is acceptable, and I look forward to hearing from you soon.

Sincerely yours

Yong Song
Reviewer 1

1. Was this a retrospective study on a biobank or a prospective one? This should be stated in the methods and the abstract.
Reply: Thank you for raising this important question. Our study was a retrospective study. Please see page 2, paragraph 1.

2. Did the authors measure serum osteopontin levels? They may also be of prognostic value as in the above study. If the authors have serum samples, it may be a valuable addition to the dataset. If not, the authors might state in the discussion that this may be a valid research question for future studies.
Reply: We thank the reviewer for pointing out this issue. We have not measured serum osteopontin levels. Please see page 11, paragraph 2.

3. Were pleural fluids collected during diagnostic or follow-up thoracenteses?
Reply: Pleural fluids were collected during diagnosis. Please see page 5, paragraph 1.

4. Were SPP1 data normally distributed? If not, the authors should use Mann-Whitney instead of t-test.
Reply: Yes, the SPP1 data were normally distributed.

5. Are the data shown as mean±SD or SEM? This should be stated in the methods (stats).
Reply: Please see page 6, paragraph 1.

6. The methods section should be subdivided into subheadings such as: study patients or protocol, measurements, statistics, etc.
Reply: We thank the reviewer for the advice. Please see page 4-6.

7. The SPP1 values of control and study patients should be given in a new figure in the form of a dotplot, so the reader can appreciate the overlap of data between the two groups.
Reply: Please see Figure 1.

8. Symbols should be explained in tables, and axes should be more clearly labeled in graphs (ie, spell out acronyms and indicate the units, ie progression-free survival in days).
Reply: Please see page 14.

9. What is progression-free survival for a patient with MPE? Please define in methods.
Reply: The progression-free survival for a patient with MPE was defined as the time from pleural effusion diagnosed to significant progression. Please see page 5, paragraph 1.
Reviewer 2

**Major Compulsory Revisions**

The background section could be significantly shortened as it currently discusses malignant pleural effusions at length without a clear direction. The discussion of SPP1 protein is good, but also could be shortened. It is not clear how these 6 paragraphs add to the main point of the paper – 2 or three paragraphs may be sufficient.

**Reply:** We thank the reviewer for the advice. Please see page 3-4.

Please describe the features of both the study patients and the case controls in a table. This is done for the study patient’s but the case controls are neglected, and no comparison is made between case-controls and study patients. These features should not be in the methods section.

**Reply:** Please see table 1.

You mention larger sample sizes needed in your discussion – was a power calculation performed to define your enrollment numbers? If not, could you perform one and show that the study was appropriately powered?

**Reply:** Our enrollment numbers was not defined by a power calculation. According to the ratio of the case group and the control group model(4:1), final calculation of the total sample size $n = 114$; case group=91; control group=23, with the formula $n = \left( (Z_\alpha + Z_\beta) \times \text{SD} / |\text{Mean}_1-\text{Mean}_2| \right)^2 \times (4+4/3)$. ($Z_\alpha = 1.96$, $Z_\beta = 1.28$, SD=1320.93 Mean1=1568.9 Mean2=644.12 according to the study of Moschos et al[25] and this study.

Please define the pathologies of the case-control group.

**Reply:** Please see table 1.

Please describe how pleural effusions are collected (bottle, additive solution, etc).

**Reply:** Please see page 4, paragraph 2.

A pilot experiment is mentioned in the methods section. Can more details be given about this, or a summary of the results? It seemed to influence the way the assay was run, (dilution) differently from how the assay directions state, although you mentioned that the manufacturers instructions were followed

**Reply:** Please see page 4, paragraph 3.

The positive/negative disease state is not stated for the ROC curve analysis. (it can be inferred, but it would be better if it was stated).

**Reply:** Please see page 14, paragraph 2.

It might be helpful to had a box plot/dot plot of concentrations of SSP1 vs. malignancy so the actual concentrations can be compared, in addition the the means and standard deviations.

**Reply:** Please see Figure 1.
The results and discussion says that SPP1 is correlated with prognosis and extra pulmonary metastases – could you include these correlation graphs and coefficients?

**Reply:** The K-M survival analysis showed that SPP1 was correlated with prognosis. (Figure 3 and 4). And the chi-square test demonstrated that SPP1 was correlated with extra pulmonary metastases (Table 2).

The paragraph about pleural fluid analysis on page is confusing and contains many grammar mistakes. I think you mean that to diagnosis a malignant pleural effusion you need to perform thoracentesis or pleural biopsy, but it currently reads that a physician need to do these things to diagnosis any effusion.

**Reply:** We thank the review’s recommendation. We have deleted this paragraph.

Could you describe why your results on SPP1 concentration in malignant vs. non-malignant differ from 1 of the other studies which did the same sort of study you performed? Also discuss the study with results that agree with your results?

**Reply:** Please see page 9, paragraph 2.

The discussion talks about the case control patients, but does not give a pathogenesis of why SPP1 is elevated in these patients.

**Reply:** Please see page 10, paragraph 2.

Your conclusion says that ‘may provide an auxiliary diagnostic modality” and an increased level ‘may be an indicator of poor prognosis, when your in your results section you say that the differences in survival were statistically significant. These statements are contradictory, and deal with the main theme of your paper.

**Reply:** Please see page 11, paragraph 3.

**Minor Essential Revisions**

Many formatting/typos in abstract – mainly missing spaces.

**Reply:** We thank the reviewer for pointing out this issue. We have modified these.

No need to use ‘however’ on page 4 in the last paragraph of the discussion.

**Reply:** We thank the reviewer for pointing out this issue. We have modified this.

In the first paragraph of the methods section it mentions pleural biopsy, but then discusses that all pleural fluid had positive cytology. This is confusing.

**Reply:** Please see page 4, paragraph 2.

Overall the language is acceptable, but not fluent. Perhaps a native English speaker with knowledge of the topic could be recruited to assist with the flow.

**Reply:** This manuscript has been edited by native English-speaking experts of BioMed Proofreading.
The review of pathogenesis of Malignant pleural effusion is very good, however, I’m not sure how it directly relates to results of this study, or is derived from the results of this study. While good, it may not be needed.

Reply: We have deleted it.

Please write out what you mean by PFS and CUM Survival and survival functions on your survival curves.

Reply: Please see page 14, paragraph 3.

Discretionary revisions:

Keywords of ‘prognosis’ and ‘auxillary diagnosis’ may be over-broad.

Reply: We thank the reviewer for pointing out this issue. We have modified this.

Was the serum levels of SPP1 measured for this study (or could biorepository/historic samples be used to assess it in the study patients?) It would be very good to compare serum vs. pleural concentrations.

Reply: We thank the review’s recommendation. We have not measured the serum levels of SPP1. This may be a valid research question for future studies.

Page 10 - word “effusate” – not sure it is a real word (google has very few instances of it being used)

Reply: We thank the reviewer for pointing out this issue. We have modified this.