Author's response to reviews

Title: Serum soluble ST2 is associated with ER-positive breast cancer

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Author's response to reviews:

Dear Prof. Dafne Solera
Editor-in-Chief Journal Editorial Office BioMed Central

We thanks very much to the reviewers’ comments. We have edited our manuscript according to that.

Reviewer: Jürgen Radons
Reviewer's report:
In the manuscript entitled “Serum soluble ST2 is a new potential immune-related marker for breast cancer” the authors analyzed the correlation between serum soluble ST2/IL-33 and VEGF as well as clinicopathological parameters together with the prognostic impact of serum sST2/IL-33 in 150 female breast cancer patients in comparison to 90 healthy individuals. This is an interesting paper which provides evidence for the putative diagnostic role of sST2 in breast cancer. However, there are several concerns that should be addressed.

Major Compulsory Revisions:
1. The manuscript needs the inclusion of cut-off values for any of the serum parameters tested. The authors must declare when a sample was regarded as being sST2/IL-33/VEGF high/low expressed and how the walking cut-offs values were evaluated.

During the detection of sST2, IL-33 and VEGF, we diluted the standard according to the instruction of the kit (Detection limit is 33pg/ml, 23pg/ml and 33pg/ml, respectively). We have added it in the Patients and Methods. When a sample was regarded as being sST2/IL-33/VEGF high/low expressed, serum sST2/IL-33/VEGF levels in ER-positive breast cancer patients were bifurcated at mean value (103.6pg/mL/162.0pg/mL/248.5pg/mL). We have added it in the Result part,
2. The paper lacks any data on the correlation between serum parameters and patient survival. For survival comparisons, Kaplan-Meier together with Cox regression analyses should be included in the manuscript. We have used Kaplan-Meier together with Cox regression analyses for survival comparisons, and added it in the manuscript.

3. In the Discussion (4th paragraph, line 4-7) the authors state that “The present study showed that [...] disease-free and overall survival rates [...] were statistical significantly higher [...]” without presenting the data. To strengthen the findings the inclusion of survival data is strictly recommended. Ok, we have added survival data.

4. There are several contradictory results reported in the literature on serum levels of VEGF in breast cancer patients. While certain studies (including the one presented here) demonstrate elevated VEGF serum levels in cancer patients compared to healthy controls, others do not (e.g. Hodorowicz-Zaniewska et al., Pol. J. Pathol 63, 255, 2012). Moreover, in the latter study VEGF levels did not correlate with clinicopathological factors. These findings argue against a positive correlation of VEGF with breast cancer. Moreover, the composition of the study cohort obviously determines the quality of the outcome measure. The authors are asked to comment on this aspect in the manuscript.

We have commented on this aspect in the manuscript and add the reference (Hodorowicz-Zaniewska et al., Pol. J. Pathol 63, 255, 2012).

5. It is well known that sST2 is also increased in other pathologies including asthma, cardiopathy etc. Therefore, upregulated sST2 levels might also be the result of mechanisms independent of the malignant process. It is therefore inexplicable why the authors believe that sST2 “may be a new potential immune-regulated marker in breast cancer.” The conclusion that sST2 might be a promising target in breast cancer therapy is thus too speculative and requires further experimental support.

Thank you! We have deleted the conclusion “may be a new potential immune-regulated marker in breast cancer.”

6. sST2 functions as an antagonistic decoy receptor which serves as a ligand sink by competing for IL-33 with membrane-bound ST2. Therefore, it is not surprising that elevated levels of IL-33 are accompanied by an sST2 upregulation. However, its pathophysiological importance is not addressed in this manuscript. Please discuss it.

We have discussed it in this manuscript.

7. Gillibert-Duplantier et al. (Ref. 10) already described elevated sST2 serum levels in patients with metastatic but not localized breast cancer. The authors should therefore critically discuss the impact of their own findings.

We have discussed it in this manuscript.
8. The finding that sST2 levels in breast cancer patients did not correlate with the Her-2 status confirms previous results by Gillibert-Duplantier et al. (Ref. 10). The authors interpretate this finding with “limiting cases or other reasons”. In order to make the article more convincing the authors should discuss possible reasons in the context of the actual literature.

We have discussed possible reasons in this manuscript.

Minor Essential Revisions:
1. Any figure should be supplemented with a detailed legend as given in the instructions for authors. Moreover, the short titles in Fig. 1D+E as well as in Figs. 2A-F need stylistic changes.

We have added a detailed legend after manuscript. The short titles in Fig. 1D+E as well as in Figs. have been changed.

2. After inclusion of additional survival data, the association of sST2/IL-33 expression levels with clinicopathological factors should be arranged in the form of a table to make it more comprehensible.

Ok, we have made a table for survival data (Table 2)

3. In the introduction, a more recent reference on the cellular source of IL-33 and its capacity to induce production of proinflammatory cytokines in pancreatic cancer cells should be mentioned (Schmieder et al., Cytokine 60, 514, 2012).

We have added the reference (Schmieder et al., Cytokine 60, 514, 2012) in the introduction

4. In the Results section the authors state that “Serum levels of sST2 in increasing tumor size […] were gradually elevated”. Since there is obviously no statistically significant difference in serum sST2 between T1 and T2, this sentence should be rephrased.

We have rephrased this sentence.

5. The sentence “Serum levels of sST2 in these sizes of breast cancer were associated, and […] needs grammatical correction.

We have corrected the sentence.

6. Discussion, last sentence: replace IL-27 by IL-33(?)

Sorry, we have corrected it.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being Published

Yes, we have extensively edited the written English already.

Statistical review: No, the manuscript does not need to be seen by a
statistician.

Declaration of competing interests:
I declare that I have no competing interests

Sincerely yours
Yunfei Wu