Peer Review File

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Reviewer A

The authors submitted the level of serum soluble ST2 in the prognostic assessment of advanced breast cancer patients. The importance of ST2 levels in breast and other cancer patients has been determined in the literature. Therefore, the novel contribution of this article is limited. Additionally, the number of patients is so limited for the evaluation of biomarkers potential of ST2 in TNBC patients. There is no information of treatment of patients. The clinical data of patients should be more explained (neoadj therapy etc.)

Reply: I would like to thank you very much for your suggestion and I think it is important to add the clinical treatment options for patients with advanced breast cancer to the "Methods" section of the article.
While most of the previous studies in the literature have focused on ST2 in predicting cardiac damage due to anthracycline chemotherapy, the innovation of this study is to focus on ST2 in the survival of advanced breast cancer patients with different molecular typing, with the aim of finding a biomarker for clinical prediction in advanced breast cancer patients. In other words, ST2 as a conventional biomarker to find new clinical value in advanced breast cancer.
Changes in the text: We added treatment regimen for advanced breast cancer patients (see Page 6, line 29-31).
All patients were treated with the NACT regimen (4 cycles of doxorubicin 60mg/m2/cyclophosphamide 600mg/m2 followed by 12 cycles of weekly paclitaxel 80mg/m2). In our study, HER2-positive patients were administered adjuvant trastuzumab.

Reviewer B

The article “Clinical value of serum soluble ST2 in the prognostic assessment of advanced breast cancer patients” investigates the differences in sST2 expression in patients with different molecular subtypes of breast cancer and assesses its clinical value in the prognostic evaluation of advanced breast cancer.
The manuscript is written adequately in all its parts. However, some obstacles must be corrected before the manuscript is suitable for publication.
These, among others, include:
Line 5-9 This section belongs to the materials and method section.
The Ethical approval number should be mentioned in the manuscript text.
Was any correction for multiple testing (e.g., Bonferroni correction) applied?
Results line 12-13 – the authors have written, “…however, sST2 levels were significantly higher in those with triple-negative breast cancer compared to the other three groups (Table 1)”. However, in Table 1, no data illustrates higher sST2 levels and associated p-value for triple-negative breast cancer patients compared to other BC subgroups.
The term Clinical phage in Table 2 should be replaced with the correct one.
The p-values corresponding to ** and *** should be mentioned in the legend of Figure 1.
The authors have written: “The 91 included breast cancer patients had never undergone

mastectomy or breast-conserving surgery, or received anti-cancer drug treatment, and were followed up for a certain period and recorded" and "and (III) serum was collected from patients admitted for the first time." So, if I understand correctly, the data presented herein correspond to baseline (before treatment and treatment-associated cardiotoxicity) sST2 levels. If that is the case, having data related to sST2 levels measured after therapy treatment will be helpful. If not, this should be clearly stated in the materials and method section.

(1) Reply: Thank you for your suggestion, which is very important for research integrity, I added the Ethical approval number l (NO. BC2018024) in materials and method section.
Changes in the text: We added Ethical approval number (NO. BC2018024) (see Page 7, line 1-2).

(2) Reply: Subgroup analysis of the different molecular typologies was performed using Bonferroni correction.
Changes in the text: W added Bonferroni correction in methods section. (see Page 8 line 3-4).

(3) Reply: Your review comments are important for my study, we added the triple negative breast cancer group (n=28) and the non-triple negative breast cancer group (n=63) in Table 1 and we found that ST2 expression was significantly higher in the triple negative breast cancer patients than in the non-triple negative breast cancer group ($p<0.01$).
Changes in the text: We added the triple negative breast cancer group (n=28) and the non-triple negative breast cancer group (n=63) in Table 1. (see Page 16 Table 1).

(4) Reply: The term Clinical phage in Table 2 should be replaced with Clinical prognostic stage.
Changes in the text: We have modified our text as advised (see Page 15, Table 2).

(5) Reply: The p-values corresponding to ** and *** had been marked in the legend of Figure 1.
Changes in the text: We have modified our text as advised (see Page 17, Figure 1).

(6) Reply: Your review comments are important for my study, the data corresponds to baseline (before treatment and treatment-related cardiotoxicity) sST2 levels
Changes in the text: We have modified our text as advised (see Page 17, line 18-19).

Reviewer C

1) First, the title is unclear and vague. Please clearly indicate the focus of this study, i.e., the prognostic role of sST2 and the clinical research design of this study, i.e., a retrospective cohort study.
2) Second, the abstract needs some revisions. The background did not indicate the knowledge gap on and the potential clinical significance of this research focus. The methods need to describe the inclusion of subjects, the assessment of baseline clinical factors, follow up procedures, and outcome measures of bone metastasis, cardiotoxicity, and OS. In the results, please describe the baseline clinical characteristics of the study sample and quantify the independent prognostic roles of sST2 by reporting HR and accurate P values. The conclusion should not repeat the main findings and please have comments for the clinical implications of the findings.

3) Third, in the introduction of the main text, the authors need to review what has been known on the effects of sST2, explain why it is associated with the prognosis, bone metastasis, cardiotoxicity, and other clinical and pathological characteristics, and have comments on the potential clinical significance of this research focus.
4) Fourth, in the methodology of the main text, please clearly describe the clinical research design, sample size estimation, the assessment of baseline clinical factors, follow up details and the measurements of outcomes. In statistics, the authors need to describe the Cox regression analysis to ascertain the independent prognostic role of sST2 and describe how the adjustment analysis was performed. Please indicate $P<0.05$ is two-sided.

(1) Reply: Your review comments are very important to my research. The title of the article has been changed to: **Prognostic role of serum soluble ST2 of advanced breast cancer patients: A retrospective cohort study.**
Changes in the text: We have modified our text as advised (see Page 1, line 3-4).

(2) Reply:
1) Description of subject inclusion, assessment of baseline clinical factors, follow-up procedures and outcome measures of bone metastasis, cardiotoxicity and OS, which are important in my study and I have modified accordingly.
2) I comment on the clinical significance of the findings and I find that baseline ST2 as a traditional biomarker of cardiac injury can be used for survival assessment in advanced breast cancer

Changes in the text: We have modified our text as advised (see Page 3, line 12-20, 34.)

(3) Reply: sST2, a "decoy" receptor, is mostly found in peripheral circulating blood and binds to IL-33, competitively inhibiting the IL-33/ST2 signaling pathway, thereby exerting the opposite effect of this signaling pathway, which was an important point cited in many articles. ST2 is widely used clinically in the assessment of cardiotoxicity caused by anthracycline chemotherapy drugs. The association of ST2 with prognosis, bone metastases, and other clinical and pathological features is less well studied and has not been reported in my review of many relevant publications.

(4) Reply: This study is a small retrospective study due to the relatively small number of patients diagnosed with advanced breast cancer for the first time, as well as the relatively high cost of the ST2 test required, which prevented many patients from being enrolled. You mentioned that the clinical study design, sample size estimation, assessment of baseline clinical factors, follow-up details and outcome measures are very important to my study. As this study is a small sample, the experimental design is not perfect and needs to be improved, and a large sample population study will be conducted subsequently.
Changes in the text: We have modified our text as advised (see Page 1, line 12-13)

Reviewer D

1. Figure 1
a) Please explain CEA, DD, TPSA, TNBC, and NS in the legend.

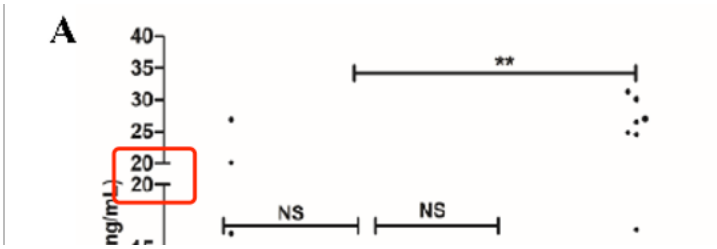
CEA, DD, TPSA, TNBC

Reply: TNBC are expressed in full in the previous articles,

Explanations of CEA, DD, TPSA, and NS in the legend have been provided

Changes in the text: We have modified our text as advised (see **Figure 1**).

b) Please check if the y-axis is correct as there are two “20”.



Reply: We have modified our text as advised (see Figure 1A).

2. Figure 2

Please explain CEA and TPSA in the legend.

Reply: Explanations of CEA and TPSA in the legend have been provided.

3. Table 1

Please explain SD, ER, and PR in the table footnote.

Reply: We have modified our text as advised (see Table 1).

4. Table 2

Please explain ER, PR, DD, TPSA, and CEA in the table footnote.

Reply: We have modified our text as advised (see Table 2), and DD replaced by D-dimer.

5. References/Citations

a) Please double-check if citations should be added as you mentioned “studies”.

31 Studies have reported that the soluble growth-stimulated expression gene 2 protein

b) Please double-check if more studies should be cited as you mentioned “studies”. OR use “study” rather than “studies”.

3 extracellular domains, while sST2 is the soluble form of ST2. Studies reporting on the
4 other two isoforms are lacking (4). ST2 is mainly expressed by immune cells, including

Reply: We have modified our text as advised. (see Page 4, line 31), and the second sentence of "studies" has been deleted.