Author’s response to reviews

Title: The kinetic profile and clinical implication of SCC-Ag in squamous cervical cancer patients undergoing radical hysterectomy using the Simoa assay: a prospective observational study

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Author’s response to reviews:

Dear editor and reviewers:

Thank you so much for your letter and the reviewers’ constructive suggestions. We’d like to extend our sincere gratitude to the reviewers for your patience and dedication to reviewing our manuscript. Your suggestions and comments enabled us to improve the quality of our manuscript. Comparing the current manuscript to the previous one, we can clearly see great changes. We really appreciate the peer-review process, as it is a precious opportunity for us to learn how to present our work effectively, and to communicate with experts all over the world. Thank you so much again for your great help! Changes made were highlighted in the manuscript. A point-by-point response to the comments was listed as follows. We uploaded a file named 'response to reviewers' comments' due to the fact that pictures included could not be shown here.

Reviewer reports:

Catharina M. Korse (Reviewer 1):
This is an extensive performed study in which the Simoa SCC-ag was compared with the Architect assay. Furthermore, the SCC-ag was investigated in a longitudinal study.
A few comments.

1. The description of the methods is incomplete. The method comparison is missing in the abstract
Answer: Thanks for figuring this out. This really helps us to improve this manuscript. A sentence as
‘The kinetic change in SCC-Ag levels and their associations with clinicopathological characteristics
were studied.’ was added in the methods of abstract (Please refer to Line 40-41, Page 3). In addition,
the result of two methods comparison ‘SCC-Ag levels measured by the Simoa assay were highly
correlated with the Architect assay’s levels (Pearson’s correlation coefficient = 0.979, Passing-Bablok
regression slope 0.894 (0.847 to 0.949), intercept -0.009 (-0.047 to 0.027))’ was added in the abstract
(please refer to Line 42-45, Page 3). Thanks again!

2. The technical validations is well done, but before using the new assay, there must be done some
more validations: There are no reference values, no Simoa SCC-Ag levels were measured in healthy
volunteers. If the reference value is known, the sensitivity could be established. ROC-curves are
recommended. So what do the authors mean with sensitivity?
Answer: Thanks for the valuable comments. We totally agree that clinical validation of the assay is
important. However, in the current work, Simoa SCC-Ag was not intended for diagnostics application.
This is the major reason why we did not provide a reference value as you kindly recommended. The
major objective of this manuscript was to employ the newly developed Simoa assay to titrate the
kinetic evolution of SCC-Ag in cervical cancer patients, which to our knowledge, still remains to be
investigated. To this purpose, a reference value of healthy control is dispensable. Instead, the method
comparison on patient tests was performed. Comparison results showed there was high correlation
(Pearson’s correlation coefficient = 0.979) between Simoa SCC-Ag assay and the most commonly used
SCC-Ag assay in the clinical setting (Architect SCC-Ag assay). The slope and intercept for the
Passing-Bablok regression were 0.894 (0.847 to 0.949) and -0.009 (-0.047 to 0.027) respectively,
indicating that Simoa SCC-Ag assay is qualified for SCC-Ag test and the two methods should present
similar SCC-Ag values.
Regarding ‘sensitivity’ in this manuscript, we’d like to clarify the ambiguity. Sensitivity in this
manuscript referred to the lower limit of quantification (LLOQ). It means the sensitivity of the Simoa
SCC-Ag assay to detect SCC antigen. In order to make it more clear, we added the definition of
’sensitivity’ in the Materials and Methods part (please refer to Line 118, Page 7). Thank you so much
again for your great dedication and suggestions.

3. Comparison between men and women is missing, comparison with age.
Answer: Thanks for the valuable suggestions for more comparisons. When used for cervical cancer
patients, only female patients are relevant. In the current cohort, we have compared Simoa SCC-Ag
measurements with age using both scatter plot and statistical test for differences between age groups
(Figures as below). It showed that there is no obvious relationship between SCC-Ag and age for each
time point. The results analyzed from Simoa and Architect data are almost the same, which supports the
consistency of two methods again. In addition, when we analyze the associations between SCC-Ag
levels and categorical clinical factors, the factor of age has been controlled. We have mentioned it in
the Method and Results part. Thank you so much again for your great comment.

Simoa SCC-Ag levels in different age groups (pictures can not be shown here, please refer to the file
we uploaded as supplementary file 'response to reviewers' comments)

Architect SCC-Ag levels in different age groups(pictures can not be shown here, please refer to the file
we uploaded as supplementary file 'response to reviewers' comments)
4. It's known that SCC-Ag is false positive if samples are contaminated with saliva or skin particles. Is that also shown in the Simoa assay?

Answer: Thank you so much for your comments. We totally agree with you, especially given that Simoa technology is an ultra-sensitive detection method. Therefore, in the whole process of the experiments, all the technicians were required to wear lab coat, gloves and gauze mask to prevent any contamination. In addition, all the operation concerning to the patient samples must to be done in the laboratory fume hood. We did not see any contamination event in the experiment. The Architect tests were performed independently in the routine lab of the hospital. The comparison between Simoa and Architect also shows the results obtained in the laboratory is normal. Thanks again!

5. I recommend the authors to consult a statistician for using the right words.

Answer: Thank you so much for the kind recommendation. We have checked the manuscript with our biostatistician. We spotted a typo for Kruskal-Wallis test and corrected this in the revised manuscript (Line 177, page 10). We have changed the description of the purpose of Kruskal-Wallis from ‘test the correlation between continuous variables (Age tumor size)’ to ‘test whether SCC-Ag measurements at different time points were different between patient groups’ (Line 178, page 10), which is more specific and clear. Besides, the current manuscript has been edited by American Journal Experts (AJE) and the editing certificate was also submitted. We believe the professional editor has a good master of both language and scientific writing. Thank you so much again!

6. This kind of validations are mostly presented in 2 papers: one technical (including reference values, et cetera)

Answer: We’re afraid that we don't fully understand this question. Is some important information missing in the question? We wonder if it is the similar topic as question 2 regarding the reference values of Simoa SCC-Ag assay. Usually as part of the Simoa homebrew procedure, 5-10 healthy samples would be tested when a prototype is done. We have tested our SCC-Ag assay in several healthy and benign samples and the results is presented as below. We admitted this is only a preliminary data. Its objective is to prove the tests is normal and Simoa SCC-Ag assay has ability to detect samples with low SCC-Ag concentrations. In order to evaluate the reference values for cervical cancer diagnosis, more healthy and benign controls need to be tested in multi-laboratory in the future. However, again, the objective of this work is to track the kinetic profile of SCC-Ag on cervical cancer patients and the quality of Simoa SCC-Ag assay is validated by method comparison to the Architect assay. If we misunderstood you, please don't hesitate to tell us. We’ll definitely answer your question as soon as possible. Thank you so much again!

Simoa SCC-Ag levels on healthy and benign controls:

(pictures can not be shown here, please refer to the file we uploaded as supplementary file 'response to reviewers' comments)

7. Abstract: are the presented concentrations concerning the Simoa assay?

Answer: Yes. To make it more clear, a modification was made in the abstract as ‘The median values for each time-point detected by the Simoa assay were 2.49, 0.66, 0.61, 0.72, 0.71 ng/mL, respectively.’ Please refer to the manuscript Line 45-46, Page 3.

8. What are the intermediate and high-risk factors?

Answer: Thank you so much for your question. The definition of intermediate and high-risk factors was well accepted by gynecologic oncologists. Therefore, we only mentioned that ‘with intermediate and high risk factors according to Sedlis criteria’ in the original manuscript. However, for target readers of BMC Cancer (a broader cancer-related journal), we should clarify the definition to avoid
misunderstanding. That’s why we’d like to show our sincere gratitude to you. We added the definition in the manuscript (please refer to Line 167-169, Page 9). As to the Abstract, we made certain changes as well (please refer to Line 47-48, Page 3). It is a bit complicated that the pre-surgery SCC-Ag level was related to FIGO stage, stromal invasion, and lymph node metastasis with statistical significance, but not to lymphovascular space invasion (LVSI). After surgery, we noted that patients with positive LVSI and lymph node had higher SCC-Ag levels at the time points Day 4 and Week 2-4 than those without. Therefore, we only mentioned certain risk factors in the Abstract without the specific details considering the word limit. Thanks again for your comment.

9. What are similar tendencies?
Answer: It means that similar kinetic patterns of SCC-Ag levels were observed in the lymph node positive and negative groups. To make it clear, a modification is made as ‘Furthermore, although patients with positive lymph node had sustained higher SCC-Ag levels compared to those negative counterparts, similar kinetic patterns of SCC-Ag levels were observed after surgery.’ (Please refer to Line 51, Page 3)

10. SCC-Ag is also available at other well-known platforms, such as the Cobas assay from Roche. Add to references
Answer: Thanks for the suggestion. A description is added into the Introduction part as ‘SCC-Ag assays are also available on other well-known platforms, such as the Elecsys® SCC assay used on the Roche Elecsys and cobase analyzer (Roche Diagnostics, China)’ (Line 70-72, Page 5). A corresponding reference is also added.

Gian Franco Zannoni (Reviewer 2):
Authors firstly described the kinetic profile and clinicopathological implications of SCC-Ag before and after radical hysterectomy within six months' duration, in 92 cervical cancer patients prospectively enrolled, by a self-developed SCC-Ag Single Molecule Assay (Simoa) prototype immunoassay levels. In the second part of work, they looked at the relationship between SCC-Ag values and clinicopathologic features. Not surprisingly, the pre-treatment SCC-Ag level was related to tumor aggressiveness as indicated by advanced stage, deep stromal invasion and lymph node metastasis. A new finding was that patients with intermediate and high risk factors had higher SCC-Ag levels postoperatively while the difference became insignificant six months after surgery. Patients with positive lymph nodes before surgery showed sustained elevated levels of SCC-Ag compared to those negative counterparts. In contrast, although patients who received adjuvant therapy had raised baseline SCC-Ag level, no difference existed at completion of treatment. They also postulated that the absolute levels of SCC-Ag might be determined by the disease severity, while the dynamic change was possibly influenced by post-operative adjuvant treatment.

As declared by the Authors, the study has several limitations. Firstly, not all patients completed the five points' blood collection. Secondly, the sample size is not so large. Lastly, given the short-time follow up, no survival outcome was analyzed in the current work.

The text is well written and the work well conceived, with an accurate statistical analysis Authors should better stress some important concepts in Discussion sections, avoiding repetitions. I retain that this article can be considered suitable for publication, after minor revision.
Minor Compulsory Revisions

1. The clinical and scientific relevance of the paper is very important. Anyway, an accurate revision of the literature is necessary.

Answer: Thank you so much for pointing out this problem. We’re truly sorry for the inappropriate mistake. We carefully checked the reference and made corresponding changes in the manuscript. Thank you so much again!

2. Please, enrich references section.

Answer: Thank you so much for your suggestion. We searched the PubMed and updated several recent publications in the manuscript. Please refer to the Paragraph 2 in the Introduction section for reference modification. We also made changes in the Discussion section (please refer to the Paragraph 2 and 4 in the Discussion section). Thank you so much again for your dedication. Your comments and suggestions really enabled us to improve the quality of our manuscript. Many thanks!

3. Please, add exclusion/inclusion criteria

Answer: Thank you for your suggestion. The major inclusion criteria were mentioned in the original manuscript (please refer to Line 154-157, Page 9). We added major exclusion criteria as “Patients with any skin disorder or past cancer history were excluded.” (please refer to Line 157-168, Page 9). If you think it is necessary, we can provide the complete list of inclusion/exclusion criteria in the trial proposal as a supplementary file. Thank you so much for carefully reviewing our manuscript.

4. Check for English language in all the paper and improve text style and format

Answer: Thank you so much for carefully reviewing our manuscript. The current manuscript has been edited by American Journal Experts (AJE) and the editing certificate was also submitted. Thank you so much again!