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Dear Dr. Lian-Sheng Ma
Editor-in-Chief
Baishideng Publishing Group Inc
World Journal of Clinical Oncology

RE: Manuscript ID 64356: "**A biomarker study in Peruvian males with breast cancer"** by Carlos Castaneda *et al*.

Thank you for the opportunity to respond to the reviewers on the above referenced manuscript. Enclosed please find our revised manuscript which has taken into account their comments and suggestions. In addition, we have uploaded a copy of the revised manuscript with changes highlighted as a supplemental file.

Our specific responses to the reviewers are as follows:

REVIEWER #1

- **Comment 1**: Title: the title did not reflect the result of this study. it's very general and could be written sharper based on the endpoint of the study (the prognosis).
  Response 1: We have changed "Biomarkers in Peruvian males with breast cancer" to "A biomarker study in Peruvian males with breast cancer" in title section.

- **Comment 2**: Abstract: Many of the abbreviations appear for the first time in the abstract, thus what it is stand-for needs to be written in the abstract. The statistical method written in the abstract not consistent with Method section line 155 and Result line 181 (intraclass or interclass?)
  Response 2: All abbreviations were described in the abstract. some abbreviations that were not essential in the abstract were withdrawn. We have corrected in the Method section to intraclass (Line 202).

- **Comment 3**: Methods: The authors should explain how to get OS data and categorize it (less than 5 yrs, 5 ysr, and more than 5 yrs?), in the Methods section and not in the Discussion section.
- **Response 3:** We added a sentence in the method section: "Live status of patients with not accurate follow-up was obtained from the Peruvian national registry (https://www.reniec.gob.pe) through the Epidemiology Department of the institution".
  We added median disease-free survival in early disease group and overall survival in the whole population to understand the categorization. It was added in Results section: "Patients characteristics".

- **Comment 4**: The author should explain what is included in "routine histopathological data" (line 113) and biomarkers, either in the Methods section or Introduction section.

- **Response 4:** We have changed to "TIL and histological grade was prospectively evaluated…" the Methods section (Line 138).

- **Comment 5**: In the Immunohistochemistry Sub-section (line 120-123), the authors should be consistently write the protein marker first, followed by the primary antibody and its manufacturer inside the brackets.

  Response 5: The order was homogenized as indicated (Line 143-147).

- **Comment 6**: For statistical analysis, what interclass correlation test that you used (Pearson?)? And how did you conduct the interclass correlation test on categorical (discrete) variables with different numbers of frequency, for example between ER (n=49) and AR (n = 35)? What is (JS) in line 133?

  Response 6: Changed to intraclass correlation test. Correlation of cell count in similar areas between ER and AR was performed in 34 cases (with available and accurate tissue). It was indicated in the Methods section and reaffirmed in the Results section: "Evaluation of similar areas for ER and AR in 34 lesions found that a median of 3738 cells". We indicate the initials of the pathologist who performed the evaluation (Joselyn Sanchez: JS).

- **Comment 7**: Result: The reader will be easier to understand if the patient's characteristics are shown in the form of Table. For lines 183-184, which stated that "No association between the median of ER-positive tumor cell count proportion and MMR-loss was found", the association is not between the median of both variables but the variables themselves.

  Response 7: We added the association between the AR and MMR-loss variables in results (Line 278).

- **Comment 8**: The wording of the subsection should be clearer, e.g. "Patients" (Patient's characteristics)," Biomarkers and clinical-pathological features" (Biomarkers, clinical-pathological features, and the correlations), "Prognostic clinical-pathological factors" (The association of prognostics and clinical-pathological factors).

  Response 8: Changed as indicated.

- **Comment 9**: Discussion Line 202 needs further explanation about what the authors mean by AR-positive because based on Table-1 there are 5,9% of AR 1-9% expression, other than 85,3% of AR >9% expression. The term of large and small series are unfamiliar, it is better to use the term of large or small sample size study.
- **Response 9:** AR positive was defined in Methods section (Line 150). Category 1-9% was grouped as negative in Table 1.
  We used the term of large or small sample size study (Line 283 and line 295).

- **Comment 10**: Line 228-230 should be elaborated more, to bridge the association of MMR-loss with anti-PDL1 and endocrine therapy. Is it increase the resistance? Line 238-239 are better written: Additionally, a recent study on drugs targeting the PIK3CA pathway has demonstrated promising effect in breast cancer…

  Response 10: We expand the Discussion section:
      -Line 300: "We found that MMR-loss was associated with the previously described marker of endocrine response [3,4], the lower AR expression. Furthermore, this association appears to

be specific for this steroid marker and not for ER, despite AR and ERexpression were co-related. A publication suggests that alteration in DNA damage repair genes could produce endocrine resistance because of the finding that defects in MMR pathway genes doesn't allow an accurate suppression of CDK4 by endocrine therapy".

-Line 365: "Additionally, a recent study demonstrates that Alpelisib, a drug targeting the PIK3CA pathway, has activity in breast cancer cases with presence of the mutation".

- **Comment 11**: Line 256 – 258 are better written: Our study serves as the largest male BC study to complement male BC knowledge in the South American population, which has been understudied.
  Response 11: Changed "Our results also increase the knowledge in the South American population that is under-studied in the largest male BC series" to "Our results serve to complement male BC knowledge in the South American population, which has been under-studied in the largest male BC series.

- **Comment 12**: The authors should add sample illustration of TIL Table: The title of the table should mention about association or correlation.
  Response 12: We have added the Figure xx contrasting high vs low level TIL (Luminal B and Luminal A groups).

- **Comment 13**: The header that states "n-54" is not accurate because it is only applied for age. The authors should add table captions that explain briefly about statistical test for p.

  Response 13: We have changed "n-54" to "n" in table 1. Statistical test was indicated for each p value. We have provided a Word file t with the table 1 in the attachment.

- **Comment 14**: Quality of manuscript organization and presentation. The authors should reconsider the form or the choice of the verb, noun, or preposition in some sentences such as line 1" varies"(vary), line 65 "slides" (slide), line 96 "few" (a few), line 97 "subtype"(subtypes), line 104 "over" (on or overall?), line 131 and 138 "were" (was), line 162 "placed" (located), line 166 "sere" (was), line 196 "survival" (overall survival?), line 210 "similar to in the female BC" (similar to the female BC), line 231 "is" (are), line 242 "methodology" (method), line 248 "Finally, our finding …." (+shows), line 251 "a weakness" (the weaknesses).
  Response 14: Changed as indicated.
- **Comment 15**: The authors should consistently use a hyphen for ER- or AR-positive and tumor-infiltrating lymphocyte, such as in lines 52, 64, and 77. The authors should add "degree symbol" before C (celsius) in lines 145-146.
  Response 15: Degree symbol was added.

- **Comment 16**: The author should use consistent terms for variables, such as prognostics or OS?, clinical-pathological features or clinical-pathological factors?
  Response 16: The terms were homogenized.

REVIEWER #2

- **Comment 17**: Title: the title seems very general to me. In my opinion, it would be useful to clarify that this is a study relating to a particular sampling (like: "a study of biomarkers in Peruvian males with breast cancer").
  Response 17: We have changed "Biomarkers in Peruvian males with breast cancer" to "A biomarker study in Peruvian males with breast cancer" in title section.

- **Comment 18**: Abstract: at line 64, define ER (estrogen receptor) like in the introduction.

  Response 18: ER was defined.

- **Comment 19**: Page 4, line 112: Explain what "TIL" means (tumor infiltrating lymphocytes, I guess) and also explain what H&E (hemalun-eosin) means to readers who are not familiar with oncology or histology.
  Response 19: Abbreviations were described.

- **Comment 20**: Page 5, line 117: "Tissue sections of 4 mm thickness were cut from 35 available tissue paraffin blocks". It would be useful to specify which fixative was used for the tissues studied.

  Response 20: Immunohistochemical method was detailed in Method section (Lines 142-155).

- **Comment 21**: Page 5, lines 118 to 125: As the manuscript is largely based on the detection of specific biomarkers with an immunohistochemical method, this part needs to be more detailed. Explaining that the immunohistochemical study was performed according to the manufacturer's instructions is not sufficient, so it seems necessary to detail the technique used. What was the method used for the detection of each antibody: a direct method? Indirect with a first antibody and a second with peroxidase or another enzyme? In this case, specify the substrate and the chromogen used. Were the negative controls by omission of the antibody?

  Response 21: Immunohistochemical method was detailed in Method section (Lines 142-155).

- **Comment 22**: Line 352: figure 1: the micrographs are of very good quality. Add a scale bar on the pictures. Give the picture of a control in an insert.

  Response 22: We have added a scale bar and a control for each staining on the Figure 1. We have provided a PowerPoint with the original picture in the attachment.

EDITORIAL OFFICE'S COMMENTS

- **Comment 23**: (1) Science editor: 1 Scientific quality: The manuscript describes a retrospective study of the biomarkers in peruvian males with breast cancer. The topic is within the scope of the WJCO. (1) Classification: Grade C and Grade C; (2) Summary of the Peer-Review Report: The authors evaluate the molecular biomarkers and the impact on survival of breast cancers in Peruvian males. It is interesting and particularly useful, breast cancer in males being a rare affection and ultimately little studied. However, the questions raised by the reviewers should be answered; and (3) Format: There is 1 table and 1 figure. (4) References: A total of 20 references are cited, including 3 references published in the last 3 years; (5) Self-cited references: There are 3 self-cited references. The self-referencing rates should be less than 10%. Please keep the reasonable self-citations that are closely related to the topic of the manuscript, and remove other improper self-citations. If the authors fail to address the critical issue of self-citation, the editing process of this manuscript will be terminated; and (6) References recommend: The authors have the right to refuse to cite improper references recommended by peer reviewer(s), especially the references published by the peer reviewer(s) themselves. If the authors found the peer reviewer(s) request the authors to cite improper references published by themselves, please

send the peer reviewer's ID number to the editorialoffice@wjgnet.com. The Editorial Office will close and remove the peer reviewer from the F6Publishing system immediately.

Response 23: We deleted one self-cited reference (Castaneda CA, Cortes-Funes H, Gomez HL, Ciruelos EM. The phosphatidyl inositol 3-kinase/AKT signaling pathway in breast cancer. Cancer Metastasis Rev 2010;29:751–759). The self-referencing rate is less than 10%.

- **Comment 24**: Language evaluation: Classification: Grade A and Grade B. 3 Academic norms and rules: The authors provided the Biostatistics Review Certificate, and the Institutional Review Board Approval Form. Written informed consent was waived. No academic misconduct was found in the Bing search. 4 Supplementary comments: This is an unsolicited manuscript. The study was supported by 1 grant. The topic has not previously been published in the WJCO. 5 Issues raised: (1) The title is too long, and it should be no more than 18 words.

  Response 24: We have a title with nine words: A biomarker study in Peruvian males with breast cancer.

- **Comment 25**: (2) The authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy of any approval document(s); (3) The authors did not provide original pictures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor;

  Response 25: We provided the funding agency copy of the approval document (No. 198-2015-FONDECYT, through the grant "EF041: Basic research projects and applied research projects".)

- **Comment 26**: The "Article Highlights" section is missing. Please add the "Article Highlights" section at the end of the main text.

  Response 26: We have added the "Article Highlights" section at the end of the main text.

We hope that you will find the revisions satisfactory and that our manuscript is now acceptable for publication in the World Journal of Clinical Oncology.

Sincerely,

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