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August 18, 2017

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

RE: Manuscript ID 35215: "**Clinico-pathological predictors of long-term benefit in breast cancer treated with neoadjuvant chemotherapy**" by Marco Galvez *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 02725974

- **Comment 1**: Introduction: "More than 50% of all breast cancer patients will receive neoadjuvant chemotherapy (NAC)…": More than 50% is very high for all Breast cancer. It is not the usual practice in our country.

  Response 1: Changed for "Neoadjuvant chemotherapy (NAC) is the standard therapy for locally advanced breast cancer"

- **Comment 2**: Patients: "Stromal lymphocytic infiltration (sTIL) was prospectively evaluated in pre-NAC core biopsy and was defined as percentage of stromal area covered by lymphocytes[16].": Different sTIL cut of rates are reported (ref 1, 28): could you explained the choice of 60% cut-off

  Response 2: Changed to 50% because it is the cutoff indicated in the article Salgado R, *et al*. Annals of oncology. 2014; 26: 259-71.

- **Comment 3**: Results: "There were 435 patients included for this study, median age at diagnosis was 49 years (range 24–84), median tumor size was 6.5 cm (range 1.0–24.0), T3 was found in 27.8% and T4 was found in 63.9%.": Are inflammatory breast cancer included. If yes, how many patients and rate?

  Response 3: Inflammatory breast cancer was present in 29.2% of the cases (added to the text).

- **Comment 4**: "Luminal A, Luminal B, HER2-enriched and TN phenotype was found in 48.3%, 14.5%, 17.7% and 19.5%, respectively.": Near 50% of patients were Luminal A: this is very

different in comparison with the majority of NAC studies. We know that Luminal A tumors had poor pCR rate. An explanation and discussion about this rate is mandatory.

Response 4: Luminal A and Luminal B cases were reviewed taking account of PgR and grade, and frequency of subtypes were modified: Luminal A (24.6%), luminal B (37.9%), HER2 enriched (17.7%), TNBC (19.8%)

- **Comment 5**: "Complete pathological response (pCR) was observed in 48 (11%) patients.": It is a very low pCR rate which could be explained by the high rate of Luminal A BC Clinicopathological factors associated to pCR according to Breast Cancer Subtypes: Predictive factors of pCR could be analyzed by multivariate binary regression analysis in order to determine independent factors of pCR for all population and respectively for each subtype.

Response 5: Logistic regression analysis finds that only TILs remained associated to pCR in the whole series (p=0.031) and had a trend to be associate (p=0.054) in Luminal A subset.

					95%CI for EXP(B)	
	Variables	**Odd Ratio (OR)**	**p**	**Exp(B)**	**LCI**	**UCI**
Población Total	%sTIL	OR (sTIL>=50% / sTIL<50%)	0.031	1.98	1.06	3.70
Luminal A	%sTIL	OR (sTIL>=50% / sTIL<50%)	0.054	3.20	0.98	10.50
Luminal B						
Her2						
TN						

- **Comment 6**: Prognostic clinicopathological factors according to Breast Cancer Subtypes: Multivariate analysis (Cox model) results could be contributive Multivariate analysis, without pCR but including sTIL could be interesting in order to determine if sTIL rate is an important prognostic factor for DFS and OS Figures are not cited in the text

Response 6: Cox model results for OS, regarding to sTIL did not achieve significance in the whole population (p=0.14) nor subtypes.

Cox model results [Total]

				95%CI for EXP(B)	
Variables	**Hazard ratio (HR)**	**p**	**Exp(B)**	**LCI**	**UCI**
Histological grade	HR (G1-G2 / G3)	**0.01**	0.65	0.46	0.91
Relapse	HR (No / Yes)	**<0.001**	0.10	0.07	0.15
Molecular subtype	HR (LumA / TN)	**<0.001**	0.50	0.33	0.76
	HR (LumB / TN)	**0.01**	0.52	0.31	0.87
	HR (Her2 / TN)	**0.06**	0.63	0.39	1.02
%sTIL	HR (<50% / >=50%)	0.14	0.77	0.54	1.09

Cox model results [LumA]

				95%CI for EXP(B)	
Variables	**Hazard ratio (HR)**	**p**	**Exp(B)**	**LCI**	**UCI**
Clinical Stage	HR (II / III)	**0.04**	0.12	0.02	0.92
%sTIL	HR (<50% / >=50%)	0.46	1.46	0.53	3.98

Cox model results [LumB]

					95%CI for EXP(B)	
	Variables	**Hazard ratio (HR)**	**p**	**Exp(B)**	**LCI**	**UCI**
Modelo 1	Age	HR (age>=50/age<50)	**0.040**	0.59	0.35	0.99
Modelo 2	Relapse	HR (No/Yes)	**<0.001**	0.16	0.09	0.27
Modelo conjunto	Relapse	HR (No/Yes)	**<0.001**	0.15	0.08	0.27
	Age	HR (age>=50/age<50)	0.130	0.67	0.40	1.13
	%sTIL	HR (sTIL<50%/sTIL>=50%)	0.230	0.72	0.41	1.24

Cox model results [Her2]

				95%CI for EXP(B)	
Variables	**Hazard ratio (HR)**	**p**	**Exp(B)**	**LCI**	**UCI**
Relapse	HR (No/Yes)	**<0.001**	0.10	0.04	0.24
%sTIL	HR (<50% / >=50%)	0.87	1.07	0.49	2.35

Cox model results [TN]

				95%CI for EXP(B)	
Variables	**Hazard ratio (HR)**	**p**	**Exp(B)**	**LCI**	**UCI**
Relapse	HR (No/Yes)	**<0.001**	0.10	0.04	0.25
%sTIL	HR (<50% / >=50%)	0.10	0.59	0.32	1.10

- **Comment 7**: Discussion “Pathological complete response is lower in Luminal-A (7.1%) subtype than Luminal-B (15.9%), HER2-enriched (13%) and TNBC (15.3%)”: pCR rates and patient’s number are different between Table 1 and 2…

  Response 7: Changed.

- **Comment 8**: “Pathological complete response is also associated to longer survival in the whole population as well as in Luminal A (100% vs 59.6% 5 year OS, p<0.001) and TNBC (92.3% vs 33% 5 year OS, p=0.006) (and trend in Luminal-B and HER2-enriched) phenotypic subsets of our series.”: These results are not presented in Chapter Results.

  Response 8: Changed.

- **Comment 9**: “Contrary to our results, Minckwitz et al. found pCR was not associated to prognosis only in Luminal-A tumors in 6377 patients with Anthracycline-Taxane-based NAC from 7 randomized trials and some authors claim it is related to the observed continuous tumor shrinkage occurred in their ER-positive tumor group during extended NAC different than early and short-period tumor shrinkage observed in the ER-negative group[6, 18-24].”: Population of

BC patients is different with this study (very high level of T4 tumors and probably including some inflammatory BC).

Response 9: I agree.

- **Comment 10**: “Pathological complete response was more frequent in small tumor in both the whole population and in Luminal-A subtype in our series.”: But only in Luminal A subtype (which represent near 50% of patients)

Response 10: Proportion of Luminal A subtype was reduced (because added PgR and HG to the classification criteria) and the current association between pCR and small size is not significant (now only a trend).

REVIEWER 00739752

- **Comment 11**: The abbreviation passes is clearly written. These corrections have been marked on the attached file

Response 11: It was corrected.

REVIEWER 00724492

- **Comment 12**: Please rewrite aim in abstract. It is not clear.

Response 12: It was re-written.

- **Comment 13**: It should be noted that neoadjuvant chemotherapy is applied after treatment.

These treatments are effective on the OS and PFS.

Response 13: I agree: Neoadjuvant chemotherapy is followed by surgery.

- **Comment 14**: In material and method, statistical analysis rewrite (PFS ?, RECIST criteria, etc)

Response 14: Added to material and method [Lines 172-174].

- **Comment 15**: Please add follow-up criteria?

Response 15: Added in material and methods [Lines 160 and 161].

REVIEWER 02510166

- **Comment 16**: Did all patients undergoing neoadjuvant chemotherapy proceeded to receive surgery (how large is the population from which the 435 cases were selected)?

Response 16: The whole population went to surgery. Population We reviewed to find the 435 cases was much larger.

- **Comment 17**: Numbering of the Tables does not match, "Table 2" not referred to in the text.

  Response 17: Corrected.

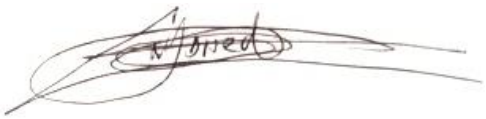
REVIEWER 00289387

- **Comment C**: There were two tables Table 2 and 3 were redundant in some parts, therefore, it should be combined to be one table.

  Response C: We combined table 2 and 3.

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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Aug 18, 2017

Scientific Research Process

Name of Journal: World Journal of Clinical Oncology

ESPS Manuscript NO: 35215

Manuscript Type: Observational Study

Title: Clinico-pathological predictors of long-term benefit in breast cancer treated with neoadjuvant chemotherapy

Authors: Marco Galvez, Carlos A. Castaneda, Joselyn Sanchez, Miluska Castillo, Lia Pamela Rebaza, Gabriela Calderon, Miguel De La Cruz, Jose Manuel Cotrina, Julio Abugattas, Jorge Dunstan, Henry Guerra, Omar Mejia, Henry L. Gomez.

Correspondence to: Carlos A. Castaneda, MD, MSc, Research Department, Instituto Nacional de Enfermedades Neoplasicas, Av. Angamos Este 2520 Surquillo, Lima, Lima15038, Peru. ccastaneda@inen.sld.pe

1 What did this study explore?

This study explores differences among the four molecular breast cancer subtypes (Luminal A, Luminal B, Triple-Negative and HER2-enriched), regarding pathological response to chemotherapy and tumor infiltrating lymphocytes in addition to other standard clinicopathological features.

2 How did the authors perform all experiments?

We worked along with epidemiology department in a comprehensive system to detect every patient with breast cancer who came to the institute from 2003 to 2014 and selected patients who received neoadjuvant chemotherapy by reviewing every patient file.

An excel with relevant clinicopathological information obtained from every patient file was developed and completed.

We looked for those with available pathology material at Institute archive and an Institute Pathologist prospectively reviewed every hematoxylin- eosin slide in order to determine Tumor Infiltrating Lymphocyte levels (this biomarker is not usually determined in standard form).

3 How did the authors process all experimental data?

Clinicopathological information was analyzed by Institute Statistical and every result was discussed by the authors/ coauthors in order to define if more statistical tests are needed.

4 How did the authors deal with the pre-study hypothesis?

Our pre-study hypothesis is based in the fact that TIL density is a prognosis biomarker in the whole breast cancer.

Based in previously published data, we completed information in the formula for sample size calculation regarding a single proportion.

$$n = \frac{Z_\alpha^2 \times p \times q}{d^2} = \frac{1.96^2 \times 0.516 \times 0.484}{(5\%)^2} = 384$$

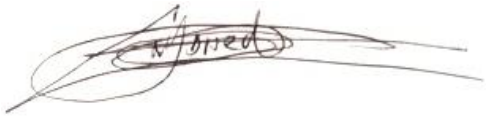
where;
n = sample size
Z_α^2 = confidence level at 95%
p = Proportion of high-sTIL patients alive at 5 years
q = 1-p
d = margin of error

We conclude that we need to evaluate almost 400 cases to find an association between TIL level and survival.
We classified our series in Luminal A, Luminal B, Triple-Negative and HER2- enriched subtypes, and evaluated the effect of pCR and high TIL levels in every subtype over survival.
Additionally, we evaluated predictive role of clinicopathological features for having high level of TILs and pCR in every breast cancer subtype.

5 What are the novel findings of this study?
This is the first series evaluating tumor infiltrating lymphocytes in Hispanic Breast Cancer cases. We found that pCR was associated to early stage in Triple-Negative breast cancer and had a trend to be associated to TIL in Luminal A but not in other subtypes. TIL was associated to grade III in Luminal B and Triple-negative, and to node involvement in Luminal B.
Survival was associated to age in Luminal B, to clinical stage II in Luminal A, to absence of node involvement in Triple-negative and to pCR in Triple-negative.

Thank you again for evaluating to publish our manuscript in the World Journal of Clinical Oncology

Sincerely yours,



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CERTIFICATE OF BIOSTATISTICS

To whom it may concern

Title of study: Clinico-pathological predictors of long-term benefit in breast cancer treated with neoadjuvant chemotherapy

Chief investigator: Carlos A. Castaneda
Research Department, Instituto Nacional de Enfermedades Neoplasicas, Lima 15038, Peru.

This letter is to confirm that I have read the ethics application prepared for this study, and that in my opinion the statistical methods and techniques mentioned are appropriate for the research.

Please contact me if you have any queries.

Sincerely,

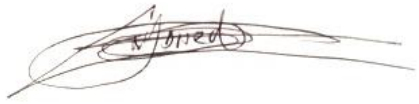
Monica Lizares

Date: 21 Nov 17

Email: monica.lizares106@gmail.com

Data sharing statement

No additional data are available.



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