Author’s response to reviews

Title: HER2 overexpression and correlation with other significant clinicopathologic parameters in Ivorian breast cancer women

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Version: 1 Date: 24 Oct 2018

Author’s response to reviews:

October 24, 2018

Dear Editor

I am writing to submit our revised manuscript entitled, “HER2 overexpression and correlation with other significant clinicopathologic parameters in Ivorian breast cancer women” (CPAT-D-18-00042) for consideration for publication in BMC Clinical Pathology.

I highly appreciate the consideration that this excellent journal gives to our manuscript in which our findings strongly indicate that not only HER2 status is highly associated with grade tumor in young women which highlights the aggressiveness of this breast carcinoma subtype but also a significant number of patients could benefit from trastuzumab in Côte d'Ivoire.

In addition, we provide some responses to the specific comments of the reviewers as follows:
Reviewer #1: Response to specific comments:

Point 1: The work is limited by the absence of the study of the expression of Ki-67 and the study of the Her2 equivocal ones. These deficits should be explained as they have an impact on the treatment decision.

Answer: The three biomarkers (ER, RP, and HER2) are now routinely tested together to predict response to chemotherapy. We have started the immunohistochemistry (IHC) with only hormonal receptors and HER2 in June 2013. We are newly done the routine IHC test of ER, PR, and HER2 of breast cancer based on the available means of our sponsor, Roche Laboratory. In addition, some well-known authors in our manuscript reference list (Lal P et al., Al-Ahwal MS, Liu X, Ayadi L, Ali EM) used the hormonal receptors and HER2 for patients’ management in their studies. Moreover, our study goal was to determine the prevalence of breast cancer patients overexpressing HER2 since 1% of breast cancer patients overexpressed HER2 before the advent of the IHC in our country. The Ki67 index was not considered in our study as mentioned on lines 16 to 19 on page 4 under subheading: Immunostaining assessment even if it is required to define the molecular classification of breast cancers for better treatment strategies; however, we could not afford it to the limited budget and this study did not highlight the molecular classification of breast cancers. The HER2 equivocal were not analysed because we did not have FISH in our laboratory as mentioned on line 9 to 12 on page 4 under subheading: Immunostaining assessment. We mentioned them in our data to search for a sponsor which will equip our laboratory with FISH technique.

Point 2: I could not understand how the molecular profiles (Like), namely Luminal B, were determined because they need to know the rate of proliferation (KI-67) (Table I). This aspect has not been described. I believe it should be described.

Answer: Correct, and we added clear description of the molecular subtypes based on ER, PR, and HER2 under the sub-heading: Immunostaining assessment of ER/PgR on lines 22 to 36 on page 4. We did not considered the rate of Ki67 in our study as mentioned early in the answer of point 1.

Point 3: In Table 2 does not understand what RO / RO and RO / RP- is?

Answer: Correct. It is a spelling error. " ER /PR+ and ER / PR- " should be the appropriate words in Table 2 on line 36 to 39 on page 8.
Point 4: I believe that in the discussion I should discuss the results a little better in the light of other more recent African studies such as: ecancer 11 763 / https://doi.org/10.3332/ecancer.2017.763

Answer: Correct, and from lines 21 to lines 28 on page 9 Under discussion in the revised manuscript, we discussed the prevalence of HER2, the most important part of our study goal, with other recent African studies done in Angola, Tunisia, Nigeria, Mali, Uganda, Ghana, and Senegal. However, these African studies did not provide any correlation of HER2 with other clinico-pathological parameters. These recent African studies with other studies were added and highlighted in yellow under the reference section.

Point 5: I also consider that the authors should argue that in countries with few resources it is possible to accept and consider that the study of tumors with these 3 ER antibodies is already a great advance; PR and HER2, knowing that there are patients who will not be spared chemotherapy (initial tumors Luminal A, but that are few given the delay in diagnosis) and HER2 equivocation.

Answer: Correct and we mentioned this point at the beginning of the discussion on lines 6 to 12 on page 9.

Reviewer # 2: Response to specific comments:

Point 1: One of major concern is the molecular sub-typing criterion which is not described in the method part but molecular sub-typing result is presented in table I. Therefore, I would suggest that authors either remove this data from table I or clearly indicate the sub-typing criterion used.

Answer: We added a detailed description of the molecular subtypes based on ER, PR, and HER2 under the sub-heading: immunostaining assessment on lines 22 to 36 on page 4. We did not considered the rate of Ki67 in our study as mentioned on line

Point 2: Another concern is the reliability of the association study. The authors reported that HER2 overexpression is associated with high tumor grade. However, their data on table II page 6 indicates the opposite. According to the result summarized on table II, HER2 negative tumors had high tumor grade or grade III (21.8%) than HER2 overexpressing tumors (11.7%).

Therefore, I suggest authors look at this discrepancy and correct the manuscript.
Answer: Our data in the table II are valid and reliable. We used the Chi-Square Test to analyse the correlation of HER2 status with tumor grade as reported on lines 45 to 48 on page 4 under the Sub-heading: Statistical analysis. We found a p value =0.007 in the Table II (on lines 21 to 22 on page 8) which was statistically different from 0.05. This result strongly suggests that there is reliable association between HER2 status and the tumor grade. Moreover, the combined results of HER2+ grade II and III tumors (86.2%) is slightly greater than that of HER2- grade II and III tumors (78.8%) was added to the sub heading: Relationship between HER2 status and clinicopathologic factors on lines 10 to 15 on page 7. Therefore, we could suggest that the HER2 status is associated with grade as we mentioned that this study revealed that the HER2 overexpression was closely associated with the Nottingham grade, and thus, suggesting the aggressive pattern of the HER2+ patients with breast cancer. on line 30 to 37 on page 10 (discussion).

Point 3: I also have a concern on the title of the manuscript "HER2 overexpression and significant clinicopathologic parameters in Ivorian women with breast carcinomas"

The reason for the emphasis on HER2 overexpression while the other two standard hormone receptors were also assessed in the study is not clear. It would have been acceptable had there been a unique finding in the expression of HER2 among Ivorian than patients elsewhere in the continent or worldwide.

I would suggest that authors report on all of the hormone receptors and modify the title accordingly.

Answer: The title of our manuscript mainly came from our study objective which was to assess the HER2 status and its association with clinicopathologic factors in women breast carcinomas for better treatment strategy and prognostic prediction on lines 48 to 53 on page 2. In addition, we clearly reported the components of clinicopathologic factors which included the classic or standard clinicopathologic items (age, histologic type, ...) and the hormonal receptors and HER2 status as mentioned on lines 20 to 25 on page 3 (Patients). We found that the title should be "HER2 overexpression and correlation with other significant clinicopathologic parameters in Ivorian breast cancer women" reported on lines 3 to 6 on the page linstead of "HER2 overexpression and significant clinicopathologic parameters in Ivorian women with breast carcinomas" since Our main interest of this study was HER2 prevalence, and hormonal receptors were included into the other significant clinicopathologic parameters.

Point 4: HER2 equivocal cases are considered as negative by previous authors in the absence of FISH to confirm the results hence it would be good to include these cases under the HER2 negative results. Answer: The HER2 equivocal cases were not analysed because we did not have
FISH in our laboratory as mentioned on lines 9 to 12 on page 4 (immunostaining assessment). We mentioned them in our data to search for a sponsor which helps us equip our laboratory with FISH technique. In addition, some authors considered the HER2 equivocal cases as positive. These cases are really debatable so we only wanted to show their rates in our manuscript.

Point 5: The discussion on page 8 second paragraph line 25 to line 45 describes methodological issues related to HER2 immunohistochemistry which is unrelated to the work presented by the authors. Therefore, remove this part and rewrite the discussion part with only supporting studies related to your current work.

Answer: We used the IHC to examine the status of HER2 from the paraffin-embedded breast tissue blocks. We found a low value of HER2 compared to other recent African studies as mentioned on lines 21 to 28 on page 9 (discussion). In contrast, the authors of these regional or African studies have not demonstrated the mechanisms or reasons underlying the low or high proportion of HER2 overexpression found in their studies. In our laboratory, we encountered several issues such as the fixation, the storage, the IHC issues as thoroughly described by Mitchell and Varga on lines 30 to 46 on page 9 (discussion). These relevant findings highlighting the importance role of the preanalytical factors such as fixation, the storage conditions, and technique type of HER2 detection as mentioned on lines 45 to 51 on page 9 (discussion) could explain our HER2 positivity rate since we did not efficiently control these factors. Therefore, their contributions are considerable in order to improve the rate of HER2 positivity for our laboratory.

Point 6: The conclusion sentence should be restated once the association between HER2 overexpression and high tumor grade is confirmed.

Answer: The association between HER2 overexpression and tumor grade is confirmed as explained in the answer of Point 2 (review 2). Thus, we could restate that the HER2 overexpression is associated with relatively high grade breast carcinoma on lines 18 to 21 on page 11 (Conclusion).

Point 7: Typos and editing:

- Page 2 background paragraph 3 line 42-45 has a language error

Answer 7.1: We corrected the language error as follows: The immunohistochemical analysis of HER2 with ER and PR is a routine clinical practice [14-16] which has recently been integrated into the management of breast cancer patients in Ivory Coast, where the data on HER2 are unknown on line 42-48 on the page 2 under subheading: Background.
Page 3 methods paragraph 1 (patients) "needle core biopsies" should change to "core needle biopsies".

Answer 7.2: Needle core biopsies was change to core needle biopsies in Patients section (page 3, lines 15 to 16)

Page 5 table I line 18 IDC-NST percentage should be 84.1 not 841

Answer 7.3: we changed 841 to 84.1% in the table I (lines 18 to 18, page 6)

Page 6 table II line 48 and 49 has a typing error ER/PR+ and ER/PR- are the correct abbreviations

Answer 7.4: Correct and changes were made in the table II (lines 36 to 39, page 8).

Page 7 discussion paragraph 2 (patients) line 23 "the type of used antibody" should change to "the type of antibody used".

Answer: Correct and we changed the type of used antibody to the type of antibody under subheading: Discussion paragraph 2 (patients) lines 28 to 29 on page 9.

This manuscript describes original work and is not under consideration by any other journal. All authors approved the manuscript and its submission to BMC Clinical Pathology.

Sincerely,

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