Author's response to reviews

Title: Distinct distribution and prognostic significance of molecular subtypes of breast cancer in Chinese women: a population-based cohort study

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Author's response to reviews: see over
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Judith Gorton  
BMC-series Journals  
BioMed Central  
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London, WC1X 8HL

RE: Distinct distribution and prognostic significance of molecular subtypes of breast cancer in Chinese women: a population-based cohort study

Dear Dr. Gorton:

Thank you for your email of 08 April 2011 inviting us to resubmit the above referenced paper. We have carefully considered the comments and suggestions of the reviewers and revised the manuscript accordingly. Our point-to-point responses to reviewers’ comments are summarized below. Changes in the manuscript are highlighted.

Reviewer 1

Comment 1: In Table 1, variables, ¥ should be changed to CNY¥ or RMB¥ or CNY avoiding to be confused to JP¥.

Response: We have changed ¥ to CNY¥ in Table 1.

Comment 2: The abbreviation BC (breast cancer?) in Table 2 should be added in the list of abbreviations.

Response: We have spelled out “breast cancer” instead of using “BC” in Table 2.

Comment 3: The number of cited references is too many for this type of paper. Please cite the important literatures only.

Response: We have reduced the number of references from 43 to 36.

Comment 4 (Discretionary): A table summarized the distribution of molecular subtypes of breast cancer in different ethnic groups should be added. It would help easy understanding of the differences between Asian, European and African-American populations.

Response: We thank the reviewer for this excellent suggestion. A new table (Table 4) has been added to summarize the distribution of breast cancer subtypes among different races/ethnicities and in different geographical areas of China.
Reviewer 2

Comment 1: It would be of interest to the readers to know the case selection from the SBCSS: now many institutions, any potential selection biases?

Response: Cases in the SBCSS were identified through the Shanghai Tumor Registry, a population-based tumor registry. It is mandatory for all hospitals in Shanghai to report incident cases of cancer to the Shanghai Tumor Registry. The overall response rate for the study is 80%. Thus, the potential selection bias, if any, should be low. We have included this information in the last paragraph of the Discussion section.

Comment 2: The impression given was that all cases had HER2 immunostaining, but only 200+ cases were done for ER and PR. Most of the results of ER and PR appeared to be obtained from the medical charts. Were the diagnostic criterion uniform among all the participating centers? More detailed information on ER and PR staining need to be provided for those patients with data obtained from medical charts in M&M. For those patients whose ER and PR status could not be obtained, double staining for PR/HER2 and ER#/ER# were conducted. Are different protocols used for these two groups? If yes, it is not clear how comparable are the data generated with the different protocols. The authors mentioned ER staining in the manuscript but they did not illustrate what is the role of this staining in the evaluation of breast cancer subtype.

Response: For the vast majority of the study participants, information on ER and PR status was obtained from medical charts. Hospitals in urban Shanghai began to apply the immunohistochemical method to determine ER/PR status in the early 1990s. By the late 1990s, almost all major hospitals had adopted this method. However, misclassification of ER/PR status, as measured by multiple institutes, is unavoidable. Unfortunately, in order to preserve the limited number of tumor slides collected by the study, our lab at Vanderbilt only measured ER/PR status for cases whose ER/PR status could not be obtained from medical chart reviews; this prevented a direct assessment of misclassification. We have acknowledged this limitation in the Discussion section. For the 243 and 222 cases whose ERα and PR status was evaluated at Vanderbilt Molecular Epidemiology Lab using double staining protocol[s1], we used >10% cut-off value for ER and used a lower value (>1%) for PR, due to a slightly decreased PR sensitivity of HER2/PR double staining. We performed additional analysis by excluding cases whose ER/PR status was measured at Vanderbilt and observed no appreciable changes in the study results, indicating the staining protocol did not affect our study results. We have now included this information in the Discussion section in the revised manuscript.

Comment 3: The author used a 10% cut-off for ER positivity while a 1% cut-off for PR positivity. ER, PR staining is now generally accepted to use 1% cut-off. It is not clear how these differences in cut-off affecting the prevalence of the different molecular subtypes.
Response: Please see our responses to comment 2, where the effect of different cut-off values on the prevalence of molecular subtypes is discussed.

Comment 4: In the materials and methods, the authors mentioned that ‘demographic characteristics, reproductive history, disease history, medication use, selected lifestyle factors, diet, use of complementary and alternative medicine, and quality of life,’ were evaluated. Please explain in greater details as to what exactly were evaluated? And also what anthropometric measurements were taken?

Response: The SBCSS was designed to investigate the association of lifestyle factors with breast cancer survival. The anthropometric measurements taken included height, weight, and circumferences of the waist and hips. In addition, we collected detailed information on demographic characteristics, reproductive history, disease history, medication use, selected lifestyle factors, diet, use of complementary and alternative medicine, and quality of life. We have previously published our findings on soy food consumption, vitamin supplement use, weight change, quality of life, tea consumption, and physical activity in association with breast cancer (see below references). None of these papers, however, have focused on the molecular subtypes of breast cancer. The current work of the current paper opens a new opportunity to further investigate the association of lifestyle factors on the prognosis of subtypes of breast cancer.


Comment 5: There was no mention of the results of the histologic (WHO) types of the tumors in this study cohort. It would be of interest to the readers, and also to assess the relationship of the histologic and molecular typing results.

Response: We thank the reviewer for this suggestion. We have added new data in the Results section and added a new paragraph in the Discussion section under “Prognostic significance of breast cancer subtypes in Chinese women”, as well as two new related references, to describe the correlation between histologic types and biomarker subtypes. The relatively small sample size of each of special histologic type prevented us from carrying out more detailed analyses.

Comment 6: Although the author adopted the ASCO guideline, they used IHC staining 3+ only to identify HER2+ subtype. The HER2 borderline group, which comprised of 8% of the total breast cancer could not be readily classified into a recognized subtype. A minor proportion of these cancers having HER2 amplification would belong to HER2+ group as suggested by the authors while those remaining could be either luminal A or triple negative subtypes. It was hard to properly interpret the present data with the unclassified HER2 borderline group and compare the results with the published data. The unclassified population could affect the prevalence in particular the rare subtypes. What were the PR and ER status of the HER2 borderline cases?

Response: We agree with the reviewer that not being able to further characterize the HER2 borderline group is a limitation for this study. It is likely that the prevalence of HER2+ tumors in our study population was underestimated. As suggested by the reviewer, we compared the ER and PR status for the HER2 borderline group with HER2+ and HER2- groups, and found the ER/PR positive rates for the HER2 borderline group are very similar to that of HER2- group, suggesting that the vast majority of cases with borderline positivity of HER2 likely belong to the HER2- group (see table below). Thus, the HER2+ rate for our study population should be very close to what we reported and the conclusion that “The HER2 subtype was more prevalent in this Chinese population compared with Western populations” should remain true.

<table>
<thead>
<tr>
<th>Distribution of ER, PR positivity by HER2 staining</th>
</tr>
</thead>
<tbody>
<tr>
<td>HER2 (+)</td>
</tr>
<tr>
<td>ER (+)</td>
</tr>
<tr>
<td>PR (+)</td>
</tr>
</tbody>
</table>

Comment 7: The authors stated that ‘Women with triple negative breast cancer also more frequently reported a family history of breast cancer than did women with other subtypes. This suggests that genetic factors may play a more important role in this molecular subtype of breast cancer.’ This statement has to be discussed in light of the known association of triple negative cancers with BRCA1 mutations.
Response: As suggested, we have added more discussion on this topic and also cited two important new references [26, 27].

Comment 8: What were the significance levels of different subtypes in comparison to the reference group for cox regression analysis for the survival data?

Response: In the Cox regression analysis, any HR with a 95% confidence interval (CI) that does not include 1 indicates that the association is statistically significant at P-value of <0.05

Comment 9: In the discussion, the author suggested that triple negative, HER2+ and luminal B subtypes were associated with patients of younger age. Although it was in concordance with published data, the mean age at diagnosis from the present study were not very different. One possible reason was the presence of a small subgroup of patient either very young or old could skew the mean. How was the distribution of patients in different cancer subtypes stratified by age groups?

Response: As suggested, we now present the distribution of breast cancer molecular subtypes by more finely graded categories of age. This information is included in the revised paper (Table 2).

Comment 10: The distribution of molecular subtypes could be affected by environmental factors as well as ethnicity. More discussion on the comparison of breast cancer subtypes with other Asian populations and other Chinese populations could be of interest to the readers.

Response: We concur with the reviewers that the distribution of the molecular subtypes could contribute to both genetic and environmental factors and have added more discussion on this topic. We added a new table (Table 4, suggested by another reviewer) to summarize the distribution of breast cancer subtypes among different races/ethnicities and in different areas of China.

Comment 11: Some patients were subjected to immunotherapy. What kind of immunotherapy did they receive? Were there any differences in treatment with patients belonging to different subtypes? It would be interesting to briefly discuss the cancer prognosis of different subtypes in relation to the treatments.

Response: We only collected general information about immunotherapy by asking patients whether they had received certain immunotherapies, such as IL-2, Lak cell, and interferons. We found that use of immunotherapy was related to improved overall survival and disease-free survival among women with luminal A breast cancer but reduced disease-free survival among women with the HER2 subtype (results presented below). We now include this information in the Results section and in the Discussion section of the revised manuscript.
<table>
<thead>
<tr>
<th>Subtype</th>
<th>Overall survival</th>
<th>Disease-free survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal A</td>
<td>0.27 (0.10-0.69)</td>
<td>0.41 (0.20-0.82)</td>
</tr>
<tr>
<td>Luminal B</td>
<td>1.44 (0.47-4.35)</td>
<td>0.79 (0.24-2.60)</td>
</tr>
<tr>
<td>HER 2</td>
<td>1.50 (0.71-3.19)</td>
<td>2.21 (1.09-4.48)</td>
</tr>
<tr>
<td>Triple negative</td>
<td>0.47 (0.16-1.41)</td>
<td>0.42 (0.17-1.04)</td>
</tr>
<tr>
<td>Combination</td>
<td>0.63 (0.15-2.55)</td>
<td>0.56 (0.14-2.25)</td>
</tr>
</tbody>
</table>

We greatly appreciate the reviewers’ critical comments on this paper. We hope that our revised manuscript has adequately addressed the reviewers’ concerns and has fulfilled the publication requirements of your journal. We thank the peer reviewers for their valuable comments and look forward to hearing from you regarding journal’s final decision regarding the acceptance of our paper for publication.

Sincerely,

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