Author's response to reviews

Title: The emerging diversities in breast cancer diagnosis - a regional population based study

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Author's response to reviews: see over
Dear Ms Neilan,

Thank you for referring our manuscript for review. We Submitted, the revised version of the manuscript. The comments of the reviewers have been addressed and recommended corrections performed. The following modifications have been made:

Referee 1:

Major Compulsory Revisions:

- In our molecular classification of breast cancer, we generally used the same categorization used by others (as has already been mentioned in “Matrials and methods”) except in the classification luminal A and luminal B tumors. Because tumors that co-express hormone receptor and HER2 are endocrine (tamoxifen) resistant and are treated by estrogen deprivation in addition to blockage of HER2 pathways, including them as an integral component of endocrine sensitive tumors (LUMB) may not be justified. We, therefore, adopted Bhargava et al’s approach [20] in considering these tumors separately from pure luminal tumors, and grouped them into a separate category of Luminal–HER2 hybrids. We also followed Bhargava et al [20] in distinguishing LUMA from LUMB tumors by the intensity and extent of ER immunostaining, considering the fact that benefit from endocrine therapy is directly related to estrogen receptor level.
• However, to answer the referee justified concern, a molecular classification was attempted using the criteria previously described and used by the other studies (17,18,19) in identification of LUMA and LUMB tumors. A similar prevalence pattern with a still significantly low incidence of luminal tumors \( (p=0.001) \) was obtained. Analysis with hybrid “luminal-HER2” and “HER2-basal” cases included among luminal and HER2 classes, respectively, yielded the following results: Luminal 66 cases (28.5%), HER2 43 (18.6%), Basal 23 (10%) and Unclassified (penta negative) 99 (42.8%). However, the prevalence of LUMA increased from 3.9% to 25.1% (58 cases), while LUMB dropped from 16% to 3.4% (8 cases). Yet the prevalence of LUMA was still significantly lower than that reported in the above mentioned studies \( (p=0.001) \). This analysis has been added to the text in the “Results” and the "Discussion" sections.

*Molecular Classification:*

1. The “Introduction” section has been reduced. Paragraph 6 was deleted with part of it incorporated into Paragraph 9 in the “Discussion” section (the comment on LUMB cancer).

2. The study cases were randomly selected on the basis of the availability of representative blocks and sufficient tissue material to perform the required procedures. The time frame covered was 12 years (1997-2008). These aspects have been clarified in the beginning of the “Material and Methods” section.

3. We did not expect that the frequencies of the phenotypes would be different comparing tru-cut biopsy specimens to the others since our case selection was based on the availability of sufficient tissue material to perform the required procedures as already mentioned in the “Material and Methods” section. Tru-cut biopsies in which cancer tissue was not adequate were not included in the study.

4. In case two TMA tissue cores were scored differently, conclusive scoring was made on a conventional paraffin section from the tumor. This statement has been added to the “Immunohistochemistry” part in “Material and Methods”.

5. Descriptive characteristics of tumors (histologic type and grade of tumors) by molecular class have been included as two additional tables (now designated as “tables 3 & 4”).

6. Regarding figures 2 and 3, the total number in each of the studies has been included next to the country.

7. The comparison of the frequencies of classes between the current study and other studies is already included in the “Results” section and highlighted in figures 2 and 3. However,
it was elaborated on in the “Discussion” to emphasize the significance of the differences noted in the compared results.

The p-value presented is indeed from a chi square test. This is already mentioned in the statistical analysis section but with the “chi-” represented symbolically; the symbol has been replaced by “chi-“.

8. Typographical errors have been corrected whenever recognized with the reference mentioned referred to its right author (Brennan et al rather than Donal et al).

Figures 4, 6 and 7 have been replaced by tables 5, 7 and 8, respectively.

9. Figures 5, 8 and 9 have been deleted.

10. The legends for figure 2 and 3 have been improved.

11. The title of the article has been modified to better indicate what the current study is about. The new title is “Protein expression profile and prevalence of the molecular classes of breast cancer-A Saudi population based study.

12. Conclusions from the current study have been included in the “Conclusion”.

Referee 2:

Major Compulsory Revisions:

- All our basal cases were “triple negative” being categorized on the basis of immunoreactivity for CK 5/6 and/or EGFR unassociated with ER, PR or HER2 expression. Cases that co-expressed ER, PR or HER2 and basal markers as CK5/6 or EGFR are grouped into separate categories of “Luminal-basal” and “HER2-basal” hybrid groups.

- The statement at the end of the discussion that “the term triple negative is obsolete and should be replaced by the term “penta-negative” has been omitted.

- The 2 suggested recent references (Atchley et al 2008 and Chen et al 2009) were added and used in the comment about “triple negative” in the discussion.

NB: The Dammam campus of King Faisal University has recently been separated from the mother university and is called University of Dammam. King Faisal University has been changed to University of Dammam in the manuscript and in authors affiliation.