Author's response to reviews

Title: Women with familial risk for breast cancer have an increased frequency of aldehyde dehydrogenase expressing cells in breast ductules

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Author's response to reviews: see over
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To the Editor
BMC Clinical Pathology

Re: Women with familial risk for breast cancer have an increased frequency of aldehyde dehydrogenase expressing cells in breast

Thank you for considering this manuscript for publication. The reviewers’ comments, which were very insightful and helpful, are included below, followed by our responses to each issue.

We are looking forward to your reply.

Sincerely,

Björn L. Isfoss

Reviewer 2

Major Compulsory Revisions

1) The Background section needs to be highly improved. The authors need to describe an increased number of findings concerning ALDH expression in breast cancer, including the one previous published by the group. The text is too much fragmented, and the aim needs to be better explained.

Author response: The aims were described at the end of the Background section in the original manuscript. As suggested, we have modified the last paragraph of that section to better explain the objective of the study. Three references have been added: two recent studies performed by other investigators (Kunju 2011 and Schwartz 2013), as well as our earlier investigation (Isfoss 2012).

2) Page 6 and Table 1. The authors need to correct the text concerning the data that is presented in Table 1, in order to have coherency between both. Additionally, there is also the need of some corrections within Table 1: there is one missing case in the parity part, there are also an increased number of missing cases in the Hormonal use at time of surgery… Please correct this table according with the data described in the manuscript text.

Author response: Please note that patient groups A–F were chosen according to inclusion criteria, whereas the analysis groups were constructed to consider clinical, hormonal, and genetic characteristics of individual patients. The number of patients in each group included in the study is now given within parentheses in the main text (Patient material section). In the beginning of Results and discussion we have added a section with the heading: Division of patient inclusion groups A–F into analysis groups. Also, Table 1 is now accompanied by the following text: "Patient groups included in the study and subsequently re-grouped according to the listed parameters for analysis of ALDH+ cells." We hope that these amendments prevent misunderstanding. Criteria for exclusion were originally correctly explained in the text, and Table 1 correctly listed patients entered in the study.
3) Figure 1. The authors should include pictures showing the differences between ALDH+ positive cells located adluminally, intermediately and basally in the TDLUs. Please discuss in the “Results and Discussion section”, why the expression location can influence the risk.

Author response: In the sub-section Distribution of ALDH+ cells, we have added a description of ALDH+ cell locations by ductular level (“The ALDH+ cells occurred primarily …”), with reference to Figure 1. Near the end of the sub-section ALDH+ cells in the six patient groups, we have added an brief explanation as to why we chose to analyze ALDH+ cells according to different levels in the epithelium, which was previous data suggesting that this is associated with risk (“… together with previous evidence that …”).

4) Table 2: It is very difficult to understand the 37 patients included in the arm “pre-menopausal patients with familial history of breast cancer (without BRCA1/2 mutations)”, based in table 1. In fact, it is difficult to understand the numbers of these patients based in the previous cohort of 106 patients. Please clarify this issue.

Author response: We have improved Table 2 with a more precise label for the mentioned category, which comprises pre-menopausal patients with a family history including all BRCA1 and BRCA2 patients (NB: patients were not tested for BRCA1/2 unless they had a family history).

5) Please describe, as a Table, the numbers and statistic associations found. It is my feeling that the number of cases is too small to take some conclusions. This table should be included to complement the figures presented.

Author response: Although this study presents a comparatively large patient material, we agree that some sub-group numbers are small, since we emphasized a large breadth of patient characteristics rather than great patient volumes for few groups. We did provide, though: numbers for each included patient group together with breakdown of clinical characteristics in Table 1, and study results for each patient analysis group including notes on all significant associations and statistical level of those in Table 2.

Reviewer 1

Major Compulsory Revisions (which the author must respond to before a decision on publication can be reached)

1) Define very clearly what is meant by ‘ALDH+ cells’ (ductules only) and use this term consistently throughout the text.

Author response: We have added a short sentence in Methods — Histology and immunohistochemistry (start of 2nd last paragraph: “ALDH immunoreactive cells …”) explaining that all cells that were present in ductules and exhibited any ALDH reactivity, weak or strong, were considered positive.

2) Abstract 1st Para. The background paragraph is vague; clarify ‘patient
hormonal factors’ and ‘various risk factors for breast cancer’. Define the question posed by this research.

**Author response:** Hormonal factors previously shown to be associated with ALDH+ cells are now clarified in slightly more detail (“… menopause and hormone therapy.”), and risk factors for breast cancer are also specified (“… clinical and genetic …”). The subject of the study is now stated in the opening of Abstract - Background.

3) Describe the potential implications of the study in the conclusion.

**Author response:** We have re-written the Conclusion section, and added a sentence: “The findings suggest … should be further evaluated as a potential marker for the risk of cancer …”.

4) Background 3rd Para, last sentence. There are several recent papers that have shown ALDH+ cells in histologically normal breast tissue are related to risk factors and outcome in breast cancer, and these should be acknowledged.

**Author response:** Please see response to Reviewer 2, item 1 (above). Two relevant and recent references have been added (Kunju 2011; Schwartz 2013), as well as our earlier paper (Isfoss 2012).

5) Methods 1st Para. Be consistent with the patient group names in the abstract, methods, results and Tables. List how many patients are in each group (A-F).

**Author response:** The original Table 1 listed patient groups A–F included in the study based on major clinical parameters. We have now added information in the legend to Table 1 indicating that the patients were re-allocated to groups better suited for analysis of clinical (hormonal; genetic) associations with ALDH+ cells. We have also added a sub-section at the beginning of Results and discussion to cover this issue.

6) The archived tissue samples were collected from 1984-2010. Over this time, major changes occurred in surgical techniques and in tissue collection, preservation and storage techniques. Such factors may all impact on the quality of immunohistochemical analysis, and these issues should be addressed. Similarly, discuss and/or clarify what type of oral contraceptive was used by patients.

**Author response:** We have added a sub-section, Methodological considerations, addressing such issues. Patients treated with neoadjuvant therapy were not included in the study. In our experience, immunohistochemistry on tissue blocks from the 1980s (i.e., the pre-buffered-formalin era) is not problematic, and others did confirm this impression when we made a query to an online surgical pathology discussion forum. Two of the present authors did separate literature searches on this subject, and found that non-buffered formalin causes less masking of epitopes than buffered formalin.

7) Results, Discussion, Conclusion. These sections were weakest and need work; there was very little discussion about how these results add to current
knowledge, the biological implications of the results, or the strengths and limitations of the study. The conclusion did not give a clear explanation of the importance and relevance of the results.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

**Author response:** We have made small changes in all the mentioned sections to achieve more logical flow, more solid foundation of a concluding statement, and easier readability.

8) Abstract 2nd Para. BRCA1/2 mutation carriers (n=22?).

**Author response:** The number presented in the abstract was correct: *n=23*.

9) Background 1st Para. Only one reference cited (7) for ‘recent studies’.

**Author response:** This text is now modified to the singular. We do not consider that the cited study represents the majority of studies.

10) Background 4th Para. Clarify ‘might be related to hormonal status’.

**Author response:** Changed to "is related to menopausal state and hormone replacement therapy."


**Author response:** Corrected, and abbreviation DAPI used (first time within parentheses).


**Author response:** Corrected.

13) Results and Discussion. ALDH+ cells in relation to HRT and contraceptive pills. 2nd Para. Replace ‘induces a risk’ with ‘increases the risk’.

**Author response:** "induces the risk" has been changed to "increases the risk".

14) Consider removing the key words ‘epidemiology’ and ‘progenitor cells’ and replacing them with ‘breast ductules’.

**Author response:** Done.

15) A measure of adiposity (e.g. BMI) would have been interesting, particularly in the post-menopausal women. Is this data available for inclusion?

**Author response:** Data not available, although it would be very interesting indeed.
Additional note from Authors: After receiving your letter we employed a certified expert to improve the language. This resulted in numerous but subtle changes in the text. The expert felt that the style of writing was more like U.S. than U.K. English, so we accepted her conversions to U.S. spelling.