Reviewer’s report

**Title:** Association of Cytokeratin 5 and Claudin 3 expression with BRCA1 and BRCA2 germline mutations in women with early breast cancer

**Version:** 1  **Date:** 11 Mar 2019

**Reviewer:** Frederic Andre

**Reviewer's report:**

In this manuscript Danzinger et al. investigated the expression of claudin-3, -4 and -7 and E-cadherin in order to define the expression profile of biomarkers in BRCA1 breast cancer.

Some pictures shown in the manuscript are for me not convincing. This is a problem since these staining represent the core of the experimental procedure.

Claudin-4 (figure 1b): The picture appears too saturated: (1) there is a stromal staining (that should be negative) and (2) the staining seems to be exclusively cytoplasmic whereas claudin-4 is normally associated to cell surface. This strongly suggests a nonspecific reactivity. For nice Clauding-4 immunolocalization on breast cancer tissue sections, see for instance [https://www.proteinatlas.org/ENSG00000189143-CLDN4/pathology/tissue/breast+cancer#im](https://www.proteinatlas.org/ENSG00000189143-CLDN4/pathology/tissue/breast+cancer#im)

The use of isotypic Abs as negative controls must be added.

Figure 1c and d: As mentionned in the figure legend, the magnification is X10. However the size of the nuclei appear to be different between figure 1C and figure 1D. A scale bar for each picture will resolve this ambiguity.

Claudin 7 staining (figure 1c): the staining is too weak and the magnification too low to determine whether claudin-7 is present at the cell surface. Moreover, some staining seem to be aspecific. To ensure the specificity of the staining, a control experiment using using an isotypic control Ab must be added.

CK5 immunolocalization is not shown in the manuscript. Such a staining should be added since this molecule is mentionned in the manuscript title.

The Ab dilutions must be done in μg/ml. This will resolve the ambiguity in the sentence « the antibody for E-cadherin was used undiluted ».

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

No

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

No

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

Quality of written English
Please indicate the quality of language in the manuscript:

Acceptable

Declaration of competing interests
Please complete a declaration of competing interests, considering the following questions:

1. Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

2. Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

3. Do you hold or are you currently applying for any patents relating to the content of the manuscript?

4. Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript?

5. Do you have any other financial competing interests?

6. Do you have any non-financial competing interests in relation to this paper?

If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.

I declare that I have no competing interests.
I agree to the open peer review policy of the journal. I understand that my name will be included on my report to the authors and, if the manuscript is accepted for publication, my named report including any attachments I upload will be posted on the website along with the authors' responses. I agree for my report to be made available under an Open Access Creative Commons CC-BY license (http://creativecommons.org/licenses/by/4.0/). I understand that any comments which I do not wish to be included in my named report can be included as confidential comments to the editors, which will not be published.

I agree to the open peer review policy of the journal