Reviewer's report

Title: Mammographic density and epithelial histopathologic markers

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Reviewer: Erin Aiello J Bowles

Reviewer's report:

This is one of the first studies to evaluate the association between histopathologic markers and continuous breast density. Overall, the study results are null; however, the authors present some interesting hypotheses for future studies.

Major compulsory revisions:

1. The main issue I have with this paper is that the authors have written their results and conclusions as though the findings are statistically significant, when they are not. The authors acknowledge the findings are not statistically significant. However, in many places, they emphasize “substantial” or “large” differences in density that are small and not clinically meaningful (e.g. 3-4 % increase or decrease in % density) given the variability in continuous breast density measures (standard deviation of % density in Table 1 was 24.8%). In my interpretation of this study, there are no associations between histopathologic markers and breast density. The few associations that are significant are likely the result of chance given the large number of associations the authors have evaluated. I realize the authors are trying to generate hypotheses to be tested, but they should make substantial revisions to their abstract, results, discussion, and conclusions to reflect the true message of the paper.

2. Methods – on page 6, the authors state that 118 breast tissue samples were recategorized as benign or malignant. Why? On what basis did the authors recategorize tissue samples? This raises another question, which is – what was the timing of the collection of tumor samples relative to each woman’s diagnosis? Were they from prior benign biopsies? If so, were they more than a year pre-diagnosis (assuming that women come in annually for screening)? This is not clear in the methods. If some of these are from the breast cancer diagnosis and were recategorized as benign based on cell composition, I would consider excluding these as the marker levels in this tissue may be different than those in tissue from women who have never been diagnosed with breast cancer.

3. Methods – I have a few questions about the mammograms used in this study. The authors state that mammograms were scanned at a resolution of 98 pixels per inch. Was this for both small and large films? What conversion factor did you use to convert pixels to cm-squared? The authors state that the CC view closest to, but before breast cancer diagnoses was used. Can you provide more information on the timing of exams relative to 1) diagnosis, and 2) collection of the tissue sample? It would be helpful to see the median and range of dates for
each of these.

4. Methods – the authors have adjusted for 9 covariates in their models, which is a lot given the small sample sizes (particularly for the stratified analyses). I know all of these are associated with mammographic density, but are they all associated with the histopathologic markers? Were the adjusted and unadjusted results markedly different to warrant including these in the model?

5. Methods – page 8 – The authors state, “Given the small sample sizes, we evaluated the mammographic difference between groups based on their size and consistency when the results were not statistically significant.” What does this mean? The authors looked for patterns in the results? I think this is reasonable, but only when differences are large (>10% percent density) and the results border on statistical significance. Otherwise, there is no basis for the conclusions other than chance.

6. Table 1 – please include the histopathologic markers in this table. Were there differences in marker results in the TMA sample compared to the original study?

7. Conclusions – Please be more specific about the hypotheses you have generated for future investigations. Few results were consistent or significant in this study, and I think the conclusions need to be more focused in terms of what might be meaningful to examine in the future.

Minor essential revisions:

1. Abstract – specify that the study population includes pre- and post-menopausal women.

2. Abstract – the second sentence of the methods states that density was assessed from “prediagnostic” mammograms. Were these all screening mammograms prior to diagnosis (and not diagnostic mammograms)? Please be more specific.

3. Abstract – in the methods, please provide the sample size and explain that the sample includes benign tissue taken prior to diagnosis for women with breast cancer. This is not clear in the abstract until one reads the methods of the paper.

4. Abstract – please define TMAs.

5. Methods – on page 5 the authors state that 1,773 tissue samples were available for analysis. How many women does this include?

6. Discussion – page 12, the authors stated that they identified “benign specimens for only 120 out of 279 subjects…” I thought there were 159 samples in the study – please explain.

7. Table 1 – The numbers for age at first live birth, <30 years, cannot be right. Perhaps the columns were switched?

Discretionary revisions:

1. Table 2 does not add much to the results, and is never mentioned in the discussion. I would replace this with the information provided in the first paragraph of the results on the differences in density by race.
2. Figure 1 – I would delete as this does not provide useful information to the reader.

3. Why would results for ER-alpha and ER-beta differ? Can the authors provide any explanations for this?

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests.