Author's response to reviews

Title: Do serum biomarkers really measure breast cancer?

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Author’s response to reviews: see over
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Rikki Graham, PhD
Senior Assistant Editor
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Dear Dr. Graham,

Please find the revised version of manuscript 1153141180219503, “Do serum biomarkers really measure breast cancer?” according to the referees’ comments. My coauthors and I have addressed all referees’ comments, as described in the enclosed “Response to referees” section.

On behalf of all co-authors, I thank you for your consideration. Please let me know if any additional information would be needed.

Please note that we have reordered the author list to better reflect Dr. Lokshin’s efforts in overseeing a large majority of the project. We have moved her name to the second-to-last position.

With my best regards,

Jonathan Jesneck, Ph.D.

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Encl: Response to referees
Response to referees

We thank the referees for their insightful comments. Our responses are listed below.

Note: Added material in the manuscript is shown in underlined text and deleted material in struck-through text.

Referee 1:

Major essential revisions:

1. The Luminex assay was performed with the Luminex LabMap system. All assays do not show any cross-reactivity with proteins other than the one measured. Sensitivities of individual assays range from 0.1 to 1000 pg/ml and this information is available from the manufacturers of assays (see Supplementary Table 2) and from the Luminex Core website (http://www.upci.upmc.edu/luminex/sources.cfm). One replicate per patient sample was performed.

2. This is a good suggestion, and in fact (not reported in the paper) we did initially withhold some samples to define an independent test set. We trained all models on the training samples (optimizing with cross-validation over these training samples), and then we tested these trained models on the test set. To see what classification effect was caused by our random selection of samples for the test set, we repeated this procedure many times and also varied the size of the test set. These classification results were very similar to those for leave-one-out cross-validation. Therefore we decided to report only the cross-validation results for simplicity.

3. Biomarkers were selected based on the known literature reports about their association with breast cancer or other cancers. More information about the selected proteins has been included in Supplementary Table 2.

Minor essential revisions:

1. Yes, of course most solid tumors cause changes in the surrounding tissue. We did not mean to imply that this issue is specific to breast cancer. We have changed this section’s scope from breast cancer to solid tumors in general. See page 5, whole paragraph 1, sentence 5: “Because solid tumors cause … the cancer itself.”

2. This is an excellent point. We have added a brief discussion of this. See page 5, whole paragraph 1, sentences 1-4: “Cancer biomarkers are … any proposed biomarker.”
3. We have added more context, referencing related cancer biomarker studies. See page 5, last paragraph continuing to page 6, “Some studies have … protein microarray.”

Referee 2:

Minor essential revisions:

1. Supplementary Table 1 now contains clinical detail about the samples. The main text now refers to this table. See page 7, paragraph 1, sentence 4: “Supplementary Table 1 … histology findings.”
2. We have included a reference to the Luminex protocol. See the second half of the section “Measuring Serum Protein Levels with ELISA.”
3. Supplementary Table 2 now contains detailed information about the assayed proteins and their antibodies.
4. These classification results were done with the feature selection. We have added a sentence explicitly stating this at the end of the first paragraph of the “Classifier Performance” section.
5. Yes, “red” should have been “blue.” This has now been corrected. We thank the reviewer for catching this mistake.
6. These feature selection comparisons looked very similar for normal vs. benign as shown for normal vs. cancer. For benign vs. cancer, the classification performance was lower (as also shown in Figure 1 C), so the BMA, stepwise, and “all features” feature selection methods all had similarly bad classification performance, whereas the “Preselected” feature selection method had good (and very optimistically biased) classification performance. For benign vs. cancer, the frequency selection heatmap showed similar smears of darker bands across all the feature selection techniques. In summary, we believe that these plots were not scientifically interesting enough to show because the normal vs. benign plots were very similar to shown normal vs. cancer plots, and benign vs. cancer plots did not have enough classification performance to show the effect of feature selection methods.

Discretionary revisions:

1. We have added our thoughts about next steps: developing better multiplex protein assay technology, collecting larger datasets with complementary medical data types, and integrating protein information with imaging-based screening. See page 17, last paragraph, “The currently proposed … and medical imaging.”