Author's response to reviews

**Title:** Serum Protein Profiles Reflect Coronary Artery Disease in Symptomatic Patients Referred for Coronary Angiography

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**Author's response to reviews:** see over
Please find attached our revised manuscript MS: 1506262321742834 entitled:

Serum Protein Profiles Reflect Coronary Artery Disease in Symptomatic Patients Referred for Coronary Angiography by William A. LaFramboise, Rajiv Dhir, Lori A. Kelly, Patricia Petrosko, J. Michael Krill-Burger, Christin M. Sciulli, Maureen A. Lyons, Uma R. Chandran, Aleksey Lomakin, Robert V. Masterson, Oscar C. Marroquin, Suresh R. Mulukutla and Dennis M. McNamara

We have made changes in the manuscript specific to the reviewer requests as annotated below. We have also made 2 minor additional editorial changes. We have replaced the word “Reflect” in the title to “Predict” since Reviewer 1 focused his insights specifically toward the predictive power of our study. Consequently, we hope the title now better befits the context of the study.

We have added substantial information to the first paragraph of the Methods, sentences 2, 3 and 4 to elaborate on the various requirements we met for acquisition of the blood samples in an ethical manner.

We added the word “reducing” to the line “reducing the economic burden” in the last line of the abstract.

RESPONSE TO REVIEWERS

We appreciate the insightful comments of the two Reviewers and have made minor revisions to the manuscript based on their suggestions.

Response to Reviewer 1:

The reviewer had one overarching criticism/request:

“One key point here is why the authors have not calculated the CAD/CV risk for these patients. Were these patients referred for coronary angiography free of past history of CAD events, beyond the symptoms that led them to the catheterization lab? One reason for the authors not providing the CAD/CV risk may be the fact that many lipid profile data is missing in near 50% of participants.”

The reviewer is correct. We did not include CAD risk calculations because many patients lacked a full compendium of clinical data to make the calculations. So our approach was to leave out any attempt to compare our results with an established risk calculation. However, the reviewer specifically asked for this comparison and we have performed it as requested according to his request below:

“In my view, and according to AHA recommendations the effect biomarkers should be tested when added to the risk function (typically that developed by the Framingham Heart Study) in terms of reclassification and improvement of AUC. I understand that the authors address the prediction of coronary artery lesions requiring PCI in symptomatic patients, but adding this information would greatly improve the message of the manuscript given the fact that patients were referred to cath lab for symptoms suggestive of CAD but without previous diagnosis of CAD event. Therefore we are here confronted with primary prevention: in this scenario risk function use is mandatory in my opinion.”
We calculated the Framingham Coronary Heart Disease Risk Scores at the reviewer’s request for those patients where the data were available. This consisted of 154 patients with 91 requiring revascularization after coronary angiography studies and 63 who had no significant coronary artery disease after catheterization. The Framingham scores were significantly different between these two groups (No CAD= 10.2 +/- 6.7 vs. CAD= 14.9 +/- 8.5; p=0.0003) as the reviewer suspected. However, the large distribution of values among these two patient groups restricted the ability to correctly classify members of each group. If we set a cutoff value at which the Framingham scores correctly called 95% of the patients in the CAD group, it also called 53 patients without CAD as false positives. Thus the Framingham scoring system correctly called only a total of 10 of 63 or 16% of the patients without CAD while our scoring system correctly classified 40 of 63 patients or 63%.

The issue for us is how to best present these results. We designed this study to determine whether an efficacious multiplex classifier for CAD existed. We believe it is the purview of the “next study” to determine how it may be improved with clinical values or contribute to a clinical scoring system. But that will require greater patient numbers from multiple sites in a prognostic paradigm. Nevertheless, the Reviewer asks for a very important comparison and we have closed the Discussion with an entire paragraph devoted to that issue including the Framingham CHD Risk Scores, addition of a reference to the original Framingham study presenting their scoring system, and a comparison of the predictive strength of the Framingham CHD scores to our scoring function algorithm. We thank the reviewer for this insightful comment and hope that we have modified the paper in an appropriate manner at his request without reinventing our study design.

Reviewer 1 requested 2 Minor Revisions
1) P-values should be described as <0.05, <0.01, <0.001 etc and not as power of 10
2) The authors may wish to change the units of some biomarkers to prevent more than 5 or 6 significant digits on table 2.

1) We agree that P values are better presented as decimal numbers but were reticent because we have many cases where 6 to 12 zeroes will precede the first number and make both tables very unwieldy.

2) We have changed the units of Table 2 as requested.

Response to Reviewer 2:

Reviewer 2 had two minor but informative requests and we have made revisions based on these insights:

“1. Osteopontin also can have an impact on immune function which is not described in this manuscript. This could be important with regards to the results.”

1) We did not make this clear although we cited the correct reference. We have revised our Results on Page 15, Paragraph 1, sentence 2:

“Only osteopontin, which acts as a negative regulator of calcification in bone remodeling, was elevated within this category with the rejoinder that OPN also may act as a chemokine in the cell mediated type 1 immune response associated with inflammatory cell accumulation rather than as a substrate for cell adhesion.”
“2. Some discussion should be given to "false negatives" For example, the list of proteins to be assessed was chosen was based on availability of reagents and historical evidence of involvement and many others proteins that are known to be involved in atherosclerosis were not assessed. While the positives will be useful, further evaluation of the negatives would seem needed. Authors should comment.”

We agree and have modified the last sentence in the same paragraph to make this point:

“However, we recognize that the domain of proteins susceptible to interrogation in this study was limited to analytes for which high affinity antibody pairs precisely characterized to two different epitopes were available. The involvement of additional proteins and pathways associated with CAD will likely be reinforced and/or revealed as the inventory of immunoassays becomes more comprehensive.”

We hope that the manuscript is now acceptable for Publication in BMC Medicine.

Sincerely,

William A. LaFramboise and his fellow authors