Author's response to reviews

Title: The influence of persistent pathogens on circulating levels of inflammatory markers: a cross-sectional analysis from the Multi-Ethnic Study of Atherosclerosis

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Author's response to reviews: see over
Dear Editor,

Enclosed please find a revised version of our manuscript entitled "The influence of persistent pathogens on circulating levels of inflammatory markers: a cross-sectional analysis from the Multi-Ethnic Study of Atherosclerosis" for consideration as an original research article in BMC Public Health. We thank you and the reviewers for helpful feedback and review of our manuscript. In this revised version, we have responded to each of the reviewer comments and made appropriate changes directly in the manuscript. We have addressed the reviewer comments point by point below.

Dr. Aiello will serve as the corresponding author for all pre- and post-publication purposes. Many thanks for your consideration.

Sincerely,

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Reviewer 1

1) In table 3b, the fully-adjusted model 4 (adjusted for age, gender, race/ethnicity, education level plus BMI, alcohol intake, smoking, diabetes, medications and self-rated health) failed to show high antibody response to multiple pathogens had a positive association with all 3 inflammatory makers of IL6, CRP and fibrinogen by multiple linear regressions. Only in model 1 adjusted by age and gender, we observed an association of high antibody response with 3 inflammatory makers. Therefore, the results might have limited significance and applicability.

RESPONSE: Yes, fully adjusted models did not show a statistically significant result. Nonetheless, the magnitude of the association was not diminished to the null value, suggesting that multiple adjustment likely reduced the statistical power but not the magnitude of the association between antibody response and inflammation. We agree this is a statistical/power limitation and addressed it in the results and limitations section as follows:

Results: “Positive associations between seropositive pathogen burden and IL-6 and fibrinogen were modest in model 1 and not statistically significant in fully adjusted models. (Table 3a).”

Discussion: “Limited sample size, as well as strong associations between antibody levels and variables such as BMI and smoking may have hampered our ability to detect associations after multiple adjustments. Larger studies are needed to
determine whether associations are independent of other risk factors.”

2) Still in figure 1 and 2; the trend tests did not adjust for any of the variables (age, gender, race/ethnicity, education level plus BMI, alcohol intake, smoking, diabetes, medications and self-rated health). Both figure simply showed crude trend test without any adjustment. Still the results might restrict its interpretation, significance and applicability. 

**RESPONSE:** The text now includes a clear designation of the analytical approach employed in the figures and tables. Unadjusted and adjusted results should aid the interpretation of findings by readers since it shows the impact of adjustment on crude associations.

3) Conclusion “high antibody response to pathogens was a more consistent predictor of inflammatory outcomes compared to seropositivity alone. High antibody response to multiple pathogens was the strongest predictor.” The conclusion might need to be significantly modified.

**RESPONSE:** Both statements are compatible with our findings, but as suggested here and below, the language has been revised to more closely reflect the study design and analytical strategy.

4) Because of inadequate adjustment in the analysis, the conclusion and discussion need a major revision.

**RESPONSE:** In line with reviewers’ suggestions, both the conclusion and discussion now reflect a more precise analysis of our findings.

**Minor comments:**
1) It seems to me that “marker” is a better term than “predictor” due to the cross-sectional study in design.

**RESPONSE:** Agreed. Changed throughout the text. However, most of these infections are acquired early in life and the inflammatory markers are usually more evident as individual’s age. Thus, the temporal relationships are likely prospective in nature.

2) Is it appropriate to define hepatitis A infection as persistent infection?

**RESPONSE:** Although HAV is considered acute relative to other Hepatitis viruses, such as HCV, HAV is found in the blood from 1-2 weeks after primary infection and persists for up to 14 weeks (this is quite a long time when compared to other acute infections such as influenza). The presence of antibody in the blood is an indication that the acute period of the illness is past and the individual becomes immune to another HAV infection. However, the individual will show life-long indications of past exposure to HAV through antibody measures. Although it is not a latent infection such as the herpesviruses, antibodies to HAV have also been implicated in chronic diseases, such as cardiovascular disease. Since HAV may have long lasting and persistent effects on chronic disease and inflammation, we feel that it is acceptable to consider HAV as persistent because of it’s potential systemic and life-
long effects along with the other pathogens evaluated here. Hypotheses surrounding the impact of this infection on cardiovascular disease have been discussed in earlier research linking HAV and cardiovascular disease. See PMID: 12105156, PMID: 11069227- both utilized as references:

3) Page 7 line 6 HAV below a standardized calibrated rate should read HAV above
**RESPONSE:** Total serum antibodies to hepatitis A virus (anti-HAV) are detected using the IM®x HAVAB qualitative microparticle enzyme immunoassay (MEIA) (Abbott Laboratories; Abbott Park, IL) at the Collaborative Studies Clinical Laboratory at Fairview-University Medical Center (Minneapolis, MN). The MEIA rates is compared to a cutoff rate obtained from each run by a calibrator, as described by the kit manufacturer (Abbott Laboratories; Abbott Park, IL). Values greater than the cut-off rate are considered non-reactive by the criteria of the IMx HAVAB assay; values less than the cut-off rate are considered reactive by the criteria of the IMx HAVAB assay. Therefore, a positive HAV rate is “below” a standardized calibrated rate (ie. Lower antibody levels are seropositive in this type of assay- unlike a typical ELISA).

4) Page 11. “When seropositive pathogen burden and high antibody response to multiple pathogens were included in models simultaneously (data not shown), results were similar to those in Table 3b.” This statement is unneeded and could be deleted.
**RESPONSE:** Removed.

**REVIEWER 2**

Discretionary Revisions:
- Social economic position (SEP) is a predictor of both infectious disease status and inflammatory biomarker levels. Are there any other measured factors for your cohort that may help adjust for residual confounding by individual SEP? Or any family or community level SEP markers?
**RESPONSE:** The MESA cohort does have neighborhood level SEP markers, but the small N of this study would prevent effective statistical analysis taking into account confounding associated with clustering with respect to our findings. Please see:
Sanchez et al. Combining data from primary and ancillary surveys to assess the association between neighborhood-level characteristics and health outcomes:
- In your analysis, you adjust for non-specific self-rated health status, but you do not make any distinctions recent viral infections (such a common cold or flu) that may impact inflammatory biomarker profiles. Do you think acute infection may influence observed associations?

**RESPONSE:** This is possible, and is now discussed in the limitations.