Author’s response to reviews

Title: Evaluating clinical periodontal measures as surrogates for bacterial exposure: The Oral Infections and Vascular Disease Epidemiology Study (INVEST).

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Author’s response to reviews: see over
We are pleased to have the opportunity to respond to the important points raised by reviewers and thank the reviewers for their careful and thoughtful evaluation of our manuscript. The reviewer comments have helped us to focus our writing and in turn clarify the manuscript. We feel that our specific responses to reviewer comments, below, can serve to both clarify any misunderstandings in our methodological approach and build on the strengths and utility of our manuscript as acknowledged in the review.

Response to Reviewer 1 Comments

General comment: The topic of this article is interesting and important. Because large epidemiological studies do not have microbiological measurements there is a need for evidence of which clinical measurement best reflects periodontal infection. This article is well written and I have only few comments and suggestions:

Minor Essential Revisions

Comment 1: The title could be rephrased better to correspond the content of the article. There are only few references to CVD, (abstract 0, discussion 1). Moreover, authors themselves justify the selected bacteria (EB, etiological burden) because these bacteria are possible causative bacteria to periodontitis. Authors also repeat this in the beginning of the discussion section. The fact that these bacteria also associate with CVD (along with other bacteria) is not a justification for this study. The article would be clearer and more readably if authors focus the relation between etiological burden (EB) and clinical measures of periodontal infection.

Response: We have now rephrased the title as follows: “Evaluating clinical periodontal measures as surrogates for bacterial exposure.”

Comment 2: Also, there are contradiction between the aim of the study and ‘We defined …’ sentence (in the intro).

Response: We have now substantially revised the introduction and hope that this has removed the noted contradiction.

Comment 3: Percentual and absolute measures seem to be modified by the number of teeth.

Response: It seems the reviewer is noting that in the situation of high tooth loss, absolute measures are more likely to underestimate disease than percent measures. We agree that percent measures are generally less susceptible to underestimation of periodontal disease and we have previously shown in INVEST that percent measures of periodontal disease positively correlate with tooth loss despite few sites being available for measurement[1]. Although we
expected percent measures to do better, Table 4 shows that most of the correlations presented for absolute measures were similar to the % measures so it seems that absolute and percent measures performed about equally depending on the definition of interest. We conducted exploratory analyses based on the reviewer comments and confirmed that these findings were independent of tooth number.

**Comment 4:** Authors could discuss on the nature of these different measures. The main problem with AL and BOP is that they no not specifically refer to the destruction of soft tissue. For example, that BOP is mainly the measure of infection of gingiva, which is one sign of periodontal infection. Due to that fact, it is not unsurprising that correlation with periodontal infection is high. It could also be mentioned that AL can be due to trauma, often related thin phenotype of gingiva. It can also be a sequela of treated periodontal infection

**Response:** We have made the suggested additions to the introduction on page 3 and in the discussion on page 13.

**Comment 5:** It is also worth pointing out that these associations between EB and different measures may vary between populations. This variation may depend on treatment of the periodontitis, age of the population and the commonness of smoking habit, for instance.

**Response:** In responses to this point, we have modified the results and discussion on pages 13 & 15 respectively.

**Comment 6:** Page 3. ‘for this hypothesis’ Authors could be more specific. Specify ‘this hypothesis’. Is the sentence where authors refer their own study really needed?

**Response:** We have now substantially revised the introduction to focus on the specific aim of this manuscript. In doing so, we have removed the reference to our previous work along with the ambiguous phrase noted by the reviewer.

**Comment 7:** Table 1 % and # should be explained

**Response:** We have made the requested changes.

**Discretionary Revisions**

**Comment 8:** Page 4 ‘relationship’: the direction of the association could be said

**Response:** We have made this change.

**Comment 9:** Page 5 ‘see below’ this reference in unclear
Response: We have now corrected this oversight.

Comment 10: Page 3 the term 'cross-sectional association'. Rephrase

Response: We have now reworded and clarified this sentence.

Comment 11: Page 5, first paragraph. Reference could be provided, as it is provided in page 6.

Response: We have now added two references in regard to the dental exam procedures.

Comment 12: Results: If the properties of the study are provided as results, a subheading could be used. Alternatively, information about the study population should be provided in the methods section.

Response: We can place this information under a subheading if the editor agrees with this formatting approach.

Comment 13: In Result section, page 9: ‘Pair-wise comparisons…’. Since p-value is a confounded measure, I suggest that authors do not emphasize the results based on p-values. Is this sentence really needed?

Response: We have left the p-values for the readers’ information but added a caveat to the methods section beginning on page 8 encouraging caution in the interpretation of p-values from this analysis.

Comment 14: Information on the distributions could be presented in the form of figures

Response: If the editor finds it acceptable, we would prefer to present the explicit numbers.

Comment 15: ‘Conclusion’ could be changed to ‘Conclusion and Implications’, because the conclusion starts with an implication

Response: We would be happy to accept this suggestion if it conforms to The Journal’s formatting standards.

Comment 16: Authors could also mention as a limitation that no combinations of different clinical measurements were studied.

Response: The reviewer raises a good point that we failed to address. In fact in our original analysis we did study whether a combination of the top clinical definitions would enhance the observed correlations but found that this post hoc definition did not substantially outperform the original definitions studied. We
have now added this information on pages 7, 8 and 10.
Response to Reviewer 2

This cross-sectional study examined patients of the INVEST study. Four bacteria are defined as being the “etiologic burden” and correlated with clinical periodontal parameters. The clinical parameters that show the highest correlation coefficients with the defined “etiologic burden” are then suggested to be the primary exposure variables for cardiovascular disease studies.

Comment 1: The definition of the "etiologic burden" is arbitrary and creates a circular argument. What is the evidence that these bacteria represent the “etiologic burden” for either periodontal disease or CVD? The authors indicate that this is based on "previous research". Evidence could be presented that this "etiologic burden" is indeed related to periodontal disease in the INVEST study after one takes into account smoking and blood glucose metabolism. This would offer weak evidence, but would be better than evidence.

Response: It seems that there is a misunderstanding of our methodological approach, possibly due to a lack of clarity on our behalf. We have outlined several points below that we hope will clarify our approach.

First, our aim is not to demonstrate etiology of the four species we include in the “etiologic burden”. It is simply to demonstrate which whole mouth clinical periodontal constructs correlate best with an underlying bacterial burden (whether the burden is “etiologic” or not cannot be determined in these data).

Second, we agree with the reviewer that the evidence for causality of the agents included in our “etiologic burden” is incomplete and after careful consideration, we can understand how our use of the term may be misleading. To address this concern, we have removed the term “etiologic burden” and replaced it with the more generic and less suggestive term, “bacterial burden”.

Finally, we feel it is important for us to more clearly delineate for the reviewer our conceptual framework and rationale for including the four aforementioned species in our “bacterial burden score”. Our decision to use these four species as a marker of pathological biofilms was based on the 1996 Consensus Report on Periodontal Diseases: Pathogenesis and Microbial Factors[2] which deemed there to be “Strong Evidence for Etiology” in regard to *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Tannerella forsythia* (formerly, *B. forsythus*). The committee that authored the report included many of the top experts in regard to the microbiology of clinical periodontal disease. Our addition of *Treponema denticola* to the etiologic construct is based on the aforementioned literature published since the 1996 consensus report; specifically, the important paper by Socransky *et al.* in 1998 describing the “Red Complex” in which T. denticola covaried strongly with T. Forthysia and *P. gingivalis*[3]. Since that time, the scientific evidence in support of this concept has grown stronger (for original research see[3-11]; for comprehensive reviews
Accordingly, we have previously published data on the association between *A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythia*, and *T. denticola* and clinical periodontal disease in INVEST[16]. We have now noted these previous findings in the discussion in accordance with the reviewer’s suggestion. However, we want to reinforce the fact that although these four species were strongly related to clinical periodontal disease in INVEST, we did not create the concept based on INVEST findings which minimizes the potential for our approach to be circular or *post hoc*. Rather the general idea of the “etiologic burden” was conceptually developed at the time of the original INVEST design and based primarily on the 1996 Consensus Report described above.

In addition, we wish to clarify with the reviewer that our conceptual framework does not require any of these bacterial species to be causal of periodontal disease (or CVD for that matter). Rather, we believe that the combined burden of the four species (*A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythia* and *Treponema denticola*) is likely to be highly correlated with truly underlying causal species. As such, these four species serve as epidemiologic markers of pathological biofilms, even if these species are not themselves causal. While we acknowledge this to be an assumption, it is an assumption based on a substantial body of literature regarding bacterial associations with clinical periodontal disease.

Whether *A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythia*, and *T. denticola* are truly causal is a question to be resolved by future intervention designs and laboratory studies as the reviewer notes. However, the fundamental idea that relatively elevated levels of these species are likely to be highly representative of an “etiologic” or “pathological” biofilm – either as causal agents themselves or as strong correlates of the true causal agents – is well supported by the literature as noted above. Importantly, we have emphasized once in the abstract and twice in the discussion (pages 11 & 14) that we cannot know for certain if these species are truly etiologic but rather, that we view them as strong correlates of the underlying causal agents. Since the true causal species are unlikely to be determined soon (if ever) we believe our strategy to be an appropriate compromise between current scientific ambiguities concerning bacterial causes of periodontal disease which require more research, and the need for methodological research regarding periodontal infections and cardiovascular disease to move forward.

Therefore, our focus on the aforementioned four species is meant to be reflective of our conceptual framework determined *a priori* over 10 years ago in the initial design and enrollment of the INVEST cohort. It was then, and continues to be now, supported by the scientific literature demonstrating an *association* between these species and clinical periodontal disease in the INVEST population and others. For this reason, while the concept might be controversial, we do not
believe our logic to be circular, unjustified or inappropriate.

**Comment 2:** Many systematic reviews have indicated that factors such as smoking and diabetes are etiologically related to both periodontal disease and CVD and this information is not included in the analyses of this manuscript.

**Response:** As noted above, we have previously published on the relationship between bacterial species and clinical periodontal disease[16]. The design of our previous analysis[16] was such that individual periodontal sites (up to 8 per person) were included as the unit of analysis allowing us to determine the within person association between bacterial colonization level and clinical periodontal disease. In doing so, we were free of any residual confounding due to between person characteristics such as smoking status, blood glucose levels, gender etc.

In addition, we have also previously published data demonstrating that in INVEST, “etiologic burden” levels are not related to smoking status (or pack years)[17]. In fact, this is one aspect of the “etiologic burden” that is so appealing to us from an epidemiological standpoint because it is an exposure variable intimately linked to the underlying biofilms related to clinical periodontal disease while at the same time having virtually no correlation with smoking. In large part due to research conducted by the reviewer, it is now well established that smoking is one of the most prominent threats to validity of published findings regarding periodontal disease/systemic disease associations (Hujoel et al. 2002)[18]. We fully agree with the importance of accounting for smoking induced bias in this research area and believe that the nonassociation between smoking and the etiologic burden in the INVEST population is a notable advantage to our approach. Thus our concept is that factors like smoking, diabetes, and adverse bacterial pattern work together to cause (or to mark causes of) periodontal disease. This does not imply anything about whether smoking, diabetes and adverse bacterial pattern cause or are caused by each other.

For the reviewer’s information, we also provide here, results from the current analysis among the subgroup of participants without diabetes as well as among the subgroup of never smokers.

**Diabetes:**
Among participants without diabetes, the best clinical definition was the % of sites with BoP (r=0.62) followed by %PD≥3 mm (r=0.59) and total BoP sites (0.59); all p-values <0.0001.
Among participants with diabetes, the best clinical definition was %PD≥3 mm (r=0.66) followed by the % of sites with BoP (r=0.60) and total BoP sites (0.56); all p-values <0.0001.

**Smoking status:**
Among never smokers, the best clinical definition was %PD≥3 mm (r=0.62) followed by the total number of sites with BoP (r=0.60) and the % of sites with BoP (r=0.59); all p-values <0.0001. Among former smokers, the best clinical definition was the % of sites with BoP (r=0.63) followed by %PD≥3 mm (r=0.58)
and total BoP sites (0.55); all p-values <0.0001. Finally, among current smokers, the best clinical definition was the % of sites with BoP (r=0.69) followed by total BoP sites (0.65) and %PD≥3 mm (r=0.64); all p-values <0.0001.

Moreover, the same three clinical constructs noted above were consistently the strongest correlates of “etiologic burden” among gender subgroups. Therefore, this demonstrates a lack of any strong evidence for either effect modification or confounding in regard to our reported associations between clinical periodontal constructs and bacterial burden.

We now report these general findings in the results section on page 10.

We now hope that we have clarified for the reviewer our conceptual approach and the fact that it was established a priori in an attempt to better focus our scientific hypothesis and remove bias. We feel that our approach can help in more closely modeling periodontal infection as an exposure for systemic disease.
References


