Author’s response to reviews

Title: Pro-Adrenomedullin predicts 10-year all-cause mortality in community-dwelling patients: a prospective cohort study

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Reviewer reports:

Alexander Limkakeng (Reviewer 1): Title: Pro-Adrenomedullin predicts 10-year all-cause mortality in community-dwelling patients: a prospective cohort study

In this paper the authors conduct secondary analysis of the PARTI intervention trial (Procalcitonin-Guided Antibiotic Use vs. a Standard Approach for Acute Respiratory Tract Infections in Primary Care). They attempt to determine the prognostic capability of serum levels mid-regional pro-adrenomedullin (ProADM) at baseline and 7 days after enrollment into the PARTI trial. In this substudy, they analyze serum levels of ProADM in patients in primary care practices who are being treated for upper respiratory infection. They then determine 10 year rates of all-cause mortality, and secondarily of major adverse events.

The question is an important one for primary care physicians and those interested in novel biomarkers generally. Unfortunately, the relatively small sample size and low event rate does not
allow for strong confidence in the validity of the findings, even though the authors note these limitations.

- Major Compulsory Revisions

1. ProADM has been studied in a variety of conditions, but there is no plausible mechanistic link advanced as to why higher proADM levels would be linked to mortality 10 years down the road. There are so many intervening events that it really calls into question the true validity of this association. The low N and the seemingly selected hazard ratio comparisons further support that this may be a coincidental, spurious finding.

Reply: Thank you for your comment. We agree that the pathophysiology of ProADM is incomplete understood and thus more research is needed. Still, several reports are in line with our which strengthen the rational of our analysis and make our results more plausible. We discuss this point as follows in the revised manuscript: “Our results are in-line with previous research reporting associations of ProADM and short-term mortality in inpatients as well as in primary care populations (25, 43-49).”

“Although there is no clear understanding why an increase in proADM points to increased mortality risk, existing data suggests that elevated ProADM levels reflect disease severity and endothelial and cardiovascular dysfunction (11-19). Also, higher ADM levels increases cardiac output, induces hypotension and vasodilation, and increases glomerular filtration rate and fractional sodium excretion (10, 19, 53), thereby inducing a reduction in cardiac pre- and afterload (20).”

Because its a general risk marker we hypothesize that also in a long-term follow-up over 10 years there’s a prognostic value. Based on this study (with low power as mentioned in the limitations), ProADM is as well a long-term valid prognostic marker for patients from the community who may benefit from preventive measures. Our study suggests that ProADM should be evaluated in future long-term studies in outpatients assessing the accuracy i.e. in combination with clinical scores (Pneumonia Severity Index, CURB65 or Framingham score) and novel markers to predict long-term outcome as mentioned in the discussion.

2. Almost 20% of patients were missing followup and excluded. They perform an analysis of differences of this excluded group from the included group, and there are significant differences between groups in age, proportion of males, and median ProADM. This is not commented on, and certainly could impact results. It's possible that many patients with higher ProADM levels actually did not suffer mortality and this could weaken or dilute the effect that they see. Additionally, this proportion missing from follow up is sizable compared to the proportion suffering an event.

Reply: Thank you for your point. For 63.5% patients no data concerning ProADM blood levels was available, because blood sampling was only done in a sub-fraction of the overall cohort and, although we rther assume a missing at random, selection bias is possible. We now incorporate your point and changed accordingly and mentioned in the results part:
1. “For 291 (63.5%) patients of the initial cohort no data concerning ProADM blood levels was available.”
2. “A comparison between the initial cohort and the patients available for 10-year follow-up is presented in Table 5 (see Appendix) and present significant differences between age, gender and even ProADM blood levels.”

Further we state the dilemma of an incomplete follow-up in the discussion:

“…we are aware of several limitations. First, this is a secondary analysis of a previous trial and baseline risk assessment is incomplete as is the availability of ProADM levels in the cohort. For 291 (63.5%) patients no data concerning ProADM blood levels was available, because blood sampling was only done in a sub fraction of the overall cohort during a certain time period. Selection bias is thus possible. Second, due to the long follow-up period, a recall bias has to be considered. Further, no information was available on the cause of death, when patients were tracked through the register of deaths. Third, our sample was small and we observed only few events for the analysis of the relationship between ProADM levels and adverse outcomes.”

3. The authors didn't control for which arm of the PARTI trial in analyses. I'm not sure if antibiotic usage had significant impact on short term outcomes, but even if not, it's theoretically possible that it would impact the outcomes studied in this paper (perhaps antibiotic usage in the short term alters microbiome impacting long term outcome?).

Reply: Thank you for your point. We added two new multivariable models adjusting for age and randomisation arm (PCT group) and adjusting for age, randomisation arm (PCT group) and smoking history. There was no significant effect modification by randomisation arm (PCT group) or a positive history of smoking.

4. I'm curious about this patient population. I get that this is a secondary analysis, of a study of respiratory infections, but why would one hypothesize that this biomarker would be ideal to use in patients with respiratory infections? It's true that ProADM has been studied in a wide variety of conditions, but it gets back to the primary problem that there is no plausible link between ProADM levels and the outcomes seen, at least not in this timeframe.

Reply: Thank you for your point. There are several reports showing that ProADM is an outcome predictor for short-term mortality (e.g., Albrich WC, et al. Enhancement of CURB65 score with proadrenomedullin (CURB65-A) for outcome prediction in lower respiratory tract infections: derivation of a clinical algorithm. BMC infectious diseases. 2011;11:112.) and long term mortality (Alan M et al. Clinical risk scores and blood biomarkers as predictors of long-term outcome in patients with community-acquired pneumonia: a 6-year prospective follow-up study. Journal of internal medicine. 2015;278(2):174-84) in respiratory infection patients. For primary care – there is very little data and our study thus provide novel insights for this population. As mentioned in the discussion it is still not entirely clear why an increase in ProADM points to increased mortality risk. The elevation of blood levels in a broad spectrum of disease (COPD, acute or chronic heart failure, community-acquired pneumonia, sepsis, acute coronary syndrome, metabolic syndrome and its component and dyspnea or even nonspecific complaints) suggest that
elevated ProADM levels reflect disease severity and probably the burden of comorbidities such as endothelial and cardiovascular dysfunction.

5. There is a difference in results between all-cause mortality and composite outcome that includes death. 5 more patients had these other results. Could this largely be due to small sample size? A change in direction of results after addition of such a small number of events calls into question the strength of association.

Reply: Thank you for your point. The results for mortality and the composite endpoint go in the same direction, but associations for mortality are stronger. We agree that the power is limited, but the results still indicate that ProADM maybe an interesting mortality marker with less enthusiasm for other cardiovascular endpoints. We agree with the reviewer that further validation, however, is needed.

6. Confounders - Pack years of smoking. 45 in nonsurvivors vs. 10 in survivors. This was not controlled for in analyses and may be the real variable that explains differences in survival

Reply: Thank you for your point. We added two new multivariable models adjusting for age and randomisation arm (PCT group) and adjusting for age, randomisation arm (PCT group) and smoking history. There was no significant effect modification by randomisation arm (PCT group) or a positive history of smoking.

Minor Essential Revisions

1. Hazard ratios compared 4th quartile of ProADM vs. 1-3 quartile. Why? Would it be more robust to compare 4th quartile vs all 3?

Reply: Thank you for your point. For the visualization of the Kaplan Meier curve in the figure we compared the 4th quartile of ProADM vs. quartile 1 through 3. Thus, this is in line with the reviewers suggestion.

2. Model - they don't describe in detail what variables were included in their models, or how variables were selected.

Reply: Thank you for your point. Due to the small sample size we only included age, due it is the most important mortality predictor. Based on your recommendation we now also added randomisation arm and smoking status. Further we describe in detail what variables were included in the models in the methods part e.g: “We used STATA 12.1 (STATA Corp, College Station, TX; USA) and created univariable and bivariable (adjusting for age) as well as a multivariable model (adjusting for age, randomisation arm (PCT group), smoking history) cox regression analysis to calculate hazard ratios (HR) and area under the receiver operating characteristics curve (AUC) to investigate the predictive accuracy of Pro-ADM.”

Further the adjusting variables are stated in every table and the results part.
3. Why this composite outcome? "death, pulmonary embolism, and major adverse cardiac or cerebrovascular events (MACCE)".

How is this related to ProADM physiology?

Reply: This endpoint was predefined because some studies found ProADM to be an indicator for cardiovascular events (e.g., AtheroGene study). As our secondary outcome we thus focused on MACE and MACCE – two “standard cardiovascular outcomes” used in many trials.

4. "For 291 (63.5%) patients no data concerning ProADM blood levels was available."

Selection bias is thus possible.

Yes- why was this- blood not drawn? Not enough leftover samples? Not available for follow up?

Reply: Thank you for your point. Blood sampling was only done in a sub fraction of the overall cohort during a certain time period. Selection bias is therefore unlikely but possible. We included in our discussion: “For 291 (63.5%) patients no data concerning ProADM blood levels was available, because blood sampling was only done in a sub fraction of the overall cohort during a certain time period. Selection bias is thus possible.”

S Di Somma (Reviewer 2): This is paper is acceptable for publication

Reply: Thank you for your positive answer.