Author's response to reviews

**Title:** Plasma procalcitonin is associated with all-cause and cancer mortality in apparently healthy men: a prospective population-based study

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**Author's response to reviews:** see over
Dear BMC Medicine editor,

We would like to thank you for considering our manuscript and the reviewers for their constructive feedback. Please find our point-by-point answers below in italics. The corresponding changes are highlighted in red in the attached revised version of the manuscript.

Sincerely,

Alexandru Schiopu and Olle Melander

Reviewer 1:

In table 1: as PCT levels may be influenced by renal dysfunction, was there any association between cystatin C concentrations and PCT quartiles? Please indicate in the table if so.

- As the reviewer suggested, there is a significant correlation between PCT quartiles and plasma cystatin C levels in our population (Spearman correlation coefficient 0.304, P<0.001). These results have now been included in the manuscript text, in the results section, paragraph 3.

The difference between table 2 and 3 is not clear. Please add in the table's title the footnote that explains which variables were adjusted for in the cox regression analysis, respectively in table 2 and table 3.

- The titles of tables 2 and 3 have been modified in the revised version of the manuscript, according to the suggestion of the reviewer.
In the discussion, the pathophysiological hypothesis to explain the association between PCT level and all-cause and cancer mortality is that PCT may be a marker of chronic inflammation which in turn may participate to cancer development (at least in several localizations). However, according to me, PCT is rather a sepsis-induced inflammatory response biomarker rather than really a global inflammatory biomarker as CRP. This is illustrated by the fact that PCT is able to discriminate between sepsis and inflammatory syndroms of non bacterial (viral for example) origin, like in auto-immune disease flare were CRP is elevated and PCT not. Could the authors comment this?

- We completely agree with the opinion of the reviewer. As this is the first study linking PCT with mortality and cancer in healthy individuals, the stimuli of PCT secretion other than bacterial endotoxin are currently unknown and our discussion of the possible link between PCT and mortality in men is speculative. We have commented on this in the revised version of the manuscript, at the end of paragraph 3 of the “Discussion” section.

Lung small cell carcinoma has been associated with "false positive" serum PCT level. Although the number of lung cancer was to low in the cohort to allow the identification of a putative association between PCT baseline levels and the development of lung small-cell carcinoma, could the authors comment this in the context of their hypothesis?

- None of our study participants had a known previous history or a current diagnosis of cancer upon inclusion. However, as a pulmonary radiography examination was not included in the baseline examination, we cannot with certainty exclude the hypothesis that some of the participants might have had an undiagnosed lung cancer upon inclusion, which might have influenced plasma PCT. The relationship between baseline PCT and the total incidence of lung and bronchi cancer in our cohort (43 cases) was far from significant. Unfortunately our dataset does not allow us to specifically analyze individuals that later developed lung small cell carcinoma, as all types of lung and bronchi cancer were included in the same category.
Reviewer 2

The main concern relates to the actual performance of the PCT sensitive LIA. The functional sensitivity (FS) quoted from reference 21 does not correspond to other studies (see Becker KL et al Crit Care Med 2008;36:941) where the FS is about 50 pg/mL and healthy controls average ~ 13 pg/mL. Although there is a statistical difference between quartile 1 and quartile 4, there is great uncertainty when all the procalcitonin concentrations fall below the FS. Moreover, could this be part of the explanation for the lack of significance in women? Unless the authors have additional information regarding the assay used, this uncertainty should be acknowledged for the benefit of future investigators.

We would like to thank the reviewer for a very good comment, which led to an important change in the Methods section of our manuscript (“Data collection” paragraph). The ProCa-S assay was used for PCT measurements in our paper and not the PCT sensitive LIA, as it wrongly appeared in the first version of the manuscript. According to the BRAHMS company (currently part of Thermo Scientific) where all PCT measurements have been performed, the lower detection limit of the ProCa-S assay has been determined as being 10 pg/mL and FAS as being 17 pg/mL. This allowed for reliable measurements of plasma PCT levels in the lower range in healthy individuals. However, whereas PCT values in most men lie above the 17 pg/mL limit, PCT concentrations in most women are below this value. We have now acknowledged this limitation of the assay in the “Study limitations” section included in the discussion.
Reviewer 3

It is unclear whether PCT could be a useful marker in a prediction of an increased risk of cancer or total mortality in a clinical practise. I propose to use time dependent ROC analysis or a similar methodology and try to find a cut-off value, which could serve for the long-term stratification of risk of total and cancer mortality, respectively occurrence of colon cancer, in healthy men in clinical practise.

I would suggest change the title, as the results confirmed relationship between increased level and procalcitonine, but there is no clear cut-off for procalcitonine predicting pure prognosis.

- We would like to thank the reviewer for a very good point. As suggested, we have run ROC analyses testing the relationships between PCT and total mortality, cancer mortality and incidence of colon cancer in men. Unfortunately, the areas under the ROC curves were modest and clear cut-off values could not be established. These findings suggest that PCT measurement in healthy men has limited clinical value with respect to long-term stratification of mortality risk. We have now changed the title of the manuscript accordingly.