Author's response to reviews

Title: Serum levels of selenium and smoking habits at age 50 influence long term prostate cancer risk; a 34 year ULSAM follow-up

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Author's response to reviews:

To the Editor
Christina Chap, PhD, Executive Editor
BMC-series Journals
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Dear Madame,

Thank you for giving us the kind opportunity to clarify questions and thereby improve our manuscript: “Serum levels of selenium and smoking habits at age 50 influence long term prostate cancer risk; a 34 year ULSAM follow-up” (MS: 1201584612541311).

We have made revisions to the manuscript in line with the constructive comments from the two reviewers. For point by point details on changes made please see responses below. We are also providing a revised manuscript with the proposed changes highlighted for clarity.

Referee 1 text:

Thank you for constructive questions and comments on our manuscript, addressed point by point below:

Question 1.1 This is an interesting exploratory study in an area of importance to prostate cancer prevention. There are a few areas that require attention. First, the recent paper by Penney KL, et al., in Cancer Prevention Res April 2010, which found an interaction of plasma selenium levels and the SEP 15 gene, with prostate cancer risk, and survival should be included and discussed.

Response 1.1. Thank you for making us aware of the, for our manuscript most relevant paper by Penney et al which now has been included as reference #24 and discussed in the manuscript in lines 56-57 and 277-280 as follows:

“However, selenium may still be protective in a subset of men with specific
genetic polymorphisms of the MnSOD-gene[2] or in men with low baseline levels of serum selenium [21, 22, 23] which was not studied in SELECT but could be relevant to study further in light of recent findings[24].”

“A recent nested case-control study within the Physicians Health Study [24] analyzing polymorphisms within the selenoprotein gene, SEP15 found genetic variants associated with prostate cancer mortality and also modifying the association of serum selenium with prostate cancer survival.”

Question 1.2 The Abstract requires some clarification. The sentence, "Smokers with se-Se....had a pronounced risk for developing PrCa" should specify this risk.

Response 1.2 Thank you for making us aware that the abstract and the results section of the proposed article were not congruent on this point. The abstract has been changed to match the results section of the manuscript proper and additional data has been included in the results section of the article, in lines 31-35 of the abstract and lines 230-232 and 289-290 of the manuscript proper.

“Smokers with s-Se in the two lower tertiles (#80µg/L) experienced a higher cumulative incidence of PrCa than smokers in the high selenium tertile (Hazard Ratio(HR) 2.39; 95% CI: 1.09-5.25).”

“Taking serum selenium levels into account, presence of the A- allele of the SNP rs125701 in the OGG1gene, was observed to protect from prostate cancer (fig 3) in the high tertile of serum selenium compared to lower tertiles (p=0.029). This was almost solely an effect in smokers with a HR of 5.8 (95% C.I. 2.13-16.1) with the caveat of this being a small subgroup of the whole cohort (n=120).”

“Due to the limited size of our study, the impact of genetic variation in relation to smoking being a risk factor for prostate cancer was not possible to analyse further.”

Question 1.3 The Introduction notes that variable that may influence serum selenium, including cholesterol and sedimentation rate, were measured. How these variables influence serum selenium is not explained. The biology should be explained to the reader in a few words.

Response 1.3 Thank you for making us aware of this information gap. The relationship between ESR, total cholesterol and serum selenium levels has now elaborated in lines 74-76 and with a further clarification in lines 100-103(a similar clarification in lines 104-106 regarding the relation between the drug clozapine and serum selenium levels has also been made although this was not commented on by the referee) as follows:

“In this cohort we also had information on factors with a potential to influence serum selenium levels: Erythrocyte sedimentation rate (ESR), total serum cholesterol, and also of body mass index (BMI).”

“The baseline mean serum levels of selenium, and two factors negatively
correlated to selenium concentration: ESR and total serum cholesterol [41] did not differ between the 1005 genotyped and the full cohort of 2045 men who at baseline had their serum selenium levels determined.”

“Use of the drug clozapine may also decrease serum selenium levels [42] but none of the participants reported use of this drug at age 50, i.e. concurrently with the blood sampling for selenium.”

Question 1.4 In the Results section, the authors finding should be discussed in light of recent findings by Penney KL, et al, "A large prospective study of SEP15 genetic variation, interaction with plasma selenium levels, and prostate cancer risk and survival, in the April Cancer Prev Res.

Response 1.4 Thank you again for pointing out this recent -for our manuscript-relevant article which has been included as reference #24 and is discussed in the manuscript on lines 277-280 and 280-283 as follows:

“A recent nested case-control study within the Physicians Health Study [24] analyzing polymorphisms within the selenoprotein gene, SEP15 found genetic variants associated with prostate cancer mortality and also modifying the association of serum selenium with prostate cancer survival.”

“If, as the recent report suggest [24] and our study may be interpreted, only subgroups of men would benefit from high serum selenium levels, then beneficial effects of supplementation in interventional studies may pertain only to subgroups and therefore be difficult to detect.”

Question 1.5 Greater discussion on the discrepant results of this study and the SELECT trial should also be added to the Discussion. The topics of serum selenium and smoking, both individually and joint, continue to be an unresolved issue in prostate cancer. The effects of these variables also are likely to differ in genetically defined subgroups, as has recently been reported in several cohorts, including the Physician’s Health Study. The analyses reported in this ms. confirm that the issue of DNA repair and its possible interaction with polymorphisms in DNA repair genes is one that merits additional study.

Response 1.5 Thank you for this question and the insightful discussion on the topic. A greater discussion on the apparent discrepant results between the SELECT trial and our study has been included in lines 283-288 of the manuscript, as follows:

“There was an overall lack of effect of selenium supplementation in the SELECT trial[20]. The trial inclusion criteria did neither enrich a study population with low baseline selenium levels nor patients defined by pre-specified genetic polymorphisms. The differences in results from the interventional study, ours and another recent observational study [24] are not contradictory but rather emphasize the possibility for interventional studies in predefined groups of men with low selenium concentrations and certain genetic profiles.”

Referee 2:
Thank you for constructive questions and comments on our manuscript, addressed point by point below:

While there is a rather strong evidence for an inverse relationship between selenium and prostate cancer, the role of cigarette smoking on prostate cancer is unclear. As pointed out by the authors: it has been shown an association between smoking and selenium which makes the study biological plausible. The data material used is of good quality and especially the last author has a large experience in this type of cancer research. The manuscript is mainly clear and well written, but it is a couple of problems that should be looked upon:

Question 2.1: At the bottom of page 5 it is said "Use of the drug clozapine. none of the participants reported use of this drug". The question is: At which time do they report?

Answer 2.1: Thank you for the possibility to clarify this. The participants reported use of pharmaceutical drugs was recorded at the same occasion as the selenium samples were taken i.e. at age 50. A note of this has been added in the manuscript for clarification in lines 107-108:

"Use of the drug clozapine may decrease serum selenium levels [43] but none of the participants reported use of this drug at age 50, i.e. concurrently with the blood sampling for selenium."

Question 2.2: At the top of page 10 it is said that BMI (23.5-26.0) appeared to have a higher risk of developing prostate cancer (Table 2). Maybe, but the result is not significant.

Answer 2.2: This comment is of course relevant and the signal of the non statistical significance by choosing the word “appeared” has been emphasised by revising the wording in the manuscript in lines 198-199 as follows:

“The non-smokers and men in the middle tertile of BMI (23.5-26.0) at 50 years of age, appeared to have a higher risk of developing prostate cancer in the full cohort as well as among the genotyped men (table 2) although not to a statistically significant level for all of these groups”.

Question 2.3: I have a problem to understand Figures 2a-c. Figures 2a,b show the cumulative incidence of death without prostate cancer for smokers and non-smokers. Accordingly, Figures 2c,d demonstrate the cumulative incidence of prostate cancer. Why are the cumulative incidences 0.00 in a long period before they start increasing?

Answer 2.3: Thank you for the opportunity to clarify this most relevant issue in the manuscript on the genotyped subcohort. Figures 2a-d depict cumulative incidences of death (a,b) and prostate cancer (c,d) by tertiles 1 and 2 combined (panel a, c) and tertile 3 (panel b, d) of selenium in smokers and non-smokers in the genotyped subcohort.

Genotyping occurred at age 70 and hence no deaths occurred before this age as
seen during the period before age 70 in figures 2a-b while diagnoses of prostate cancer which for some men had occurred already before the age of 70 are included in figures 2c-d. Changes to clarify this have been included in the manuscript in lines 205, 207, 225 and in the figure legend for figure 2, as follows:

“Since the results by smoking and BMI may be influenced by the increased risk of dying early among smokers and among men with BMI over 26.0 or BMI under 23.4, we estimated the cumulative incidence of non-prostate cancer death and prostate cancer occurrence by smoking status in the full cohort, stratified by serum selenium levels in the genotyped subcohort.”

“As illustrated in figure 2a and 2b, the cumulative incidence of death without prostate cancer in the genotyped subcohort was higher in smokers than in non-smokers/ex-smokers combined, independently of tertiles of serum selenium (figure 2a low and middle serum selenium tertiles combined, figure 2b high serum selenium tertile).”

“We further explored if SNPs in the OGG1 or MnSOD gene modified the association between serum selenium levels, smoking status and prostate cancer risk presented in figure 2.”

“Figure 2a, b: Cumulative incidences of outcomes.
Cumulative incidences of death without prostate cancer for smokers (black line) and non smokers (grey line) by tertiles 1 and 2 combined (panel a) and tertile 3 (panel b) of serum selenium at baseline, 50 years of age in the genotyped subcohort. Fig 2 c, d: Cumulative incidences of prostate cancer for smokers (black line) and non-smokers (grey line) by tertiles 1 and 2 combined (panel c) and tertile 3 (panel d) of serum selenium at baseline, 50 years of age in the genotyped subcohort. Tertile serum selenium definitions; tertile 1 and 2 combined: <81µg/L, and tertile 3: >81µg/L.”

Editor’s comments:

A declaration on the lack of competing interests has been included as a separate section as requested in lines 304-305:

“Competing interests
The authors declare that they have no competing interests.”

Details of the format of the article have been revised to conform to the journal style. These changes have not been highlighted in the manuscript since they are considered minor corrections none of which have an impact on the scientific content of the article. One detail has not been changed according to the journal style and that is the format of Table 2 which we would if possible like to keep in landscape format. However if this is strictly crucial for a publication decision, we will of course change this format also.

Thank you again for the possibility to improve our manuscript.
With sincere hopes for a positive decision on your side on the publication of our manuscript, best wishes

Birgitta Grundmark, M.D. on behalf of the authors