Author's response to reviews

Title: Use of Beta-blockers and Mortality Following Ovarian Cancer Diagnosis: A Population-Based Cohort Study

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Author's response to reviews: see over
Reply to review

Concerning manuscript number 2004917058311386 entitled "Use of Beta-blockers and Mortality Following Ovarian Cancer Diagnosis: A Population-Based Cohort Study".

We are grateful for the careful review of the manuscript. Below we reply to each comment and we have revised the manuscript accordingly. We believe that the manuscript has improved and we hope you will find it suitable for publication. We are of course willing to make further changes if necessary.

We submit a clean copy of the manuscript.

Should you have questions or concerns regarding the manuscript, please do not hesitate to contact us.

Yours sincerely,
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Reviewer 1

The authors evaluated the association between beta-blocker use and all-cause mortality in a Danish population of women (20+ years of age) with ovarian cancers during 1999-2010. They did not find any association between beta-blocker and mortality following ovarian cancer diagnosis.

Major Compulsory Revisions (which the author must respond to before a decision on publication can be reached)

1) The main exposure of interest, beta-blocker use, was defined based on use BEFORE a woman’s ovarian cancer diagnosis. The authors’ reason for doing that was “to avoid introducing immortal bias, i.e. that users would appear to survive longer because a user by definition had to survive to become a user [14].” A solution given by the cited article by Suissa was to treat the exposure as time-depending covariate in a Cox model, which would remove the bias and at the same time, capture new users during the follow-up period as some women had a relatively long follow-up time (those who were diagnosed in earlier years) and might begin using beta-blocker as they got older, etc. Defining the exposure only based on use prior to diagnosis would falsely classified women who began use after the diagnosis as non-users.

Reply: We are grateful for the reviewer’s consideration on this matter. However, we chose to classify patients by use before/at time of diagnosis for several reasons. First, we aimed to mimic an “intention to treat” method (Lemeshow S, Sorensen HT, Phillips G, Yang EV, Antonsen S, Riis AH, Lesinski GB, Jackson R, Glaser R: β-blockers and survival among Danish patients with malignant melanoma: a population-based cohort study. Cancer Epidemiol Biomarkers Prev, 2011, 20:2273-2279), which is justified because the diagnosis of ovarian cancer should not affect use of β-blockers. Second, as mentioned in the article, experimental studies show that β-blockers inhibit progression of ovarian cancer cells. Any inhibitory effect could be exerted early in the carcinogenesis and we therefore feel that classification based on use before/up to diagnosis is the most relevant. Finally, although time-depending exposure may be an alternative solution it does also have some drawbacks. As mentioned in the reference by Suissa this technique assumes “that initiation and interruption of treatment are subject to random censoring, in the absence of which approaches such as inverse probability of censoring weighting can be considered.” In our
study, initiation and interruption of treatment would not be random. Another issue is that with time varying exposure starting after diagnosis, patients still have to survive until their first prescription to become exposed (thus immortal time bias is not necessarily eliminated).

It is correct that our method may not capture new users, e.g., women with relatively long follow-up time, and potentially results in non-differential misclassification and bias towards the null. However, long-term survival is less likely to be ovarian-cancer related than related to other causes, e.g., cardiovascular mortality.

We have now added the following sentence to the methods (page 6, paragraph 2, lines 11-13):

“We chose to assess exposure prior to diagnosis to mimic an intention-to-treat method [9] and because we hypothesized that β-blockers exert an inhibitory effect early in ovarian cancer progression.”

2) Also, at the bottom of p5, “all prescriptions for beta-blockers redeemed by study subjects before their diagnosis date” were used to define exposure status. Women were diagnosed between 1999 and 2010 but prescription records were only available since Jan 1, 1998. Thus, there was a differential length of time for defining exposure by diagnosis year, i.e. women who were diagnosed in later years were more likely to be exposed since they had a longer period or more opportunity to have a prescription record in the database, compared to women who were diagnosed in early years. A solution would be restricting to beta-blocker use since a year prior to diagnosis (and through the end of follow-up, and defining use as time-depending covariate) since everyone had a minimum of 1 year of prescription history in the database. Same is true for comedication use of the agents considered in the study.

Reply: Although left censoring would not affect the current user definition, we agree that it may affect the former user and non-user definitions. We have therefore changed the definition to only consider prescriptions redeemed within the year prior to ovarian cancer diagnosis. We then re-fit the model with this definition and the new results are presented in the article. Regarding time-depending variable assessments, please see reply to comment 1.

We now write the following on page 6, paragraph 2, lines 4-8:
“We defined three exposure categories: (1) current users were those redeeming at least one prescription within 90 days of ovarian cancer diagnosis, (2) previous users were those redeeming their last prescription in the interval 90 to 365 days prior to ovarian cancer diagnosis, and (3) nonusers were those with no prescription records of β-blocker use within 365 days of ovarian cancer diagnosis.”

3) *Consider adjusting for the comorbid conditions (listed on top of page 7) as time-dependent covariates and include information post-diagnosis.*

**Reply:** We did consider all covariates as potential confounders in the model building stage of the analyses. However, we only included those that had an impact on the estimates (please see section on statistical analyses). Regarding time-dependent covariates please see our reply to comment 1.

4) *I’m surprised that no data on cancer treatment was presented or included in the model. How many women were treated and free of cancer? It would be highly correlated with mortality.*

**Reply:** We did not include information on cancer treatment because it is not available for the entire study period (unavailable from 2004 and onwards). Also, treatment may be on the causal pathway since the hypothesis was that β-blockers affect ovarian cancer progression and thereby stage, which in turn affects choice of treatment.

We have no information on how many women were cured of their cancer. The suggestion from the reviewer is not entirely clear for us: does the reviewer suggest that cancer-free survival should be evaluated 1) as a separate outcome, or 2) as a covariate. The first option was not possible for us since we did not have data on it. Also, as the reviewer points out, such outcome would be highly correlated with the current mortality outcome and would not be appropriate as a potential confounder as it could be part of the causal pathway (Schisterman EF, Cole SR, Platt RW. Overadjustment bias and unnecessary adjustment in epidemiologic studies. Epidemiology. 2009;20(4):488-95.)

5) *Women were diagnosed between 1/1/1999 and 12/31/2010, but end of follow-up was also*
Does it mean that some women had no follow-up time contributed (those dx in Dec 2010)? Why not require a minimum follow-up time like 6 months or a year?

Reply: Yes, some women were possibly censored early in the follow-up, but this censoring is independent of β-blocker exposure and does therefore not introduce bias. Likewise, patients diagnosed in, e.g., 2005, could also be censored within a month due to emigration. The important thing is that the measure of effect was hazard ratios and not cumulative incidence proportions and thus the estimates are not dependent on a minimum follow-up time.

6) How many deaths were ovarian cancer-related? Since the hypothesis was that beta-blocker highly inhibits ovarian cancer progression, I wonder if it’d be more appropriate to evaluate ovarian cancer-specific deaths instead of all-cause mortality.

Reply: Unfortunately, we do not have information on cause-specific mortality.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1) Background, 2nd paragraph, “Previous research on the association between beta-blocker use and mortality following malignant melanoma, …”

Reply: This has been corrected.

2) That same sentence, “…, have shown consistent results between the protective effects observed ex-vivo and in a population-based setting [9].” It looks like only 1 study was cited. So where are the “consistent” results?

Reply: We thank the reviewer for noting this and we have now added the references.

3) The next sentence, “However, data on the effect of beta-blockers on mortality following ovarian cancer in a population-based setting are sparse.” Please provide reference(s).

Reply: We have revised as recommended.
4) P5, end of Study Cohort, please spell out ATC when mentioned it for the first time.

Reply: We have now spelled out the abbreviation.

5) Please justify the use of 90 days when defining exposure categories.

Reply: We have now added the following description to justify this choice (page 6, paragraph 2, lines 13-15):

“In Denmark, most prescriptions cover 30 to 90 days. To assure that we captured most current users, we therefore chose 90 days as the exposure window.”

6) What was the time axis used in Cox models? Please specify.

Reply: This has now been specified (page 8, paragraph 1, lines 3-4): “Using time since diagnosis as the time scale, we then used Cox proportional hazard regression…”

7) In the overall model, was stage at dx adjusted for? If so, how were the missing/unknown cases handled?

Reply: No, we chose to stratify on stage rather than to adjust for it because the hypothesis was that β-blockers affect ovarian cancer progression and thereby stage. Hence, stage could be on the causal pathway making adjustment incorrect (Schisterman EF, Cole SR, Platt RW. Overadjustment bias and unnecessary adjustment in epidemiologic studies. Epidemiology. 2009;20(4):488-95).

8) Was year of dx evaluated and adjusted for in the Cox model?

Reply: No it was not included, but we agree that it is relevant. We have therefore added a variable for the year of diagnosis and included it in the model. The model was re-fit with this variable and the new exposure definition (see comment 2).
9) In the analyses of the duration of use, why is the definition “plus 90 days”?

Reply: Because the time period of exposure from the last prescription also should be included.

10) Also, please clarify the duration of use analyses. Were they only among current users (vs non-users, so to exclude previous users? And when looking at months of use, that was also restricted to current users (vs non-users)? I believe the duration of use analyses should also incorporate use post-diagnosis and treat duration as time-dependent so to capture all use during the study period.

Reply: The analysis for duration of use was only among current users. The analysis for months of use was in the entire population. Nonusers provided a reference group in all comparisons. Regarding time-varying covariates please see reply to comment 1.

11) Months of use was included as a linear variable in the model. How about including it as categorical variable (e.g. 1-6 mo of use, 7-12 mo of use, etc.) which may be more meaningful to interpret.

Reply: Since information is lost when categorizing a continuous variable, we chose to include it as the latter.

12) Results for the duration of use analysis was HR=1.00 with 95% CI= 1.00-1.01. Why is the CI so much narrower than the CIs for the main analyses? Is that a typo?

Reply: The results are correct. The large cohort makes the estimates precise.

13) Table 2. Please remove the crude HR column.

Reply: The crude estimates are necessary for the reader to evaluate the confounding impact of the variables in the model. Thus, we find the crude estimates important.
Discretionary Revisions (which are recommendations for improvement but which the author can choose to ignore)

1) When assessing the proportional hazards assumption, in addition to graphically examine the log-log plots, it’d be more assuring if a formal statistical test was done (test for the interactions between time and exposure).

Reply: We consider a formal statistical test not to be as informative as a graphical approach. For example, the statistical test may turn out significant due to few events at the extremes of follow-up. Such uncertainties would be illustrated in the graphical test.

2) I wonder if a subgroup analysis on older women would show any significant findings. The study included women 20+ years of age, but the median age was 65 with 56 as the 25th percentile. Both mortality and use of beta-blocker are more prevalent among older women.

Reply: As mentioned in the statistical analysis, there were no statistically significant interactions between any of the covariates. However, after re-fitting the model with the new exposure definition (prescriptions within a year before diagnosis) and adjusting for diagnosis year, the results do suggest effect modification by age. This is described in the results (page 9, paragraph 4, lines 20-23 continued on page 10):

“There were no statistically significant interactions between any of the covariates and ß-blocker use, except for age. To examine the clinical relevance, we therefore stratified the results by age group. Although rather imprecise, the estimates indicated a tendency towards higher HRs in older age groups (Table 1 in Additional File 2: Tables for secondary analyses and stratification by age). The imprecise estimates for the age group below 40 years, made this strata inconclusive.”
Reviewer 2

The authors should be commended on a well-designed and statistically sound study that addresses an important question. The large sample size is certainly a strength of this study and the authors have done an excellent job of utilizing the resources available through the Danish medical system that allow this type of large cohort study to be performed.

Minor Essential Revisions:
1) Minor grammatical error: on page 10, line 22, should read "platinum- and taxane-based...".

Reply: This has now been corrected.

Discretionary Revisions:
1) In the first paragraph of the discussion, the authors focus primarily on data from nasopharyngeal carcinoma. Although it makes sense to include some of this data since it is their own prior work, there is also a body of similar data in ovarian cancer that they only briefly refer to in one sentence (page 10, line 12). I would recommend that the authors consider shifting the focus of this first paragraph in the discussion towards prior published pre-clinical data about this topic in ovarian cancer, specifically since that is the disease being investigated in this paper.

Reply: We agree that this change would improve the discussion. We have therefore changed the paragraph to read (page 10, paragraph 3, lines 14-24):

“Previous in vitro data on cancer cell lines suggest that β-blockers exert an antitumor effect via direct action on ovarian cancer cells [1-3]. It has been shown that treatment with adrenergic agonists could upregulate the production of matrix metalloproteinase (MMP)-2, MMP-9, and VEGF in ovarian cancer cell lines resulting in increased invasive capability [1, 3]. This effect was mediated through β-adrenoceptors and was blocked by treatment with the β-antagonist propranolol [1, 3]. Similar results have been found for other malignancies, e.g., malignant melanoma, multiple myeloma, and nasopharyngeal carcinoma [4-11]. Finally, it has been shown that presurgical ovarian cancer patients with low social stress have lower VEGF levels possibly linking stress, and hence β-adrenergic agents, to tumor angiogenesis [2].”
2) Consider rewording the sentence on page 11, lines 3-4 to be clearer. One possibility would be, "Finally, and most importantly, because the authors decided that to be considered exposed a patient had to have used beta-blockers for at least 6 months, they also had to have survived at least 6 months, and therefore immortal time bias may entirely explain the increased survival among beta-blocker users."

Reply: We thank the reviewer for this suggestion and have changed the sentence accordingly.

3) Table 1 includes a large amount of comorbidity data that makes the table a bit difficult to read. I am not sure that it is necessary to include all of it. Some of these comorbidity data are referred to in the discussion section when the authors explain that they tried to adjust partly for lifestyle factors that may have influenced outcome by using some of these comorbidities such as history of obesity, COPD, and ischemic heart disease as a proxy for lifestyle factors. I think it makes sense to include data about these comorbid conditions in the table since this data is discussed in the text of the manuscript. However, there are several other comorbidities in Table 1 that are not discussed within the manuscript and thus it is not clear why they are included in the table. Some examples are tremor, anxiety, esophageal varicies, migraines, and hysterectomy. Many of these such as tremor, migraines, and anxiety seem more like indications for beta-blocker use than actual co-morbidities that may influence survival. Since the authors also used the Charlson Comorbidity Index to report any significant difference in co-morbidities between non-users, current users, and previous users, they may consider cutting out some of the more detailed comorbidity data to make the table easier to read and more concise. If the authors do indeed feel that it is important to include all of this co-morbidity data then I would urge them to discuss its importance in the text of the manuscript.

Reply: We considered all the variables as potential confounders in the model-building stage of the analysis. Only those that resulted in a meaningful change in the log hazard ratios were considered confounders and were included in the model. Because the role of confounding was considered for all variables, we think it is important to present them in the article. Also, they provide a good description of the cohort with regard to comobidity burden and
comedication use.

4) Similarly, in Table 1 there is a large amount of data regarding comedications, however there is no discussion in the manuscript about why this data is important. The authors mention that current and previous users of beta-blockers more frequently used the comedications identified, but they do not explain why this is clinically significant. For example, does it matter if these patients were also taking anxiolytics and/or antipsychotics? The authors should make it clear why they are including this data if they choose to include it in the table.

Reply: Please see reply to comment 3.