Author's response to reviews

Title: Cardiac Glycosides Use and the Risk of Lung Cancer: A Nested Case-Control Study

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Version: 2 Date: 2 July 2014

Author's response to reviews: see over
Reviewer: Olaf H Klungel

This is a well-written paper of a robustly designed and analyses case-control study demonstrating that cardiac glycosides (CGs) are not associated with an increased risk of lung cancer. This is an important safety message, particularly in the light of previous publications that seemed to point in a direction of an increased risk.

Major compulsory revisions:

Since a major difference between previous studies and this one seems to be the selection of the base population (in this study restricted to subjects with a potential indication for CGs) I suggest that a stratified analysis that investigates the association in those with AF and those with HF separately. As the authors mention, CGs have in recent years moved down the management pathway and currently probably only have an indication in subjects with HF and AF.

As noted by the Reviewer, previous studies compared patients using digoxin to non-users, with the latter mainly composed of individuals without any of the CG-related indications. As these indications and lung cancer share common risk factors, any association would be confounded by the indication. Furthermore, there is also a possibility for detection, since patients with CHF and AF are more regularly seen in clinical setting than non-diseased individuals. For these reasons, we decided to limit the study population to patients with CG-related indications. That being said, we do not see any biological mechanism of how the indication itself (i.e. CHF vs AF) would act as an effect modifier of the association between digoxin and lung cancer, i.e. do we expect digoxin users with CHF to be at a greater risk of lung cancer than patients with AF (or vice versa)? Moreover, many patients have both CHF and AF, which then adds another level of complexity in the analyses. Thus, for simplicity and because there is no clear biological basis for such an analysis, we elected to not stratify on the indication.

The analyses of dose and duration are probably looking at similar categories. A high cumulative dose presumably is given during a longer duration. Would the authors be able to make a distinction, for instance in the duration analysis among those who are more adherent (receiving higher cumulative doses) and those who are less adherent?

The Reviewer raises an interesting point. We agree that there is likely a strong correlation between cumulative duration of use and cumulative dose, likely owing to the fact that digoxin has a narrow therapeutic index. The analysis proposed by the Reviewer is an interesting one, but unfortunately, it would require larger numbers of exposed cases and controls. With 377 exposed cases, this study is not powered to conduct such analyses, which would require a further categorization of the different duration categories.

Though numbers will be small, I wonder if a duration category of 60 months is long enough (5 years). It might be worth considering an even longer-duration category of >10 years, although I understand that numbers may be limited. Can the authors comment on this possible limitation of relatively short term follow-up?

This is a very good point which we now considered in the revised manuscript. Overall, there were 89 cases and 972 controls that were prescribed CGs more than 5 years. Among them, only 16 cases...
and 207 controls underwent a CG therapy more than 10 years. The rate ratios were 1.04 (95% CI: 0.78 – 1.39) and 0.79 (95% CI: 0.45 – 1.39) for these two duration categories, respectively. We now provide these additional analyses in the revised manuscript (Results section/ Page 8).

“A total of 89 cases and 972 matched controls and 16 cases and 207 matched controls used CGs for more than 5 and 10 years. The use of CGs for at least 5 and 10 years was not associated with an increased risk of lung cancer (RR: 1.04, 95% CI: 0.78-1.39 and RR: 0.79, 95% CI: 0.45-1.39, respectively)”

Minor essential revisions:
Page 8, Discussion. The first paragraph describes the conclusion on use of CGs and breast cancer in women (and not lung cancer). This must be an error that the authors can be trusted to correct.

This is a typo, which is now corrected in the revised manuscript (Discussion section / Page 9).

Reviewer: Jennifer Nicholas

This is an interesting and original paper that examines the association between use of cardiac glycosides and lung cancer. The study makes use of a large population based cohort of patients registered with general practices in the United Kingdom, which provides an excellent opportunity to examine this question. In general, the paper is clearly written and addresses some important clinical issues. The methods are appropriate and clearly explained. I have no major concerns with the paper, but I have given some minor revisions below.

Minor Essential Revisions

In the statistical analysis section (p6) please give details on how missing data was treated in the adjusted models. From table 1, there were around 10% missing data for smoking and 17% for BMI. It is not clear whether a complete case approach was used or if some other method of accounting for missing data was considered.

When variables had missing values, we created an ‘unknown’ category so that all patients are included in the analysis. This is now stated in Methods section (Page 6 and updated in Tables 1 and 4):

“Variables with missing information were coded with an ‘unknown’ category.”

It is not clear which time point was used to derive the mean of 73.9 years of age (p7). Was this at time of entry into the cohort?

Age was calculated at cohort entry, i.e. at the time of the CG-related diagnosis. This is now specified in the revised manuscript (Results section, Page 8):

“and the mean (standard deviation [SD]) age at cohort entry was 73.9 (11.5) years”

In table 2, 3 and 4 please indicate the numbers of cases and controls included in the adjusted analyses, if this differed to the numbers included in calculation of the crude RR. (see also previous point regarding the missing data methods).
As mentioned above, the creation of ‘unknown’ categories allowed us to include all patients in the adjusted analyses. Thus, the numbers of cases and controls did not differ between crude and adjusted analyses.

The first sentence of the discussion refers to breast cancer (p8). Should this read lung cancer?

We agree, and have corrected the manuscript accordingly (Discussion section / page 9).

In the third paragraph of the discussion, it is mentioned that sex hormones may act as a trigger in a small subset of patients, perhaps those predisposed to hormone related cancers (p8). Does this refer to those carrying genetic mutations that increase risk of hormone related cancers, or is it a more general point about multiple types of risk factors that could predispose to these sorts of cancer?

This point refers to polymorphism in some different genes related to estrogen metabolism which were found to increase lung cancer incidence. In the cited reference, the authors raised the question about a sub-population of patients which may be more “sensitive” to sex hormones. This point is detailed in the revised manuscript (Discussion section / Page 9):

“It is possible that sex hormones may act as an oncogenic trigger in a small subset of patients, possibly those predisposed to hormone-related cancers and who carry some particular polymorphisms in estrogen metabolism related genes, as was previously suggested”

The following section (p8) suggests that additional studies in never-smokers are needed to identify subgroups who may be at risk of sex hormone triggered lung cancer. Please clarify why particularly non smokers should be studied for this reason. The results of the current study do not suggest any different effect of GCs in never smokers (Table 4). So, further explanation of the reasoning behind studying the non smoker sub group would be helpful.

Since tobacco is the strongest risk-factor for lung cancer, tobacco use may override the effects of other exposures on that outcome. For this reason, a number of studies focusing on smaller risk factors of lung cancer are conducted among the non-smoker population. However, we feel that this section may be off topic, and to improve the flow of the discussion, it was removed from the revised manuscript (Discussion section / page 9).

Discretionary Revisions
If space permits, the introduction could be made clearer, on two points
- The authors state on p3 that there “has been interest in assessing whether use of CGs is associated with incidence of breast cancer”. The studies referenced here appear to be case-control studies, could the authors provide specific details on the evidence for the association between CG and breast cancer. For example, did any of the referenced studies find positive evidence for this association and how strong was the relationship?

We agree, and have added some details on these papers in the revised manuscript (Introduction section / page 3).
“Namely, two case-control studies found that the use of digoxin was associated with an increased risk of breast cancer (RR: 1.30, 95% CI: 1.14-1.48 and RR: 1.39, 95% CI: 1.32-1.46) respectively.[4, 5]”

- Please clarify what is meant by “there is evidence that female sexual hormones can play an important role in lung cancer carcinogenesis”? Is this evidence from lab studies or epidemiology? Is it purported that higher levels of female sexual hormones increase or decrease lung cancer risk?

As suggested, we have expanded that section (Introduction section / page 3):

“There has also been interest in the effect of CGs on the incidence of lung cancer. Indeed, there are data supporting a role of female sexual hormones on lung cancer carcinogenesis,[10] which raises the hypothesis that the use of CGs may be associated with an increased risk of lung cancer. The main epidemiologic argument is the dramatic increasing of non-small cell lung cancer in women over these last decades. [11] In addition, some observational studies found an association between lung cancer and some reproductive factors. [12–14] Biological rational is led by the finding that estrogen receptors are frequently and notably expressed in lung cancer tumors. [15–17]”

Reviewer: ZHEN WANG
This work demonstrated a nested case-control study between CGs medication and lung cancer risk with the use of CPRD database. In general, the big-population study is well-designed and analyzed, and provides convincing data suggesting that use of CGs is not associated with an increased risk of lung cancer. As clinical evidence with regard to the association between the medication of CGs and the risk of lung cancer is quite limited, this work added interesting evidence and compared with other related work in this field. However, more words are suggested to be added in the discussion part for the reasons as below:

1) Many studies have revealed potent anti-cancer activity of CGs in vitro, and some derivatives of CGs are under clinical trials for cancer therapy, including lung cancer, the authors need to add references to show this, and may wish to discuss this inconsistency to some extent between lab work and clinical results. (Minor essential revisions)

We agree with the Reviewer, and have added references to the Discussion section (page 9):

“Moreover, recent studies have revealed potent anti-cancer activity of CGs in vitro, and some derivatives of CGs are currently being investigated for cancer therapies in clinical trials. [18, 19] CGs may act as inhibitors of hypoxia-induced factors and inducers of immunogenic cell death, possibly through the MAP Kinase pathway”.

2) Despite lung and breast cancer, correlation of CGs with other type of cancer in clinical studies is better to be added in the discussion part, to help readers understand the whole picture for the use of CGs in the oncological indications (as seen in Menger L et al. Trial watch: Cardiac glycosides and cancer therapy. Oncoimmunology. 2013 Feb 1;2(2):e23082.) (Minor essential revisions)

We thank the Reviewer for this point, and have added the suggested reference to the revised manuscript (reference #20, discussion section, page 9):
“However, only a few clinical studies have assessed the effect of CGs on oncogenesis, with heterogeneous findings. Therefore, the potential of CGs as anticancer drugs remains to be fully evaluated. [20]”