Author's response to reviews

Title: Breast density in birth cohorts of Danish women: A longitudinal study

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Author's response to reviews: see over
June 13, 2013

Dear Editor,

We have addressed point-by-point all comments by reviewer Giske Ursin on our manuscript, and hope that you will find the comments addressed to your satisfaction. We present results on individual level data from two independent high quality screening programs. Birth cohort effects in mammographic density has not been addressed in previous studies, and we find that it is highly relevant to address possible time trends in women's mammographic density, since mammographic density is one of the strongest known risk factors for breast cancer. We hope that you will find our manuscript improved for publication.

Yours sincerely,

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Point-by-point response to Reviewer's report

Title: Breast density in birth cohorts of Danish women: A longitudinal study

Version: 3 Date: 25 April 2013

Reviewer: Giske Ursin

Specifically - major concerns:

1. Concern regarding gliding assessment over time, and the possibility that the radiologist’s previous assessment can have affected the current one: This is a limitation of the design, and should be stated as such. The fact that 31% of the mixed density mammograms were, upon a reread reclassified as BI-RADS 1 seems high, and confirms my concern.

   The possibility of this type of bias, which some might call a reporting bias, should be added as a limitation in the discussion. This is a serious limitation of the paper, and may have resulted in systematic bias, i.e. failure to find an effect of age. It therefore needs to be spelled out clearly.

   Answer: It is not correctly cited from our manuscript that 31% of the 87 women with mixed/dense mammographic density was reread as BI-RADS 1 at re-evaluation. Only 1 woman (1%) out of the 87 women with mixed/dense breasts was reread as BI-RADS 1. From the Methods section page 6, 2nd paragraph, we describe, that among 87 women with mixed/dense mammographic density, 27 women (31%) were classified as BI-RADS density code 2, 54 women (62%) as BI-RADS code 3, and 5 women (6%) as BI-RADS code 4. We find that this number seems fairly reasonable in perspective of the BI-RADS density definitions, since BI-RADS density code 1 is defined as women with glandular tissue in 0-25% of the breast, code 2 as glandular tissue in 25-50% of the breast, code 3 as 51-75 % of glandular tissue in the breast, and code 3 as glandular tissue in more than 75% of the breast. We found that only 3 women were misclassified, manually exploring these 3 women’s records showed, that they were borderline cases with changed density status over time.

   We acknowledge that qualitative measures of mammographic density are less precise than quantitative measures of mammographic density, which we have now clearly added in the section of Discussion page 12, 2nd paragraph (line 2-6): "A limitation of the study was the qualitative
dichotomous measure for mammographic density available defined as mixed/dense relative to fatty mammographic density by senior radiologists. Since qualitative measures tend to overestimate the degree of density and are less precise than quantitative measures of mammographic density, subtle changes in mammographic density in the age-period-cohort modeling could potentially be masked.

However we do believe, that the comparison between the dichotomous outcome and the BI-RADS distribution confirm, that the dichotomous mammographic density outcome overall performed well compared with the BI-RADS distribution in a subsample of 118 women with also BI-RADS measures available. Also, we restricted the analysis to the period between 1991-2001, where the mammographic density assessment is consistent. From the definition in our manuscript, we also clearly state how the dichotomous outcome is defined, and the reader can from the manuscript clearly comprehend potential weaknesses in the manuscript, which we acknowledge is important.

2. Sensitivity analysis confirming age- and birth cohort effects. It is not clear based on what is presented (or explained in the letter) why this confirmed the age-effect.

Answer: The sensitivity analysis was based on consecutive regression models in separate datasets restricting on screening period. Within each screening period, we found the same birth cohort and age pattern as in the overall analysis presented in Table 4. This means that the age and birth cohort effects cannot be explained by systematic period effects and fluctuations over time in the density assessment conducted by the same team of senior radiologists. The density assessments were conducted by highly trained senior radiologists, and by the same team of radiologists, and no major changes in screening practice was conducted in the studied time period in our study.

We have now clearly stated the purpose of the sensitivity analysis restricting on screening periods in the section of Results (page 11, 2nd paragraph, line 9-13): ”Sensitivity analysis in subgroups of women with mammographic density assessments conducted within the same screening period confirmed the age- and birth cohort patterns found in the overall data (data not shown), and it therefore seems unlikely that the age and birth cohort effects were explained by systematic period effects and fluctuations over time in the density assessment between screening periods.”
3. Potential for SES bias, in particular BMI: Since there is no data that BMI did not adversely affect drop-outs in the study, we do not know if that occurred. Again, this is a limitation that should be spelled out in the discussion.

Answer: In the section of Discussion (page 12, 2nd paragraph, line 16-20 to page 13, 1st paragraph, line 1-3) we have added the sentence: "We find it unlikely that selection bias affected our results since coverage differed between the two programs with similar results. However, we did not have information available on body mass index (BMI) for the whole cohort and systematic bias could have occurred, if obese women from younger birth cohorts were more likely to abstain from mammography screening than obese women from older birth cohorts, since obesity is inversely associated with mammographic density\textsuperscript{25}. In a subanalysis of 5134 women with available data on objectively measured BMI from the Diet, Cancer, and Health cohort study\textsuperscript{26}, we found no systematic differences across birth cohorts among obese women abstaining from mammography screening (data not shown)."

4. There is something strange with these findings. I am concerned about the classification, and in particular the proportion of fatty to mixed dense. Why this is a reasonable method should be backed up further. Also this should be commented on in the discussion - as a limitation.

Answer: We present the data, we had available from the Copenhagen and Funen mammography screening programs, both programs perform in accordance with European guidelines. As mentioned in our first point-by-point response, previous studies have been published based on the dichotomous outcome for mammographic density in the Copenhagen and Funen mammography screening programs. The qualitative dichotomous measure available was based on assessments by the same team of expert senior radiologists from the two programs in the studied time period. We believe that potential weaknesses of the dichotomous outcome is now clearly addressed in the section of Discussion as described ad 1.

5. Background: The sentence "on perinatal exposures" should be omitted. This is still rather far-fetched, and in my mind detracts from the manuscript.
Answer: We have omitted the sentence on perinatal exposures in the Background section.

6. Discussion: General comment: The findings from this study should be toned even further down. Also see 1-4 above for limitations that need to be spelled out more clearly in the discussion.

Answer: In the section of Discussion (page 15, 3rd paragraph, line 1-2) of the study, it is clearly stated, that this evidence is suggestive and should be replicated in larger individual level data. However, we did add to this sentence, that it would be preferable in a replicative study to use a quantitative measure for mammographic density: "The results of the current study warrant future replication in larger individual level data with longer follow-up and with preferable a quantitative measure for mammographic density."

7. The second sentence in the discussion is somewhat misleading. It sounds as if the authors have done a completely different analysis on individual data. What I think the authors mean is that they found that the age effect was not observable after adjusting for birth cohort.

Answer: The meaning of the sentence as it stands is correct in perspective of the analysis presented in Table 2, where we found a stable pattern in the probability of mixed/dense breasts across age within each birth cohort as depicted in each step-wise diagonal chain of squares representing birth cohorts.

8. There are several strong statements in the discussion that recall, reporting and selection biases were unlikely. The recall bias issue is not an issue and should be simply omitted. There might very well be strong reporting bias (see 1 above). This statement should be omitted, and a more thorough discussion of the possible for biases should be added. As for selection bias, see below.

We have added more discussion of potential bias in the discussion (ad 1-7) and have further added a discussion of weaknesses of the age-period-cohort modeling in the section of Discussion (page 13, 1st paragraph, line 5-8): The age-period-cohort modeling in Table 4 was not adjusted for
reproductive or life style risk factors pertaining to women's lives, since we did not have information available for the whole cohort, wherefore residual confounding cannot be completely ruled out. "

9. The sentence on selection bias should also be altered - there might very well be selection bias, the authors simply do not have that information - see SES/BMI above.

Answer: We have addressed this issue in ad 3 and ad 8.

10. Discussion - 3rd para, first sentence - needs to be reworded.

Answer: We have reworded the sentence, now standing: "The birth cohort adjusted odds of mixed/dense breasts across period remained steady in Copenhagen but increased in Funen (Table 4)"

11. Note that the absence of known systematic changes does not mean that there were none. This sentence on page 13 should be altered.

We have changed the sentence in the section of Discussion (page 13, 2nd paragraph, line 9-12): "We therefore restricted the analysis to the period 1991-2001 where no systematic changes in screening practice or mammographic density assessment took place in the two programs, to reduce the probability that systematic changes in screening practice could have caused the findings attributed to the age- and birth cohort effects."

12. The sentence that the true biological decline is not expected to be constant…is hard to follow with the double negative, and I disagree, there could be other gradual changes.

Answer: We have omitted the sentence: "Further, since the true biological decline is not expected to be constant over the age span studied, our findings are unlikely to be explained by a drift in the data caused by the late change in screening practice."

13. The discussion does not mention that the measure of density used is a rather crude measure. This should be acknowledged.
Answer: Please see ad 1, where we have addressed this issue.

Also, addressed in the section of Discussion (page 15, 1st paragraph, line 1-3): "Further, it is questionable whether the dichotomous variable in mixed/dense versus fatty mammographic density would be sensitive enough to detect a moderate decrease in mammographic density during menopause."

14. The change in % density from 44% to 34% (Verheus et al.) is not a small change. The sentence describing the "small" decreases related to menopausal status is therefore misleading and should be rewritten. The fact that no change was seen from age 50-51 to age 52-53 is a strange argument. This should be toned down as "evidence".

Answer: We have altered the "small" to "moderate" in the section of Discussion (page 14, 2nd paragraph, line 9-12): "Mammographic density decreased during menopause from 44% at premenopausal status to 34% at postmenopausal status. These longitudinal data from screening populations unanimously showed a moderate decrease in mammographic density related to changing menopausal status and moderate changes at later age.

15. Bottom paragraph on page 14: it would probably be better, or at least less controversial if the authors removed the "higher background burden...." and replace with "a number of factors". I suggest adding a comment that changes in formulation/dose of postmenopausal hormone therapy could have mattered.

Answer: We have altered the sentence accordingly in the Discussion (page 15, 2nd paragraph, line 4-9): "This difference may be explained by a higher number of hormone related exposures in younger compared with older birth cohorts of women, among others changes in postmenopausal hormone therapy over time could have had an impact. A higher number of cohort borne breast cancer risk factors in younger compared with older birth cohorts of Danish women has previously been linked to the increasing breast cancer incidence across birth cohorts of women over time in Denmark [5]."
16. The BCSC finding on page 15 is left hanging - it should be moved up or removed altogether.

Answer: This sentence has been moved to page 13, 3rd paragraph, line 3-5 to page 14, 1st paragraph, line 1: "Though birth cohort trends in women's mammographic density have not been investigated in previous studies, the largest study on longitudinal measurements from the Breast Cancer Surveillance Consortium did report a BI-RADS density distribution across age-groups consistent with the birth cohort effects found in our study."

17. There is no discussion of the general limitations with age-period cohort modeling. This should be added. Then the conclusion should sum up that this type of analysis have a number of limitations and must be interpreted with care.

Answer: In Table 2 and Table 3, we only present numbers according to the binominal distribution without further statistical modeling. With regards to the statistical regression modeling in Table 4, please find AD 8 and below, where we address this issue of limitations by the age-period-cohort modeling in Table 4.

In the Discussion (page 13, 1st paragraph, line 5-8) we have mentioned limitations of the age-period-cohort model of residual confounding: The age-period-cohort modeling in Table 4 was not adjusted for reproductive or life style risk factors pertaining to women's lifes, since we did not have information available for the whole cohort, wherefore residual confounding cannot be completely ruled out."

In the section of Discussion (page 15, 3rd paragraph, line 1-3): "The results of the current study must be interpreted with care and warrant future replication in larger individual level data with longer follow-up and with preferable a quantitative measure for mammographic density and adjustment for reproductive and life style covariates."

In the section of Conclusions (page 16, 2nd paragraph, line 1), we have added that the data should be interpreted with care due to the limitations of the age-period-cohort modelling in Table 4, where
two variables were included due to risk of collinearity: "The results must be interpreted with care and should be replicated in future studies."

**Minor:**
Suggest change mammographic density status to mammographic density category to make it easier to understand.

**Answer:** We have made the changes accordingly throughout the manuscript