PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	A Population-based Case-Control Study on Social Factors and Risk of Testicular Germ Cell Tumours
AUTHORS	Ahrens, Wolfgang; Schmeisser, Nils; Conway, David; Stang, Andreas; Jahn, Ingeborg; Stegmaier, Christa; Baumgardt-Elms, Cornelia; Joeckel, Karl-Heinz; Behrens, Thomas

VERSION 1 - REVIEW

REVIEWER	Katherine A. McGlynn Senior Investigator National Cancer Institute U.S.A. I have no competing interests.
REVIEW RETURNED	04-Mar-2013

THE STUDY	It appears that the case group is composed of gonadal and non-gonadal germ cell tumors, rather than being composed solely of testicular cancers. According to the Methods section, cases could have cancer of the testis, epididymis, spermatic cord or extragonadal germ cell tumors of the mediastinum, retroperitoneum or brain. In addition, the stratification of the cases by histology is also not entirely clear. It would be helpful if the authors would provide the ICD morphology codes used to define seminoma and nonseminoma. For example, spermatocytic seminoma (morphology code 9063) should not be included under the category 'seminoma' as it is not a classic seminoma. In the interest of comparing these findings with the other literature on social status and testicular cancer, it would be beneficial to restrict the current manuscript solely to testicular cancers.
RESULTS & CONCLUSIONS	There is not a great deal of discussion about similarities or differences with the existing literature.
GENERAL COMMENTS	Minor comments: 1) As the manuscript notes, both personal and family history of testicular cancer are established risk factor for testicular cancer. Were the study participants queried about personal or family history? 2) The first paragraph of the Discussion section indicates that the findings of this study are in line with the findings of other newer studies. Please note that the studies cited, however, are not recent publications. 3) The second paragraph of the Discussion section suggests that

	'Increased risks in agriculture and related occupations are not explained by social factors but rather with exposures such as pesticides, fertilizers or contact with farm animal and zoonotic infections…' Please note that while it has been suggested that associations with farming and testicular cancer might be related to the factors mentioned, these hypotheses are not proven. 4) Some speculation about why the association with socioeconomic status and testicular cancer may have changed over time would be beneficial.

REVIEWER	Assoc. Prof. Ladislav Dusek, Ph.D. director Institute of Biostatistics and Analyses Masaryk University Brno, Czech Republic
REVIEW RETURNED	18-Mar-2013

THE STUDY	The study addresses interesting topic which deserves research. Aetiology and risk factors of colorectal cancer are still relatively unknown and the this work brings some interesting inputs to the running discussion in the literature. The manuscript is well written, chapters are consistent and mutually corresponding. Detailed methodical section makes the section Result easy to understand. However, several outcomes should be more carefully described with respect to real statistical significance and with respect to real statistical power of the study. Otherwise, some of the findings could be misinterpreted or overestimated. It refers also to some of the conclusions written to the abstract and commented in the Discussion section.
RESULTS & CONCLUSIONS	• Please clarify the number of analysed control subjects or provide a study flow diagram explicitly stating the number of patients. For cases, it is clear that 353 – 54 – 29 – 1 equals to 269, but 2014 – 552 – 512 – 182 equals to 768 and not 797 as stated in the Abstract. • Similarly - in Table 1, please check the number (sample size) of control subjects matched to seminoma and nonseminoma cases. Obviously, 725 + 682 is not equal to 797. If the number 725 and 682 are correct, please specify the matching of control subjects for seminoma and nonseminoma cases in more detail. • In the Results section of the Abstract, the authors claim that "An increased testicular cancer risk was observed for subjects with an apprenticeship (OR=1.5 [95%-CI: 0.9-2.5]) or a university degree (OR=1.5 [95%-CI: 0.9-2.6]) relative to those whose education was limited to school." I cannot agree with such a statement because the width of the reported confidence intervals (both include value 1) suggests a lack of statistical significance of these results. Moreover, these results indicate that, in fact, there might be a 10% decrease in the testicular cancer risk in those two groups of men. So, speaking about increased risk of testicular cancer is an overly strong statement in this situation. • The same problem is in the Results section – see the text on page 8 before Table 2. Moreover, the Discussion section should be corrected accordingly. Exact estimate of proper p value should be given in all sentences concluding some risk of the cancer. • In the Results section, summarizing length of job history and age with mean +/- SD is not appropriate because the data are obviously

	highly asymmetric. Please use rather median and range …or some proper percentile range. • Formal comment - in Table 2, decimal commas should be corrected to decimal points. • The first sentence on page 9 does not make sense. No difference in what? • Page 9, the authors claim that "A modest increased risk was observed for seminoma cases where the risk increase was restricted to the lowest category (OR=1.4; 95%-CI 0.8-2.4)." Regarding the fact that the 95% CI includes 1, this is again an overly strong statement. It would be better to write: "Although not statistically significant, the seemingly higher risk was observed for...". • Regarding numerous outcomes which indicate some potential risk association, but not supported by exact statistical significance: what is the real statistical power of the study – some comment should be added to the methodical section in this sense, - the power also should be in adherence with the study experimental plan. Was the power optimized also for separated analyses of seminoma and nonseminoma cases? • The main conclusion of this study that "Occupation as farmer or farm worker entails an elevated risk of testicular cancer, possibly due to related exposures." should be more discussed in the Discussion section because the effect was different for the considered histology groups: statistically significant for seminomas (OR=2.4 95%-CI 1.1-5.0) but statistically not significant for nonseminoma cases (OR=1.6 95%-CI 0.5-4.8). What are the reasons of such difference, can it be discussed with some reasoning or is it a consequence of statistical error of the study, or lack of power (nonseminoma) ? • Please, check carefully your English. See for example page 10: "This pattern was replicated for almost all analysis presented in table 4."…there are several such typing errors.

VERSION 1 – AUTHOR RESPONSE

Reviewer Katherine A. McGlynn

Major comment

Reviewer*: It appears that the case group is composed of gonadal and non-gonadal germ cell tumors, rather than being composed solely of testicular cancers. According to the Methods section, cases could have cancer of the testis, epididymis, spermatic cord or extragonadal germ cell tumors of the mediastinum, retroperitoneum or brain. In addition, the stratification of the cases by histology is also not entirely clear. It would be helpful if the authors would provide the ICD morphology codes used to define seminoma and nonseminoma. For example, spermatocytic seminoma (morphology code 9063) should not be included under the category ?seminoma? as it is not a classic seminoma. In the interest of comparing these findings with the other literature on social status and testicular cancer, it would be beneficial to restrict the current manuscript solely to testicular cancers. There is not a great deal of discussion about similarities or differences with the existing literature.*

Reply: We agree. We restricted the analyses to testicular cancer and deleted three cases from our analyses (spermatocytic seminoma (N=1), tumours of the epididymis (N=1), spermatic cord (N=1)). Accordingly, we changed the title of the article to ' A Population-based Case-Control Study on Social Factors and Risk of Testicular Germ Cell Tumours'. In the study subjects and methods section we now report the number of cases by ICD-O and morphology codes.

Minor comments:

1) Reviewer*: As the manuscript notes, both personal and family history of testicular cancer are established risk factor for testicular cancer. Were the study participants queried about personal or family history?*

Reply: Personal and family history were indeed part of the interview (cf. Bromen et al. Testicular, other genital, and breast cancers in first-degree relatives of testicular cancer patients and controls. Cancer Epidemiol Biomarkers Prev. 2004 Aug;13(8):1316-24.). We specified this fact as follows in the section on study subjects and methods: 'The interview entailed questions about familial characteristics, family history of cancer and other diseases, medical conditions since childhood, chemical and physical exposures and an occupational biography for every job held 6 months or longer.'

2) Reviewer: *The first paragraph of the Discussion section indicates that the findings of this study are in line with the findings of other newer studies. Please note that the studies cited, however, are not recent publications.*

Reply: We agree and deleted the word 'newer' in the sentence.

3) Reviewer: *The second paragraph of the Discussion section suggests that ?Increased risks in agriculture and related occupations are not explained by social factors but rather with exposures such as pesticides, fertilizers or contact with farm animal and zoonotic infections?? Please note that while it has been suggested that associations with farming and testicular cancer might be related to the factors mentioned, these hypotheses are not proven.*

Reply: We admit that this is a strong statement and changed the corresponding statement as follows: 'It has been suggested that the observed risks in agriculture and related occupations could be associated with specific exposures such as pesticides,[33, 35] fertilizers[36, 37] or contact with farm animals and zoonotic infections[33] which were not in the scope of this study.'

4) Reviewer: *Some speculation about why the association with socioeconomic status and testicular cancer may have changed over time would be beneficial.*

Reply: In the discussion section we now speculate: "If a social gradient for testicular cancer in Germany existed in the past and exposures were associated with this gradient, this gradient may have been attenuated by an increase in exposures that do not differ by social position or for which the social gradient declined over time. The rising trends of testicular cancer in industrialised countries might be an indirect indication for such an increase of exposures that are (or have become) independent of social position."

Reviewer: Ladislav Dusek

1) Reviewer: *The study addresses interesting topic which deserves research. Aetiology and risk factors of colorectal cancer are still relatively unknown and the this work brings some interesting inputs to the running discussion in the literature. The manuscript is well written, chapters are*

consistent and mutually corresponding. Detailed methodical section makes the section Result easy to understand. However, several outcomes should be more carefully described with respect to real statistical significance and with respect to real statistical power of the study. Otherwise, some of the findings could be misinterpreted or overestimated. It refers also to some of the conclusions written to the abstract and commented in the Discussion section. My detailed comments are added in the sections below.

? Please clarify the number of analysed control subjects or provide a study flow diagram explicitly stating the number of patients. For cases, it is clear that 353 ? 54 ? 29 ? 1 equals to 269, but 2014 ? 552 ? 512 ? 182 equals to 768 and not 797 as stated in the Abstract. ?

Reply: Thank you for this comment, we corrected this sentence to "Participation was denied by 552 control subjects, 32 were excluded due to insufficient language skills, 512 moved away, died or were never reached."

2) Reviewer: *Similarly - in Table 1, please check the number (sample size) of control subjects matched to seminoma and nonseminoma cases. Obviously, 725 + 682 is not equal to 797. If the number 725 and 682 are correct, please specify the matching of control subjects for seminoma and nonseminoma cases in more detail. ?*

Reply: We added: "Controls were removed from the subgroup analyses if no matching case was available in an age-group×study region stratum." in the section describing the statistical analysis. We also added in the section on study subjects and methods: "Due to overlap of the age distribution of seminoma and non-seminoma cases, the majority of controls matched to, both, seminoma and non-seminoma cases. Thus, for the analyses by histologic subgroup, 725 controls were matched by age and region to the seminoma cases while 682 controls were matched by age and region to the nonseminoma cases'.

3) Reviewer*: In the Results section of the Abstract, the authors claim that ?An increased testicular cancer risk was observed for subjects with an apprenticeship (OR=1.5 [95%-CI: 0.9-2.5]) or a university degree (OR=1.5 [95%-CI: 0.9-2.6]) relative to those whose education was limited to school.? I cannot agree with such a statement because the width of the reported confidence intervals (both include value 1) suggests a lack of statistical significance of these results. Moreover, these results indicate that, in fact, there might be a 10% decrease in the testicular cancer risk in those two groups of men. So, speaking about increased risk of testicular cancer is an overly strong statement in this situation.*

Page 9, the authors claim that ?A modest increased risk was observed for seminoma cases where the risk increase was restricted to the lowest category (OR=1.4; 95%-CI 0.8-2.4).? Regarding the fact that the 95% CI includes 1, this is again an overly strong statement. It would be better to write: ?Although not statistically significant, the seemingly higher risk was observed for...?. ?

Reply: We agree and attenuated our statements as suggested by the reviewer. In addition, since we had deleted three cases from our analyses, the results are now a little bit different. In the case of educational training the risk estimates for the whole study sample and the seminoma study sample increased slightly since the deleted cases were in the reference group. For the analyses of ISEI the risk estimates diminished slightly. These changes did not alter the results substantially.

***4) Reviewer**: ? The same problem is in the Results section ? see the text on page 8 before Table 2. Moreover, the Discussion section should be corrected accordingly. Exact estimate of proper p value should be given in all sentences concluding some risk of the cancer. ?*

Reply: We are convinced that statistical significance alone is no proof of the existence or absence of an association between exposure and disease. We agree with the reviewer that any observed associations should be reported and interpreted with caution. However, we strive not to disregard possible associations with effect sizes that seem relevant just because they do not reach the significance level due to small numbers. This position is supported by others. As Sterne & Davey Smith stated, "in many cases published medical literature requires no firm decision: it contributes incrementally to an existing body of knowledge" (Sterne & Davey Smith, BMJ 2001). Furthermore, "epidemiologic research is an exercise in measurement. Its objective is to obtain a valid and precise estimate of either the occurrence of disease in a population or the effect of an exposure on the occurrence of disease." (Lash, Epidemiology 2007). For these reasons, we did not simply dichotomise our results according to statistical significance with an arbitrarily chosen alpha value (see also Stang A, Poole C, Kuss O. The ongoing tyranny of statistical significance testing in biomedical research. Eur J Epidemiol 2010;25:225-230).

***5) Reviewer**: In the Results section, summarizing length of job history and age with mean +/- SD is not appropriate because the data are obviously highly asymmetric. Please use rather median and range ?or some proper percentile range*

Reply: We agree and now present median and 10/90percentiles.

***6) Reviewer**: Formal comment - in Table 2, decimal commas should be corrected to decimal points. ? The first sentence on page 9 does not make sense. No difference in what? ?*

Reply: Thank you for these hints. We changed the sentence as follows: ‘No difference between cases and controls in ISEI-score was observed’. We checked the tables carefully and corrected formal errors.

***7) Reviewer**: Regarding numerous outcomes which indicate some potential risk association, but not supported by exact statistical significance: what is the real statistical power of the study ? some comment should be added to the methodical section in this sense, - the power also should be in adherence with the study experimental plan. Was the power optimized also for separated analyses of seminoma and nonseminoma cases? ? The main conclusion of this study that ?Occupation as farmer or farm worker entails an elevated risk of testicular cancer, possibly due to related exposures.? should be more discussed in the Discussion section because the effect was different for the considered histology groups: statistically significant for seminomas (OR=2.4 95%-CI 1.1-5.0) but statistically not significant for nonseminoma cases (OR=1.6 95%-CI 0.5-4.8). What are the reasons of such difference, can it be discussed with some reasoning or is it a consequence of statistical error of the study, or lack of power (nonseminoma) ? ? Please, check carefully your English. See for example page 10: ?This pattern was replicated for almost all analysis presented in table 4.??there are several such typing errors.*

Reply: The study was designed as an exploratory study as the risk factors that could explain the increasing incidence of testicular cancer are largely unknown. Thus our study was powered to detect an OR of 1.8 for any risk factor with a prevalence of 20%, and to detect an OR 0f 2.0 for any risk factor with a prevalence of 10%. The study was not powered to confirm any difference in risk for

seminoma and non-seminoma separately. This is now stated in the methods section. Regarding the discussion of the risk among farm workers see reply to the review by McGlynn above. The broad confidence intervals of the ORs for farm workers indicate statistical instability and thus limit any speculations about possible differences between seminoma and non-seminoma.

The whole manuscript was carefully language edited.